# ANTIHYPERURICEMIA ACTIVITYOF VANILLA (*Vanilla planifolia* Andrews) FRUITS ETHANOL EXTRACT TO MICE (*Mus musculus* L.) MALE

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**Abstract:** Antihyperuricemia activity test from vanilla (*Vanillaplanifolia* Andrews) fruit extract on male mice. Hyperuricemia is a disease caused by an increase in uric acid levels in the blood over the normal level. Increased uric acid levels happen because the levels of purines in the body are quite high, so that the breakdown of purines into uric acid increases. This study used dried vanilla fruit extracted using a 60% ethanol solvent. The test animals used were 24 mice which were divided into 6 groups namely group Kn (standard feed), group K- (induced with suspension of chicken liver), group K + (induced by suspension of chicken liver and given allopurinol 10 mg / kgBB), group treatments P1, P2, and P3 were induced by suspension of chicken liver and were given vanilla fruit extract with doses of 50 mg / kgBB, 100 mg / kgBB, and 200 mg / kgBB respectively. Statistical data analysis using ANOVA (Analysis of Variance) through the SPSS 15.0 program with a level of $\alpha = 5\%$ and continued with Duncan test at the level of $\alpha = 5\%$. The results of this study showed that vanilla fruit extract treatment P1, P2, and P3 had the potential to be antihyperuricemia, because it can reduce blood uric acid levels in mice induced by the chicken liver. The antihyperuricemia activity of vanilla fruit extract is comparable to the standard allopurinol chemical drug in reducing blood uric acid levels in mice statistically.

**Key Words**: Allopurinol, Antihyperuricemia, Uric Acid, *Vanilla planifolia* Andrews and Xanthine oxidase

## INTRODUCTION

Uric acid is the final production of the breakdown of purines originating from the body itself or foods containing purines [1]. Hyperuricemia is a disease caused by an increase in uric acid levels above the normal level that occurs due to excessive uric acid production and reduced uric acid excretion or a combination of both if hyperuricemia continues to occur it can cause arthritis gout [2]. A bad lifestyle such as consuming foods that contain high purine substances can increase the risk of gout. Examples of foods with high levels of purine are vegetables (long beans and spinach), *Gnetumgnemon*seeds, meat, chicken innards (liver and intestines), seafood (shellfish and shrimp) and others [3]. From each food containing purine substances, there are high purines and some are low. Foods that contain high purine substances must be avoided, or not consumed excessively because it can increase hyperuricemia. Apart from food, the body itself has produced quite a lot of purines, which are the result of the synthesis of substances in the body such as CO2, glutamine, glycine, aspartic acid, and folic acid [2].

The kidneys are associated with increased levels of uric acid in the blood, because uric acid in the blood is carried to the kidneys for excretion. One of the causes of hyperuricemia is that the kidneys are unable to excrete uric acid through urine, this can occur as a result of kidney problems such as the influence of drugs or the influence of some nutrients that can inhibit uric acid excretionurinary [1]. Normal uric acid levels in men are 3.4-7.0 mg / dL, in women 2.4 - 5.7 mg / dL and children 2.8 - 4.0 mg / dL [4].

Chemical compounds of flavonoids and alkaloids are thought to be potentially antihyperuricemic because they can inhibit the action of the xanthine oxidase enzyme, thereby reducing excess uric acid levels in the blood and treating hyperuricemia [5]. Vanilla fruit (*Vanillaplanifolia* Andrews) contains chemical compounds of flavonoids and alkaloids [6], also besides vanilla fruit contains antioxidants [7] and anti-inflammatory [8]. So far there has been no research on vanilla fruit extract as an antihyperuricemia agent. This study aimed to determine the antihyperuricemia activity of vanilla (*Vanillaplanifolia* Andrews) fruit extract on male mice and compared to standard allopurinol chemical drugs. This research is expected to be able to search scientific references for further investigation so that it can be used by the community as a standard (OHT) herbal medicine for uric acid (antihyperuricemia).

## MATERIALS DAN METHODS

This research is an experimental study using 24 mice (Mus musculus L.) test animals, aged 2-3 months with a weight of 30-40 grams. The tool used to measure uric acid levels is a measuring strip of uric acid levels and easy touch GCU (Glucose, Cholesterol, Uric Acid)

### Extraction

Making vanilla extract uses a one-stage maceration method [9]. Comparison of vanilla fruits used in this study is: 200 grams of dried vanilla fruit cut into small pieces and put into beaker glass and then added with 60% ethanol solvent as much as 2 liters for repetition of 3 times maceration, each maceration process is carried out 24 hours at 20OC- 30OC. The results of maceration are filtered and used using a rotatory evaporator at 600C, speed of 150 rpm for 14 hours.

### Making Pathological Conditions for Hperurisemia

Test animals were induced using the suspension of chicken liver to increase the uric acid level of mouse blood to hyperuricemia. On day 0 the test animal was fasted for 12 hours, then measured the initial uric acid level, as the normal uric acid level of the test animal. The treatment groups II, III, IV, V, and VI were fed a standard feed of mice and chicken liver suspension at a dose of 25 ml/kgBB given once daily orally for 14 days to approach the condition of hyperuricemia.

### Giving Treatment

The following are the details of each treatment group as follows.

Group I : Normal control (standard feed)
Group II : Negative control (induced by suspension of chicken liver)
Group III : Positive control (induced and given allopurinol suspension 10 mg/kg BB)
Group IV : Treatment 1 (induced and given vanilla fruit extract at a dose of 50 mg/kg BB)
Group V : Treatment 2 (induced and given vanilla fruit extract at a dose of 100 mg/kg BB)
Group VI : Treatment 3 (induced and given vanilla fruit extract at a dose of 200 mg/kg BB)

### Measuring Uric Acid

Measurements of uric acid levels in mice were carried out on days 0, 15 and 30. Blood collection was carried out on the mice's tail, by cutting the tip of the tail of the 0.1-0.2 cm mice using scissors that had been sterilized with alcohol, then blood is dripped on the uric acid strip which has been attached to the GCU easy touch tool and the tool will read the blood uric acid levels of mice in mg/dL. The data obtained were analyzed using ANOVA (Analysis of Variance) through the SPSS version 15.0 program at a real level of 5% and continued with the Duncan test to see the differences between each treatment.

## RESULTS AND DISCUSSION

The antihiperuricemia activity of vanilla fruit extract on male mice in this study showed that (Table 1) on day 15 all treatment groups (K-, K +, P1, P2, P3) multiplied the increase in uric acid levels compared to the normal control group (Kn) not induced. On the 30th day after treatment of vanilla P1 fruit extract treatment group at a dose of 50 mg/kgBB, P2 with a dose of 100 mg / kgBB, and P3 at a dose of 200 mg / kgBB and a positive control group (K +) decreased compared with the negative control group (K-). To clarify the average results of measurement of uric acid levels from each treatment group can be seen in Figure 1

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**Table 1. The Average of Total Uric Acid in Mice**

| Treatment | Uric Acid Levels Mice ± SD (mg / dL) | | |
|---|---|---|---|
| | Preliminary data Day- 0 | Improved data 15th day | Decrease Data 30th day |
| **Kn** | 1,40±0,00 | 1,40±0,00 [a] | 1,87±0,95[a] |
| **K-** | 1,70±0,60 | 3,65±0,69 [b] | 4,77±1,89[a] |
| **K+** | 1,80±0,80 | 3,87±0,69 [b] | 2,02±1,25[a] |
| **P1** | 1,40±0,00 | 4,05±0,61 [b] | 2,45±1,23[a] |
| **P2** | 1,40±0,00 | 3,70±0,58[b] | 2,20±0,92[a] |
| **P3** | 1,40±0,00 | 3,67±0,69[b] | 2,02±0,77[b] |

Description: Numbers followed by different superscripts show significant differences based on Duncan's test 5%

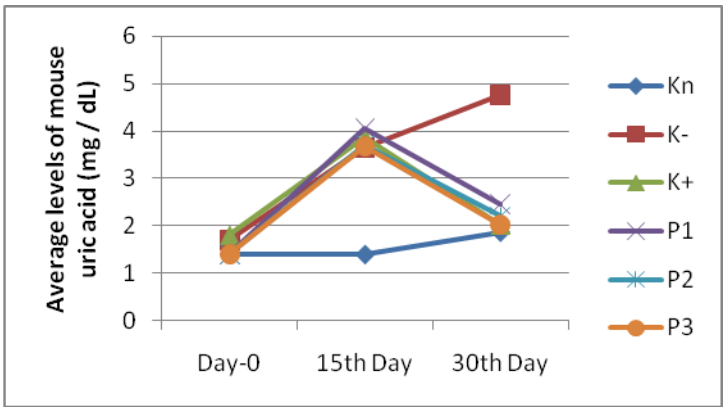


**Figure 1. Graph of mean blood uric acid levels in mice**

The difference in decline from each group (Table 2) shows that in the treatment group P1, P2, P3 and K + experienced a higher decline compared to groups Kn and K-, but each treatment group P1, P2, P3 and K + has almost the same decline in value. The following table shows the average difference between all groups in Table 2.

**Table 2. Mean Difference in Decreased Uric Acid Levels of Mice**

| Treatment | Average Decrease Difference (mg / dL) |
|---|---|
| Kn (standard feed without induction) | -0,47[a] |
| K- (standard feed after induction) | -1,12[a] |
| K + (allopurinol 10 mg / kgBB) | 1,85[b] |
| P1 (vanilla fruit extract 50 mg / kgBB) | 1,60[b] |
| P2 (vanilla fruit extract 100 mg / kgBB) | 1,50[b] |
| P3 (vanilla fruit extract 200 mg / kgBB) | 1,65[b] |

Description: Numbers followed by different superscripts show different significant based on Duncan's 5% test

In this study, the antihyperuricemia activity test carried out vanilla fruit extract (Vanilla planifolia Andrews) on male mice (Mus musculus L.) induced using chicken liver suspension. Chicken liver used for induction given once daily orally for 14 days has been shown to increase blood uric acid levels in mice. The results of the analysis (Table 1) show that the suspension of chicken liver can increase the total uric acid level of the treatment group K-, K +, P1, P2, and P3 compared to the normal control (Kn) which is only given standard feed. But the five groups of induction treatments K- and K +, P1, P2, and P3 showed statistically large increases. On the 30th day (Table 1) the treatment groups P1, P2, and P3 and the positive control group (K +) decreased compared to the negative control group (K-), although the average decrease in uric acid levels did not reach the initial level of uric acid , but the result of the decrease is close to the initial uric acid level. The results obtained in this study prove that vanilla fruit extract has antihyperuricemia activity because it can reduce blood uric acid levels in mice.

Normal uric acid levels of the test animals (Mus musculus L.) ranged from 0.5 to 1.4 mg/dL, and experienced hyperuricemia if uric acid levels in the blood reached 1.7-3.0 mg/dl [10]. The increase in uric acid levels of mice after induction varied, i.e. in the range of 2.3-7.5 mg/dl, this was due to the different conditions of individual mice. The induction of mice aims to condition mice to undergo hyperuricemia, which is done by administering High Purine Diet Foods (MDPT) in the form of suspension of chicken liver. According to Diantari and Candra [3], innards have high purine content which can increase uric acid levels in the blood of mice.

The results of the analysis (Table 2) prove that from each group P1, P2, P3 and positive control (K +) the mean decrease was almost as large, and showed that the vanilla fruit extract group P1, P2, and P3 had activity comparable to the drug chemistry of allopurinol (K +) in statistically reducing uric acid levels. The mean difference in decline for each group from high to low is as follows, in the positive control group (K +) the highest difference is 1.85 mg / dL, the vanilla P3 fruit extract group at a dose of 200 mg/kg has a difference of 1.65 mg/dL, group P1 with a dose of 50 mg/kgBB has a decrease in difference of 1.60 mg/dL, and the P2 group with a dose of 100 mg/kgBB has a mean difference in decrease of 1.60 mg/dL. Whereas in the normal control (Kn) it has a mean difference in decline of -1.125

mg/dL, and in the negative control group (K-) the mean difference in decline is -0.475 mg/dL, this occurs because in the negative control group (K-) and the normal control group (Kn) did not decrease uric acid levels, but there was an increase in the mean uric acid level.

Uric acid is the final production of the solution and disposal, which comes from purines produced by the body itself or from foods containing purines [1]. Uric acid normally functions as an antioxidant in the human body [11]. Uric acid levels in the blood can increase continuously beyond the normal limits if the levels of purine in the body increase, and trigger the plasma in the blood to become very saturated, causing hyperuricemia, which is a disease caused by an increase in uric acid levels above the normal limit caused by production excessive uric acid and reduced uric acid excretion or a combination of both. Other diseases that can be caused are gout, a buildup of monosodium urate (MSU) crystals in soft tissue, socket, and cartilage. Gout is an inflammation of the joints due to the accumulation of monosodium urate (MSU) crystals that form deposits (tofus) continuously and cause acute inflammation such as acute or chronic joint inflammation called rheumatic gout or arthritis gout [2].

Purine is a protein that belongs to the nucleoprotein group, in the body that comes from the results of nucleic acid synthesis (endogenous) or comes from food consumed (exogenous). Purines aided by the xanthine oxidase enzyme will be catabolized to uric acid which is the final product of purine metabolism [2]. The xanthine oxidase enzyme is an enzyme that plays a role in the synthesis of uric acid, which catalyzes the oxidation of hypoxanthine to xanthine and subsequently catalyzes the oxidation of xanthine to uric acid [12].

Vanilla fruit extract can act as an antihyperuricemia agent because vanilla fruit extract contains chemical compounds of flavonoids and alkaloids [6], besides that vanilla fruit has antioxidant activity [7], anti-inflammatory [8]. Based on the content of secondary metabolites, if the plants have antioxidant an activity, then they have the opportunity to have activityxanthine oxidase inhibitor [13].

Flavonoids in vanilla fruit extract [6] have a working mechanism that inhibits the action of the xanthine oxidase enzyme, which can reduce uric acid levels in the blood of mice. In addition, vanilla fruit also acts as an anti-inflammatory [8] so it can help healing due to inflammation in the joints caused by the buildup of monosodium urate (MSU), due to high levels of uric acid in the blood. Flavonoids are useful as anti-inflammatory, protect cell structures, increase the effectiveness of vitamin C, prevent bone loss and as an antibiotic [14]. According to Cos *et al.* [5], flavonoids can inhibit xanthine oxidase enzymes and have superoxide cleansers. This xanthine oxidase inhibition activity causes a decrease in blood uric acid levels.

In this study, the positive control used was the standard chemical drug allopurinol, which is one of the drugs that is often used by the public in treating gout. The working principle of allopurinol is to inhibit the activity of the xanthine oxidase enzyme to convert hypoxanthine to xanthine and then to uric acid [15]. Allopurinol inhibits enzyme activity irreversibly by being a specific inhibitor and substrate for the xanthine oxidase enzyme. The function of this drug is as an analog substrate (purine) which will occupy the active side of the xanthine oxidase enzyme. In the liver xanthine oxidase will metabolize allopurinol and produce its active metabolite, oxypurinol (alloxantine) which also plays a role in inhibiting xanthine oxidase so that it can reduce blood uric acid levels maximally [16].

## CONCLUSION

Based on the results of the study it can be concluded that:
1. Vanilla fruit extract (*Vanillaplanifolia* Andrews) has the potential as an antihyperuricemia drug for mice induced by a suspension of chicken liver.
2. Vanilla fruit extract (*Vanillaplanifolia* Andrews) has activity comparable to the chemical drug allopurinol (K +) in reducing blood uric acid levels in mice statistically.

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