# Xanthine Oxidase Inhibitory Activity of Gadung Tubers (*Dioscorea hispida* Deenst.), Common Plantain Leaves (*Plantago major* L.), Comfrey Roots (*Symphytum officinale* L.), and Asthma Weed Leaves (*Euphorbia hirta* L.)

**Ami Tjitraresmi[1*], Moelyono Moektiwardoyo[1], Evariani Dwi Wulandari[1]**
[1]Department of Biology Pharmacy, Faculty of Pharmacy, Padjadjaran University, Sumedang, West Java, Indonesia 45363
Email *: ami.tjitraresmi@unpad.ac.id

## ABSTRACT

**Aim:** This research aims to determine xanthine oxidase inhibition activity of gadung tubers (*Dioscorea hispida* Deenst.), common plantain leaves (*Plantago major* L.), comfrey roots (*Symphytum officinale* L.), and asthma weed leaves (*Euphorbia hirta* L.).

**Methods:** Each plant material was macerated with ethanol 70%. Xanthine oxidase inhibition activity of each plant extracts was carried out through in vitro assay by measuring the absorbances spectrophotometrically at 290 nm.

**Results:** Gadung tuber (*Dioscorea hispida* Deenst.), common plantain leaves (*Plantago major* L.), comfrey roots (*Symphytum officinale* L.), asthma weed leaves (*Euphorbia hirta* L.) extracts at a dose 50 µg/ml could inhibit more than 50% the action of xanthine oxidase with inhibitory values 53.49%; 62.53%; 53.49%; and 61.28%, and $IC_{50}$ of these plant extract were 47.14 µg/mL; 38.57 µg/mL; 42.93 µg/mL; And 31.57 µg/mL respectively.

**Conclusion:** Gadung tubers (*Dioscorea hispida* Deenst.), common plantain leaves (*Plantago major* L.), comfrey roots (*Symphytum officinale* L.), asthma weed leaves (*Euphorbia hirta* L.) were a good source for xanthine oxidase inhibitor and potential to be developed as anti-hyperuricemia agent. Asthma weed leaves extract had the highest xanthine oxidase inhibition activity.

**Keywords**: *Dioscorea hispida* Deenst., *Plantago major* L., *Symphytum officinale* L., *Euphorbia hirta* L., hyperuricaemia, uric acid, xanthine oxidase inhibitor

## INTRODUCTION

In recent years, hyperuricemia has become a health problem in the world, especially the elderly. Symptoms experienced by a person with gout include rheumatic pain, stiffness, and joints pain [1] In many countries, normal uric acid levels for adults are 3.5 and 7.2 mg/dL in adult males and postmenopausal women and between 2.6 and 6.0 mg/dL in premenopausal women. Disposal of uric acid in the urine is regulated by the kidneys. If the production of uric acid becomes very excessive, the level of uric acid in the blood becomes high. This condition is called hyperuricemia, while inflammation in the joint area due to deposition of uric acid is known as gout. Hyperuricemia is a risk factor for gout arthritis, kidney stone formation, and atherosclerosis [2,3].

Increased levels of uric acid can be influenced by several factors including weight, gender, age, alcohol, consumption of high-purine foods, certain drugs usage, and impaired kidney function. Types of foods that contain high purines, such as shrimp, crabs, offal, spinach and buko seeds (young coconut). Hyperuricemia prevalence tends to increase in men aged 30 years and women above 50 years old. Men are more at risk than women, because the hormone estrogen in women can control uric acid levels in the body by increasing excretion of uric acid through the kidneys [2,4,5,6,7].

In many countries, the prevalence of hyperuricemia from year to year is always increasing, as in the USA, which is 3.9% [8]. Based on the data from Global Burden of Diseases (GBD), the prevalence of hyperuricemia in Indonesia is 18%, especially in Java, which is 1.7% [9], in Taiwan the highest prevalence of hyperuricemia in men 61.5% and women 51.4 % [10].

Indonesia is a country that has the second highest biodiversity in the world after Brazil. Of the 40,000 species of flora that exist in the world as many as 30,000 species found in Indonesia and 940 species of which are known to be efficacious as drugs that have been used in traditional medicine for generations. Herbal medicines were believed to have lighter side effects compared to modern medicine. Empirically traditional medicines are able to cure various diseases, but their efficacy and abilities have not been widely proven scientifically or clinically [9].

In purine metabolism, there is a role for the xanthine oxidase enzyme in catalyzing hypoxanthine oxidation to xanthine and becoming uric acid. Inhibition of xanthine oxidase can block uric acid biosynthesis to prevent hyperuricemia. Alopurinol or uricosuric drugs such as probenezid have been used to overcome hyperuricemia however allopurinol has side effects such as diarrhea, nausea, reddish skin with itching, so it is necessary to look for plant bioactive compounds as natural inhibitors of xanthine oxidase to be used as alternative treatments that are safe to consume [11]. Compounds that have the potential as inhibitors of xanthine oxidase enzymes are tannins, flavonoids, saponins, polyphenols, and ellagic acid [11].

The prevalence of hyperuricemia in Indonesia is about 29% often occurs in Minahasa, Toraja and Batak tribes, due to habits or eating patterns, especially foods containing purines. In addition to the three tribes, hyperuricemia in the Gorontalo tribe has also become the second disease in recent years which has experienced a fairly high increase. According to the Indonesian Basic Health Research [12], there were around 8,462 Gorontalo tribal people who experienced hyperuricemia, it consisted of 5,683 women and 2,779 men.

The Gorontalo tribe still uses traditional plants as anti-hyperuricemia drugs, including gadung tuber (*Dioscorea hispida* Deenst.), common plantain leaves (*Plantago major* L.), comfrey roots (*Symphytum officinale* L.), asthma weed leaves (*Euphorbia hirta* L.). Generally, plant parts that can be used from the four plants are roots and leaves [13]. Based on the empirical efficacy of the Gorontalo tribe, the four plants are often used as antihyperuricemia, therefore, this study was conducted to prove the empirical efficacy of the four plants in inhibition of xanthine oxidase as anti-hyperuricemia based on the $IC_{50}$ values.

## MATERIALS AND METHODS

### Materials

All plant materials (Gadung tubers (*Dioscorea hispida* Deenst.), common plantain leaves (*Plantago Major* L.), comfrey roots (*Symphytum Officinale* L.), and asthma weed leaves (*Euphorbia Hirta* L.)) were obtained from Lembang, West Java, Indonesia, allopurinol, xanthine and xanthine oxidase (buttermilk) were purchased from Sigma-Aldrich, Dimethylsulphoxide (DMSO), hydrochloric acid (HCl), ethanol 70%, absolute ethanol,

Potassium dihydrogen phosphate ($KH_2PO_4$) and dipotassium hydrogen phosphate ($K_2HPO_4$) and other reagents of analytical grade were obtained from Merck (Darmstadt, FR, Germany).

**Extraction of Plant material**

Plant material is separated from impurities and dried. The dried and grountd plant materials were extraced by maceration method using 70% ethanol. The macerate was collected once every day,

followed by soaking the residue with the same solvent system. This procedure was repeated for three consecutive days. The extract is then evaporated with a rotary evaporator under reduced pressure to obtain a concentratedextract.

**Xanthine Oxidase Inhibitory Activity Assay**

Each plant extracts were analysed for xanthin oxidation inhibition activity under in vitro assays. The assay was done by measuring spectrophotometrically at 295 nm under aerobic condition, with some modifications [14,15,16,17]. A well-known xanthine oxidation inhibitor, allopurinol, was used as a positive control for the inhibition test. Xanthine oxidase enzyme from bovine milk was prepared by dilution of the enzyme to a final concentration of 0.2 Units/mL. 1 mM xanthine substrate solution was made by adding 5 drops of 1.0 M NaOH to increase the solubility of xanthine. Each ethanolic plant extracts were dissolved in 1% dimethyl sulfoxide (DMSO) and made the test concentration at 10 μg/mL, 20 μg/mL, 30 μg/mL, 40 μg/mL, and 50 μg/mL. The reaction mixture consisted of 2.9 mL of 50 mM sodium phosphate buffer (pH 7.5), 1 mL of sample solution dissolved in DMSO in various concentration, 100 μl of freshly prepared enzyme solution (2 units/ml of xanthine oxidase in phosphate buffer). The assay mixture was pre-incubated at 37°C for 15 min. Then, 1 ml of substrate solution (1 mM of xanthine) was added into the mixture. The mixture was incubated at 37°C for 30 min. Next, the reaction was stopped with the addition of 1 m of 1 N HCl. Spectrophotometer absorbance was at 295 nm, suggesting the formation of uric acid. Percent of inhibition of xanthine oxidase activity of the test sample was determined by measuring the absorbance of uric acid from the mixture without test extracts (blank samples) compared with the absorbance of a mixture of test extracts. $IC_{50}$ values were obtained by linear regression analysis of a plot a series of different sample concentrations against percent inhibition.

**Phytochemical screening**

These extracts were evaluated by phytochemical qualitative reactions for usual plant secondary metabolites. The screening was performed for alkaloids, flavonoids, phenolics, tannins, quinons, monoterpenoids and sesquiterpenoids, steroids, and triterpenoids, conducted by standard procedures [18,19, 20]. The concentrated extracts were subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures.The color intensity or the precipitate formation was used as analytical responses to these tests.

The following qualitative tests were carried out:

**Test for alkaloids**

Dragendroff's test: Two mL of extract was taken in a test tube and then 0.2 mL dilute hydrochloric acid (HCl) was included, followed by Dragendroff's reagent. A yellowish precipitation indicates alkaloid's presence.

Meyer's test: Two mL of extract was taken in a test tube and then 0.2 mL dilute hydrochloric acid (HCl) was included, followed by 1 mL of Meyer's reagent. A white precipitation indicates alkaloid's presence.

**Test for flavonoids**

One to five drops of concentrated hydrochloric acid (HCl) and Magnesium were added to little amount of ethanolic extract of the plant material. Immediate development of a red colour that can be extracted by amyl alcohol indicates the presence of flavonoids.

**Test for polyphenolics**

Five mL of extract was placed in a test tube and then 2 mL of 5 % of Ferry chloride ($FeCl_3$) solution was added. A greenish-black precipitate indicates the presence of polyphenolics.

**Test for Tannins**

Five mL of extract 1% was placed in a test tube an then gelatin solution containing 10% sodium chloride was added. Formation of white precipitate indicates the presence of tannins.

**Test for terpenoids/steroids**

Liebermann-Burchard reaction was performed to assess the presence of steroids. An ether solution of extract of each plant was placed at the porcelain crucible. The extract dried ether solution was treated with acetic anhydride and a few drops of concentrated $H_2SO_4$ were added. A blue greenish coloration indicated the presence of steroids and the purplish coloration indicated the presence of triterpenoids.

**Test for monoterpenoids and sesquiterpenoids**

An ether solution of extract of each plant was placed at the porcelain crucible. The extract dried ether solution was treated with acetic anhydride and a few drops of vanillin sulphate. A various coloration indicated the presence of monoterpenoids and sesquiterpenoids.

**Test for saponins**

Extract was diluted with distilled water to 5 mL and shaken in a test tube for 15 minutes. The formation of one centimeter layer of foam indicates the presence of saponins.

**Test for quinons**

Five mL of extract was placed in a test tube and then 2 mL of 5 % of Potassium hydroxyde (KOH) solution was added. A yellowish coloration indicates the presence of quinons.

## RESULTS AND DISCUSSION

Extraction of dried and ground Gadung tubers (*Dioscorea hispida* Deenst.), common plantain leaves (*Plantago major* L.), comfrey roots (*Symphytum officinale* L.), and asthma weed leaves (*Euphorbia hirta* L.) was done with cold maceration and followed by low pressure evaporation at minimal heating to preserve active substances contained. The four plant material tested were used to treat hyperuricemia among the Gorontalo Province in Indonesia.

**Table 1: Xanthine oxidase inhibitory activity of four medicinal plants extracts tested which are often used as remedies for gout in Gorontalo Province, Indonesia.**

| | Concentration μg/mL | Inhibition (%) | $IC_{50}$ μg/mL |
|---|---|---|---|
| Gadung tubers | 10 | 6.68 ± 1.12 | 47.14 |
| | 20 | 13.26 ± 4.11 | |
| | 30 | 30.14 ± 3.10 | |
| | 40 | 41.76 ± 5.06 | |
| | 50 | 53.49 ± 7.08 | |
| Comfrey root | 10 | 31.21 ± 3.10 | 42.93 |
| | 20 | 39.02 ± 4.11 | |
| | 30 | 42.29 ± 4.07 | |
| | 40 | 48.83 ± 6.10 | |
| | 50 | 53.49 ± 4.10 | |
| Asthma weed leaves | 10 | 35.52 ± 2.12 | 31.57 |
| | 20 | 42.83 ± 6.06 | |
| | 30 | 50.49 ± 6.05 | |
| | 40 | 54.91 ± 7.03 | |
| | 50 | 61.28 ± 9.09 | |
| Common plantain leaves | 10 | 12.51 ± 2.09 | 38.57 |
| | 20 | 29.24 ± 4.10 | |
| | 30 | 39.04 ± 6.09 | |
| | 40 | 53.44 ± 7.10 | |
| | 50 | 62.53 ± 5.08 | |
| Allopurinol | 0.1 | 15.96 ± 1.12 | 1.24 |
| | 0.2 | 19.67 ± 3.08 | |
| | 0.5 | 29.30 ± 3.06 | |
| | 1 | 47.10 ± 7,10 | |
| | 2 | 69.51 ± 6.08 | |

The results of xanthine oxidase inhibitory assay can be seen in Table 1. Gadung tuber (*Dioscorea hispida* Deenst.), common plantain leaves (*Plantago major* L.), comfrey roots (*Symphytum officinale* L.), asthma weed leaves (*Euphorbia hirta* L.) extracts at a dose 50 μg/ml could inhibit the action of xanthine oxidase with inhibitory values 53.49%; 62.53%; 53.49%; and 61.28%, and $IC_{50}$ of these plant extract were 47.14 μg/mL; 38.57 μg/mL; 42.93

µg/mL; And 31.57 µg/mL respectively. Asthma weed leaves (*Euphorbia Hirta* L.) gave the highest activity among the plant extract tested, it showed by the lowest $IC_{50}$ (31.57 µg/mL), however its activity remains not as good as allopurinol which has $IC_{50}$ 1.24 µg/mL.
The comparison of a xanthine oxidase inhibition activities from the four test extracts in various concentrates can also be described in graphical form as shown in Figure 1.

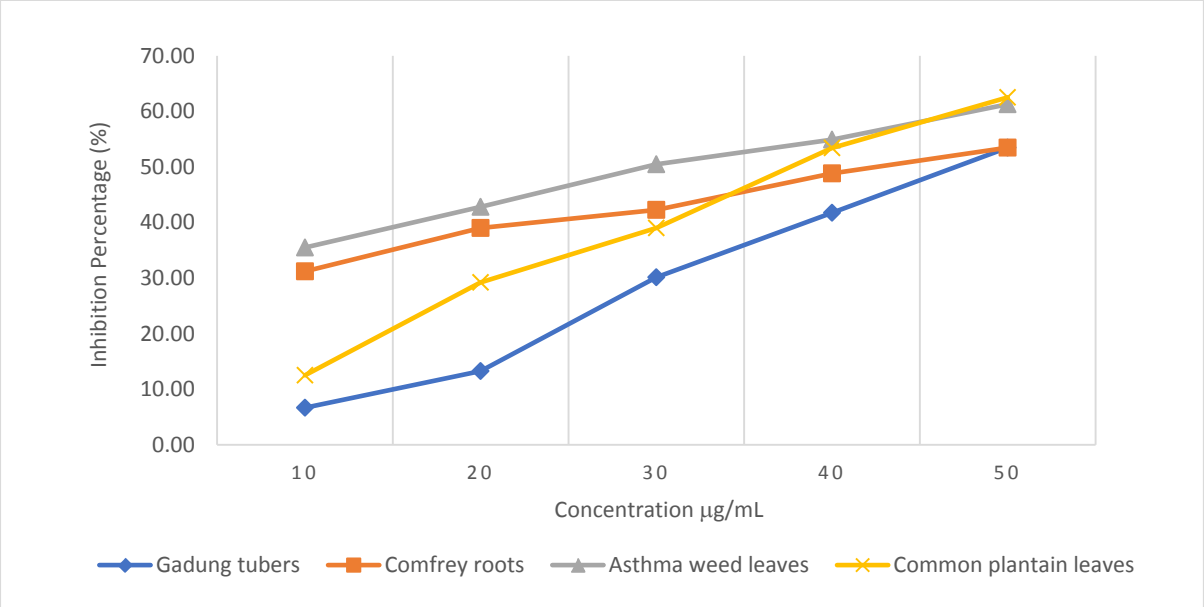


**Figure 1**: The comparison of a xanthine oxidase inhibition activities from the four plant materials extracts tested

Uric acid is a nitrogenous compound that is the final breakdown product of purine (a DNA building block) catabolism. The enzyme xanthine oxidase catalyzes the oxidation of hypoxanthine and xanthine to uric acid. Excessive production of uric acid in the body can cause hyperuricemia and high levels of blood uric acid have been associated with gout [4].

Phytochemical analysis conducted on the plant extracts revealed the presence of constituents which are known to exhibit medicinal as well as physiological activities. Phytochemical screening analysis of plant extracts revealed the presence of phytochemicals such as alkaloids, polyphenolics, tannins, flavonoids, saponins, quinons, steroids, triterpenoids, monoterpenoids and sesquiterpenoids,. The results of the phytochemical screening analysis can be seen in Table 2.

**Table 2: Results of phytochemical analysis of the four selected medicinal plants extract**

| Secondary metabolites | Extract of | | | |
|---|---|---|---|---|
| | Gadung tubers | Common plantain leaves | Asthma weeds leaves | Comfrey roots |
| Alkaloids | - | - | - | - |
| Polyphenolics | + | + | + | + |
| Tannins | - | - | + | - |
| Flavonoids | + | + | + | + |
| Steroids and Triterpenoids | - | - | - | - |
| Monoterpenoids and Seskuiterpenoids | - | - | - | - |
| Quinons | - | + | + | - |
| Saponins | + | + | + | + |

The phytochemical screening showed that the four selected medicinal plants extract with xanthine oxidase inhibition activity have polyphenolics and flavonoids, and asthma weed leaves, that has a highest xanthine oxidase inhibition activity showed that it contain polyphenolic compounds, flavonoid, saponins, tannins and kuinons. Further study is needed to investigate the chemical compound that responsible for the xanthine oxidase inhibitory activity.

## CONCLUSION

Gadung tubers (*Dioscorea hispida* Deenst.), common plantain leaves (*Plantago major* L.), comfrey roots (*Symphytum officinale* L.), asthma weed leaves (*Euphorbia hirta* L.) can inhibit xanthine oxidase activity, so they were good sources for xanthine oxidase inhibitor and potential to be developed as anti-hyperuricemia agent. Asthma weed leaves extract had the highest xanthine oxidase inhibition activity.

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