Inhibition Against Xanthine Oxidase Enzyme by *Andrographis paniculata*, *Orthosiphon aristatus*, and *Salacca zalacca* Fruit Water and Ethanolic Extracts as Antigout

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***Abstract*—Gout is a joint disease caused by the accumulation of uric salt crystals that causes an acute inflammatory response, or the accumulation of these in soft tissue (cartilage). Cat's whiskers leaves (*Orthosiphon aristatus*), bitter herbs (*Andrographis paniculata*), and snake fruit (*Salacca zalacca*) are native Indonesian plants that are commonly used to reduce uric acid levels in the body. This study tested the bioactive potency and the xanthine oxidase enzyme inhibition *in vitro* of water and ethanol extracts from these three plants to evaluate their potency as antigout. The phytochemical assay result of each extract shows the presence of flavonoid and tannin. Probit analysis results of water and ethanol extracts show a lethal concentration value of 50 (LC_{50}) below 1000 µg/mL, and the smallest LC_{50} value produced by cat's whiskers water extract is 366.34 µg/mL. The inhibitory activity of xanthine oxidase enzyme by cat whiskers leaves water extract is shown by the smallest IC_{50} value of 84.78 µg/mL, which proves that cat's whiskers leave water extract has the potency as herbal ingredient for alternative uric acid treatments.**

Keywords—andrographis paniculata, antigout, inhibitor, Orthosiphon aristatus, Salacca zalacca, xanthine oxidase

I. INTRODUCTION

Uric acid (2,6,8-trioxypurine) with molecular formula $C_5H_4N_4O_3$ is the end product of purine metabolism in the human body which comes from protein, then distributed to blood plasma, joint fluids, liver, and other internal organs, and then excreted by the kidneys in urine. Uric acid can be synthesized through the xanthine conversion process catalyzed by the enzyme xanthine oxidase (XO) [1]. Excessive synthesis or impaired elimination in the kidneys will increase uric acid levels which can lead to hyperuricemia. This situation can trigger the formation of monosodium urate crystals if the concentration exceeds 6.8 mg/dL. The accumulation of monosodium urate crystals that occurs continuously, one of which will result in excessive pain in the joints of the patient, or commonly known as gout [2]. Gout is the second most common joint disease in Indonesia after osteoarthritis. The prevalence of gout in Indonesia is in the range 1.6–13.6 per 100 000 people, which will increase with age [3].

Gout treatment strategies can be classified into two ways. The first way, namely through increasing uric acid excretion which can be done by uricosuric group drugs and the second way through inhibiting XO performance which can be done by uricostatic group drugs [4]. One of the synthetic drugs used for gout therapy is allopurinol. The use of this drug has several side effects, such as fever, chills, leukopenia, eosinophilia, arthralgia and leucocytosis. Gastrointestinal disorders can also be experienced by allopurinol drug users [5]. Therefore, it is necessary to look for drugs or other alternative materials that have no side effects but are effective in treating gout.

Natural ingredients that have been reported to have the potential to be anti-gout are sidaguri [6] and celery [7], mahogany bark [8], a combination of rosella petals and cat's root plants [9], black cumin [10] and soursop leaves [11]. In addition, other natural ingredients that have the potential to act as anti-gout include cat's whiskers (*Orthosiphon aristatus*), bitter herbs (*Andrographis paniculata*), and snake-skin fruit (*Salacca zalacca*). These three plants are plants and fruits that have many useful chemical contents [12]. There have been many studies on cat's whiskers for health. The results of research conducted by Sukisyowati [13] reported that cat's whiskers plant extracts have been used as a formula for healing diabetes, diuretics (urine shedding), anti-inflammatory (analgesic), antihypertensive, and decreased uric acid levels. In addition, the ethanol extract of the cat's whiskers has high antioxidant activity [14].

Bitter herbs plant has clinically proven its activity to influence hepatoprotective, cardiovascular, hypoglycemic,

antifertility, antibacterial, immunostimulant, antipyretic, antidiarrheal, anti-inflammatory, antimalarial, antivenom, antihepatotoxic and decreasing uric acid levels [15,16]. The antioxidant compounds in snake-skin fruit can be used to prevent and maintain the immune system, slow down the aging process, deal with stress, decrease uric acid levels, prevent degenerative diseases such as cancer, heart disease, brain disfunction, and cataracts [17,18]. However, research on the leaves of the cat's whiskers and bitter herbs and snake-skin fruit as xanthine oxidase inhibitors to reduce uric acid has never been reported. Therefore, in this study, the ethanol and water extract of the cat's whiskers and bitter herbs leaves, and snake-skin fruit were carried out for the inhibition activity test toward xanthine oxidase *in vitro*.

II. METHODS

A. Materials

The materials used in this study were simplicia of cat's whiskers, bitter herbs from the Cikabayan Tropical Biopharmaca Research Center, IPB University, and snake-skin fruit from Sumedang, aquades, 70% ethanol, chloroform, NH_4OH, H_2SO_4 2M, Meyer reagent, Wagner reagent, Dragendorf reagent, anhydrous acetic acid, concentrated H_2SO_4, Mg powder, concentrated HCl, amyl alcohol, sea water, iron (III) chloride solution, NaOH 1N, phosphate buffer 0.05 M pH 7.5, xanthine (SIGMA), xanthine oxidase (SIGMA), HCl 0.58M, allopurinol and commercial allopurinol.

B. Sample Preparation

The raw materials for cat's whiskers and bitter herbs were obtained from the Experimental Garden of Tropical Biopharmaca Research Center, IPB University. All materials are separated from dirt or other foreign materials then washed and chopped. The leaves of the cat's whiskers and bitter herbs are then dried in an oven at 50 ºC and ground until they form a powder. In contrast to the snake-skin fruit sample preparation. According to Feskanich et al [19], snake-skin fruit steamed at 60-70 °C for 15 minutes and oven-dried at 40 °C for 5 days. The dried snake-skin fruit is then ground to obtain the simplicia powder of the snake-skin fruit.

C. Water Content

The porcelain cup is dried in an oven at 105 ºC for 30 minutes, cooled then put in a desiccator for 30 minutes and weighed its empty weight. The sample was weighed about 3 grams and put into a porcelain cup. The sample and the plates were dried at 105 °C for 5 hours in the oven. After being cooled and put in a desiccat'sor for 30 minutes, the plates and their contents were weighed. The procedure is carried out repeatedly until a fixed weight is obtained by a difference of less than 1 mg. Determination of water content was carried out 3 times (triplo). The water content of the sample is calculated by the equation (1):

$$\text{Water Content (\%)} = \frac{\text{Initial Weight - Final Weight}}{\text{Initial Weight}} \text{x} 100\% \quad (1)$$

D. Extraction

The sample powder was extracted with solvent (70% ethanol or water) using the maceration method with a ratio of 1: 9. The sample and the solvent were shaken for 6 hours using a stirrer, then let stand for 24 hours. The filtrate is separated and the process is repeated 3 times with the same type and amount of solvent. All macerate is collected and evaporated by rotary evaporator at a temperature of 40 ºC until a thick extract is obtained, then dried, weighed and the yield calculated with the following formula (2):

$$\text{Yield (\%)} = \frac{a}{b\,(1-\frac{c}{100})} \text{ x } 100\ \% \quad (2)$$

Description:

a = extract weight (g)

b = sample weight (g)

c = water content (%)

E. Phytochemical Assay

1) Alkaloid: A total of 0.05 g of the extract was dissolved in 10 mL chloroform and 4 drops of NH_4OH, then filtered and the filtrate was put into a closed test tube. The chloroform extract in the test tube was shaken with 6 mL H_2SO_4 2 M and the acid layer was separated into another test tube. This acid layer is dropped on the drop plate and Meyer, Wagner, and Dragendorf reagents are added which will cause white, brown, and red-orange colored deposit if alkaloids are present (positive results in the sample).

2) Flavonoid. A total of 0.05 g of extract was added to 100 mL of hot water then boiled for 5 minutes and filtered. The filtrate obtained was then taken as much as 5 mL, added with 0.05 g of Mg powder, 1 mL of concentrated HCl, and 1 mL of amyl alcohol. The mixture is then shaken vigorously. A positive test is indicated by the appearance of red, yellow, or orange color on the amyl alcohol layer.

3) Saponin. A total of 0.05 g of extract was added to 100 mL of hot water then boiled for 5 minutes and filtered. A total of 5 mL of the filtrate was shaken in a closed test tube for 10 seconds then left for 10 minutes. The presence of saponins is indicated by the formation of stable foam.

4) Tannin. A total of 0.05 g of extract was added to 100 mL of hot water then boiled for 5 minutes and filtered. Part of the filtrate obtained is added with $FeCl_3$ solution. The formation of a greenish black color indicates the presence of tannins.

5) Triterpenoid and steroid. A total of 0.05 g of extract was dissolved with 25 mL of hot ethanol 50 ºC, then filtered into a porcelain dish and evaporated until dry. The residue was

dissolved with ether and transferred to a test tube, then added 3 drops of anhydrous acetic acid and 1 drop of H_2SO_4 concentrated (Lieberman Burchard test). Red or purple color indicates triterpenoids and green or blue color indicates the presence of steroids.

F. Toxicity Assay

Shrimp eggs *A. salina* were hatched in a beaker filled with filtered seawater. Hatching was assisted by aeration so that oxygen levels dissolved in the water were sufficient and the shrimp eggs hatch into larvae. The extract solution was made with a concentration of 2000 µg/mL, as much as 0.02 g of the extract was dissolved in 10 mL of sea water. After 48 hours, 10 shrimp larvae and 1000 µL of sea water were put into the test vial. Next, 1000 µL was added to the extract solution so that the final concentration in the vial was 1000 µg/mL. The 500 µL of extract solutions and 1500 µL of seawater were added for a concentration of 500 µg/mL, 100 µL of the extract solution and 1900 µL of seawater for 100 µg/mL, and 10 µL of the extract solution and 1990 µL of seawater for 10 µg/mL. Each concentration was made 3 repetitions. The LC_{50} was determined using a statistical software application called Statistical Product and Service Solution (SPPS).

G. In Vitro Xanthine Oxidase Inhibition

Assay for the inhibition of water and ethanol extract of the sample toward xanthine oxidase was carried out under optimum conditions as reported by Iswantini [20]. The optimum conditions were at an incubation temperature of 20 °C, at pH 7.5, the xanthine oxidase concentration 0.1 unit/ml, the xanthine concentration was 0.7 mM, the incubation time was 45 minutes, and the xanthine absorption was measured at a wavelength of 267 nm.

The extract was put into a test tube with various concentrations. The concentration variations for all sample extracts were 50, 100, 150, 200, 250, 300, 350, 400, 450, and 500 µg/mL. Then the extract was added with a potassium phosphate buffer solution of 50 mM pH 7.5 of 1.9 mL. The mixture was then added with 1 mL of 2.1 mM xanthine and 0.1 mL xanthine oxidase as much as 0.1 mL and then incubated at 20 ºC for 45 minutes. After incubation, the mixture was immediately added with 1 mL of 0.58 M HCl to stop the reaction. The absorption of the mixture was measured using a UV spectrophotometer at a wavelength of 267 nm to see how much unreacted xanthine residue was in the test sample. Inhibition was compared with pure allopurinol and commercial uric acid drugs containing allopurinol with concentrations of 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 µg/mL. Activity of xanthine oxidase (XO) was calculated using the linear equation obtained from the standard curve. The formula (3)-(6) as follows:

$$\text{Linear equation: } y = a + bx \quad (3)$$

Description:

y = Average absorbance measured

x = After-reaction xanthine concentration (remaining xanthine concentration) [xantina] which reacted

$$[\text{Xanthine}]\ \text{which reacted} = [\text{xanthine}]\ \text{initial} - [\text{xantina}]\ \text{remaining} \quad (4)$$

$$\text{Activity of XO} = \frac{[\text{Xanthine}]\ \text{which reacted (mM)}}{\text{Enzyme volume (L)} \times \text{Incubation time (menit)}} \quad (5)$$

$$\text{Inhibition (\%)} = \frac{\text{XO activity control} - \text{XO activity sample}}{\text{XO activity control}} \times 100\% \quad (6)$$

III. Results and Discussion

A. Water Content

The sample used in this study was cat's whiskers leaves, bitter herb, and snake-skin fruit in the form of dry simplicia that have been ground into a powder form and sieved. Drying the sample at a temperature of 40–60 °C aims to reduce the water content in the material so that it is not easily damaged, durable, and protected from microbial attack [21]. The purpose of grinding the simplicia into powder is to expand the contact area to facilitate and accelerate the solvent diffusion process so that the extraction can take place more optimally. Meanwhile, sieving aims to homogenize the size of the powder produced.

Powder water content was measured by indirect evaporation gravimetric method. Determination of water content aims to obtain information on the amount of water contained in the material so that the mass and the best storage method can be found so that damage does not occur due to the influence of microbial activity (fungi and bacteria). In addition, the water content also acts as a correction factor in the calculation of extract yield. The water content in cat's whiskers powder, bitter herbs, and snake-skin fruit in this study were 2.69 ± 0.12%, 2.91 ± 0.22%, and 5.46 ± 0.08%, respectively. Based on the results obtained, the risk of microbial attack can be reduced because the water content is less than 10% [22]. In addition, the powder obtained is also not moldy and has a distinctive smell like its fresh ingredients.

B. Yield of Extracts

Isolation of the compounds contained in the simplicia of the cat's whiskers, bitter herbs and snake-skin fruit were carried out by extraction by means of maceration. This extraction method is used to extract compounds that are less heat resistant, usually in samples whose compound characterization is not yet known [23]. The solvents used in this study were water and ethanol. These two solvents were selected based on the interest of the active compound which is thought to be able to inhibit xanthine oxidase which is to be taken from cat's whiskers, bitter herbs, and snake-skin fruit, namely flavonoids. The compound can be polar or semipolar, so that water which is polar in nature and ethanol which is semipolar is used. Another reason is that there is a regulation issued by BPOM [24] regarding pollutants for pharmacological purposes that only use water or ethanol.

The results of this study indicate that the yield of water and ethanol extracts of all samples can be seen in Table I. The yield of water extract is higher than that of ethanol extract of cat's whiskers, bitter herbs and snake fruit. Based on the yield value, it is known that the type of solvent used affects the amount of yield produced. The difference in yield produced from each solvent can be caused by differences in the content of the compounds of each of these simplicia which can be extracted using water and ethanol as a solvent.

TABLE I. YIELD OF THE WATER AND ETHANOL EXTRACT OF CAT'S WHISKER, BITTER HERBS AND SNAKE-SKIN FRUITS

Sample	Solvent	Yield (%)
Cat's Whiskers	Water	16.50
	Ethanol	15.88
Bitter Herbs	Water	11.50
	Ethanol	8.28
Snake-Skin Fruit	Water	16.32
	Ethanol	15.16

Zalukhu [25] stated the yield of leaves cat's whiskers extracted with water and ethanol were 27.76% and 9.27%, respectively. The yield of ethanol extract in that study was lower than this study, but the yield of water extract in that study was lower than in this study. The difference in yield can be caused by differences in the age of the cat's whiskers used. Yonanda [26] stated that the yield of bitter herbs extracted with water, 30% ethanol, and 70% ethanol were 15.18%, 14.89%, and 17.13%, respectively. Those yield were higher than the yield of water and ethanol extract obtained in this study. The difference in yield obtained in this study can be caused by the extraction method using ultrasonication and the difference in the age of the bitter herbs used and the difference in the planting location. Sahputra [27] stated that the yield of snake-skin fruit extracted with water and ethanol 70% were 51.54% and 62.61%, respectively. The yields were higher than the yield of water and ethanol extracts obtained in this study. The yield difference obtained can be caused by the research methods of extraction using reflux, the fruits age difference used, and different planting sites.

C. Phytochemistry

Phytochemical assay aims to test the existence of groups of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, steroids, and triterpenoids in the sample. This preliminary test is conducted to determine the presence or absence of flavonoids and other compounds that are thought to play a role in inhibiting xanthine oxidase.

Phytochemical assay provides a specific result for each test (Table II). Alkaloid test showed negative results for simplicia, water and ethanol extract of cat's whiskers, bitter herbs, and snake-skin fruit. A positive result is indicated by the formation of white, brown, and red deposits respectively after the addition of Mayer, Wagner, and Dragendorff reagents. Suryana [28] reported that the simplicia of cat's whiskers did not contain alkaloids. Yulianti [29] reported that cat's whiskers water extract did not contain alkaloids. Aziz [30] reported that the ethanol extract of cat's whiskers did not contain alkaloids. This study confirmed the previous studies which reported that alkaloids were not detected in simplicia, water and ethanol extract from cat's whiskers. Rivai et al [31] reported that the water and ethanol extracts of the bitter herbs contained alkaloids. Sulaksono [32] reported that snake-skin fruit simplicia contained alkaloids. Sahputra [27] reported that the ethanol extract of snake-skin fruit contained alkaloids. Different results were shown in the alkaloid test of the herbal extract of bitter herbs and snake-skin fruit in this study with the previous reported studies. This difference can be caused by different extraction methods and different planting locations.

The flavonoid test showed positive results for simplicia, water and ethanol extract of cat's whiskers, bitter herbs, and snake-skin fruit. A positive test for flavonoids is indicated by the appearance of a red, yellow, or orange color on the amyl alcohol layer. Previous research has also reported that flavonoids were found in simplicia, water, and ethanol extract from cat's whiskers [28-30]. Water and ethanol extracts of bitter herbs have also been reported to contain flavonoids [31]. Other literature also states that the simplicia and ethanol extract of snake-skin fruit positively contained flavonoids [27,32].

Saponin test showed positive results for simplicia, water and ethanol extract of cat's whiskers, while negative for simplicia, water extract, and ethanol extract of bitter herbs and snack-skin fruit. Positive result for saponins is indicated by the formation of a stable foam within 10 minutes after mixing. Suryana [28] reported that the simplicia of cat's whiskers contained saponins. Yulianti et al [29] reported that the water extract of cat's whiskers contained saponins. Aziz et al [30] reported that the ethanol extract of cat's whiskers contained saponins. The results obtained in this study are in line with studies that have been previously reported. Rivai et al [31] reported that the aqueous and ethanol extract of the bitter herbs did not contain saponins, andSahputra [27] and Sulaksono [32] reported that the simplicia and ethanol extract of snake-skin fruit did not contain saponins. The results obtained in this study are also in line with studies that have been previously reported.

The tannin test showed positive results for simplicia, water and ethanol extract of cat's whiskers, bitter herbs, and snake-skin fruit. The positive test for tannins is indicated by the formation of a black color after the addition of $FeCl_3$. Suryana [28] reported that the simplicia of cat's whiskers contained tannins. Yulianti et al [29] reported that the water extract of cat's whiskers contained tannins. Aziz et al [30] reported that the ethanol extract of cat's whiskers contained tannins. The results obtained in this study are in line with studies that have been previously reported. Rivai [31] reported that the water and ethanol extract of bitter herbs contained tannins, and Sahputra [27] and Sulaksono [32] reported that the simplicia and ethanol extract of snake-skin fruit contain tannins. The results obtained in this study are also in line with studies that have been previously reported.

TABLE II. SECONDARY METABOLITES CONTENT OF SIMPLICIA, WATER AND ETHANOL EXTRACT OF CAT'S WHISKERS, BITTER HERBS AND SNAKE-SKIN FRUITS

Phytochemical Test	Cat's Whisker			Bitter			Snake Fruit		
	Simplicia	***Water***	***Ethanol***	***Simplicia***	***Water***	***Ethanol***	***Simplicia***	***Water***	***Ethanol***
Alkaloids									
Mayer	-	-	-	-	-	-	-	-	-
Dragendorff	-	-	-	-	-	-	-	-	-
Wagner	-	-	-	-	-	-	-	-	-
Flavonoid	+	+	+	+	+	+	+	+	+
Saponin	+	+	+	-	-	-	-	-	-
Tannin	+	+	+	+	+	+	+	+	+
Triterpenoid	-	-	-	-	-	-	-	-	-
Steroid	+	+	-	+	-	-	-	-	-

The triterpenoid test showed negative results for simplicia, water and ethanol extract of cat's whiskers, bitter herbs, and snake-skin fruit. A positive result is indicated by a change in color to red or purple after adding the test reagent. Suryana [28] reported that the simplicia of cat's whiskers did not contain triterpenoids. Yulianti [29] reported that the water extract of cat's whiskers did not contain triterpenoids. Aziz et al [30] reported that the ethanol extract of cat's whiskers did not contain triterpenoids. This study confirms the previous studies which reported that triterpenoids were not detected in simplicia, water and ethanol extract from cat's whiskers. Rivai et al [31] reported that the water and ethanol extract of the bitter herbs did not contain triterpenoids, and Sahputra [27] and Sulaksono [32] reported that the simplicia and ethanol extract of snake-skin fruit did not contain triterpenoids. The results obtained in this study are in line with studies that have been previously reported.

The steroid test showed positive results for simplicia, water extract of cat's whiskers, and bitter herbs. Negative results were obtained by ethanol extract of cat's whiskers, water and ethanol extract of bitter herbs and simplicia, water extract, and ethanol extract of snake-skin fruit. A positive result is indicated by the formation of green color. Suryana [28] reported that cat's whiskers simplicia contained steroids. Yulianti [29] reported that cat's whiskers water extract contained steroids. Aziz et al [30] reported that the ethanol extract of cat's whiskers did not contain steroids. The results obtained in this study are in line with studies that have been previously reported. Rivai et al [31] reported that the aqueous and ethanol extract of the bitter herbs did not contain steroids, and Sahputra [27] and Sulaksono [32] reported that the simplicia and ethanol extract of snake-skin fruit do not contain steroids. The results obtained in this study are consistent with studies that have been reported previously.

D. Toxicity

In general, a natural substance to be used for medicinal purposes needs to be tested for its toxicity. Letha1 Concentration 50 (LC_{50}) is the concentration of the tested extract which is able to cause the death of shrimp larvae in the amount of 50% of the population after an incubation period of 24 hours. In this study, the toxicity test of water and ethanol extract on samples of cat's whiskers, bitter herbs and snake-skin fruit was carried out using the Brine Shrimp Lethality Test (BLST) method on *Artemia salina* shrimp larvae. The BSLT method is a fast and cheap way to screen the toxicity of plant extracts. The BSLT method has broad spectrum of pharmacological activity, the procedure is simple, fast and the results are reliable.

TABLE III. LC_{50} VALUE OF WATER AND ETHANOL EXTRACT OF CAT'S WHISKERS, BITTER HERBS AND SNAKE-SKIN FRUIT

Sample	Solvent	LC_{50} (µg/mL)
Cat's Whiskers	Water	366.34
	Ethanol	527.94
Bitter Herbs	Water	496.67
	Ethanol	493.30
Snake Fruit	Water	602.97
	Ethanol	535.86

Toxicity testing of water and ethanol extracts of cat's whiskers, bitter herbs, and snake-skin fruit obtained LC_{50} values below 1000 µg/mL (Table III). This LC_{50} indicates that the extract has bioactive potential because according to [33] a compound has bioactive potential if its LC_{50} is below 1000 µg/mL. The lower of LC_{50} will show a high pharmacological effect, while the higher of LC_{50} indicates that the sample has a low pharmacological effect. The difference in LC_{50} in water and ethanol extracts can be caused because the ethanol extract contains more compounds and is more toxic than water extracts. The difference in toxicity seems to be proportional to the amount of extract obtained by the amount of crude extract in the water extract of cat's whiskers which has the lowest LC_{50}. This may indicate that the number of secondary metabolites exposed to polar solvents may be higher. The difference in extracted secondary metabolite level is proportional to the level of toxicity [34].

E. Xanthine Oxidase Inhibition

Xanthine oxidase catalyzes the oxidation of hypoxanthine to xanthine and then becomes uric acid, which plays an important role in gout (Fig. 1). When reacting with xanthine to form uric acid, oxygen atoms are transferred from molybdenum to xanthine. Recast of active molybdenum centers occurs with the addition of water. During the oxidation process, oxygen molecules act as electron acceptors to produce superoxide (O_2*) and hydrogen peroxide radicals [35].

Inhibition test on xanthine oxidase was carried out to water and ethanol extracts of cat's whiskers, bitter herbs, and snake-skin fruit with various concentrations. The difference in concentration is based on LC_{50}. The LC_{50} of each extract will be used as the highest concentration limit (except for the water extract of the cat's whiskers) in determining the various concentrations of the extract in the enzymatic test of xanthine oxidase activity. This is because the drug formulation will be safer if the concentration is made below LC_{50}. Tests at various concentrations were used to see the effect of increasing the extract concentration on increasing inhibition. In addition, enzyme activity was also observed without the addition of extract (control) to see the effect of inhibition of the extract on enzyme activity.

Its working principle is that the absorbance of the xanthine substrate solution which is not converted to uric acid will be read by a spectrophotometer. This concentration can later be converted into the reacting xanthine concentration. By obtaining the reacting xanthine concentration, it will be known how much xanthine oxidase converting xanthine into uric acid. Thus, it can also be determined how much the percent inhibition of the extract tested against the activity of the xanthine oxidase enzyme. Before testing the sample, a standard curve for the xanthine substrate was made first. Standard curve creation needs to be done before the enzymatic test to determine xanthine absorption at various concentrations. It will show how much xanthine is converted to uric acid in enzymatic reactions. This absorption can later be converted into xanthine concentration based on the standard curve linear equation, namely $y = 1.047x + 0.222$ with an R^2 value of 0.997, where y is the measured xanthine absorption and x is the xanthine concentration.

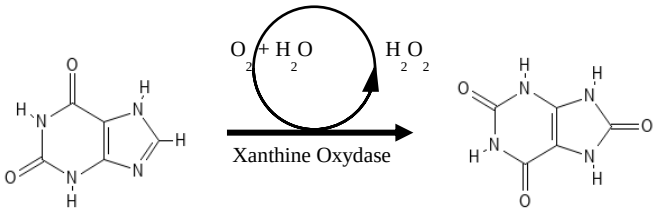

Fig. 1. Reaction of xanthine formation to uric acid catalyzed by xanthine oxidase.

The test results showed that all tested extracts had lower xanthine oxidase activity compared to the control. This indicates that water and ethanol extracts of cat's whiskers, bitter herbs and snake-skin fruit can inhibit xanthine oxidase activity. The inhibition of water and ethanol extracts of cat's whiskers, bitter herbs and snake-skin fruit toward xanthine oxidase activity increased with the increasing of extract concentration. The overall inhibition of all samples to xanthine oxidase activity has a linear R^2 value along with increasing concentrations with different inhibition values for each extract tested.

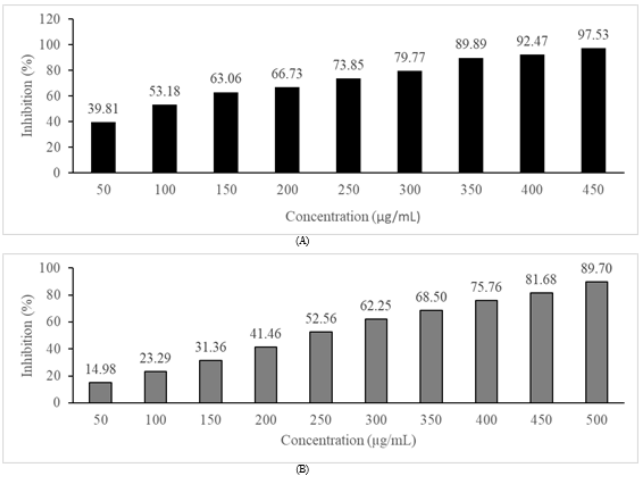

Fig. 2. Inhibition of water extract (A) and ethanol (B) of cat's whiskers.

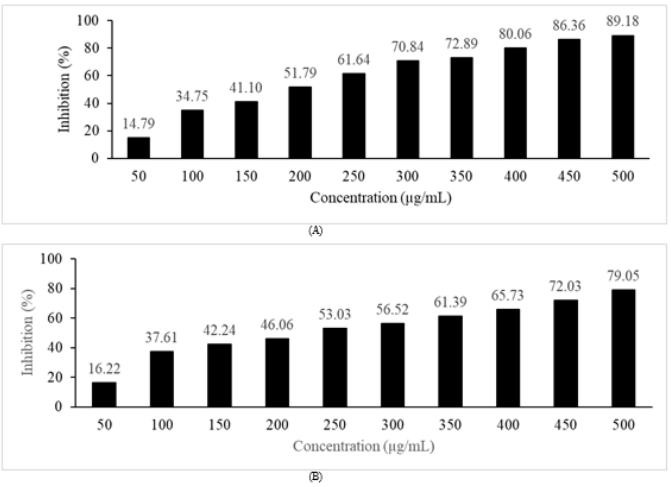

Fig. 3. Inhibition of water extract (A) and ethanol (B) bitter herbs.

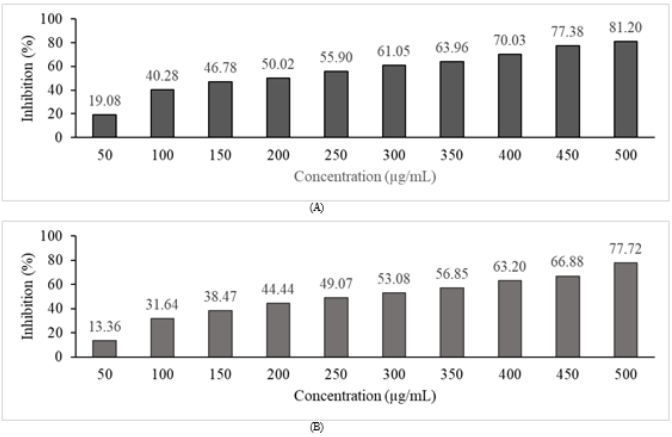

Fig. 4. Inhibition of water extract (A) and ethanol (B) snake-skin fruit.

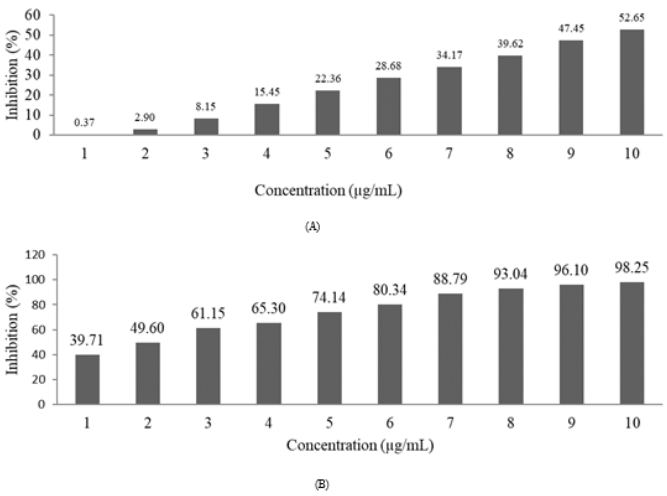

Fig. 5. Inhibition of allopurinol (A) and commercial allopurinol (B).

Referring to Fig. 2-5, the inhibition of all samples to xanthine oxidase activity have the percentage of inhibition increases with the increasing of concentration with different percent inhibition values in each tested extract. The water extract of cat's whiskers had the highest inhibition obtained at a concentration of 450 µg/mL, which was 97.53±0.22%. The ethanol extract of cat's whiskers has the highest inhibitory power obtained at a concentration of 500 µg/mL, which is 89.70±0.29%. The water extract of bitter herbs had the highest inhibition at concentration of 500 µg/mL, which was 89.18±0.30%. The ethanol extract of bitter herbs had the highest inhibition at concentration of 500 µg/mL, which was 79.05±0.46%. Snake-skin fruit water extract had the highest inhibition obtained at concentration of 500 µg/mL, which was 81.20±0.22%. The ethanol 70% extract of snake-skin fruit had the highest inhibition obtained at a concentration of 500 µg/mL, which was 77.72±0.22%. Pure allopurinol has the highest inhibition obtained at concentration of 10 µg/mL, which is 52.65±0.22%. Commercial allopurinol had the highest inhibition obtained at concentration of 10 µg/mL, which is 98.25±0.22%. The increase of inhibition occurs because at high concentrations there are more secondary metabolites from the water extract of cat's whiskers which are thought to have the ability to inhibit xanthine oxidase activity. Inhibition of cat's whiskers water extract (97.53%) was higher than all extract samples tested and approached the inhibition of commercial allopurinol (98.25%). This is presumably because the synergistic effect of secondary metabolites contained in the water extract of cat's whiskers is more potential as a xanthine oxidase inhibitor.

The IC_{50} is the concentration of the test compound giving 50% enzyme inhibition. The smaller the IC_{50}, the greater the inhibitory effectiveness of the extract toward xanthine oxidase activity. The IC_{50} of cat's whiskers leaves extract was smaller than that of all tested sample extracts. However, when compared to commercial allopurinol, the water extract of cat's whiskers is still below commercial allopurinol (Fig. 6). This is because commercial allopurinol is in its pure form, while the water extract of cat's whiskers is still a multicomponent.

IV. Conclusion

Three native Indonesian plants, i.e., Cat's whiskers leaves, bitter herbs, and snake fruit are found to reduce uric acid levels in the body. In this experimental study, the bioactive potency and the xanthine oxidase enzyme inhibition were tested. The results showed that the water extract of cat's whiskers leaves had the potency as herbal ingredients for treating uric acid, as shown by the lowest IC_{50} of all extracts.

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