Preliminary Exploration of Anti Gout Herbal Drug: A Case Study on Xanthin Oxidase Inhibitor

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Abstract

The global gout prevalence was increase sharply in recent decade. On other hand, as a mega-biodiversity country, Indonesia is a home to 11 percent of the world's flowering plant species, so it is potencial as resource of many bioactive metabolites, including herbal antigout drug. Therefore, the exploration of herbal antigout by biochemical studies is important to do. The aims of this research are to find out the juice or simplicia extract of the plants samples that have in vitro activity as xanthine oxidase inhibitor, relative to the gout medicine: allopurinol, and identifying the bioactive compounds. Samples of this study are: soursop (*Annona muricata* L.) fruit ; sugar apple (*Annonasquamosa L.*) fruit; "mlinjo" peel (*Gnetum gnemon* L.); mangosteen peel (*Garcinia mangostana* L.) ; outer peel (shells) of peanuts and inner peel of peanuts (*Arachishypogaea*L ; leaf of of daun sendok (*Plantago major* L.); and leaf of "pegagan" (*Centella asiatica* L.).This research was carried out in some stages, that are: the production of simplicia plant extracts, phytochemical tests for alkaloid, flavonoid, saponin, tannin and polyphenols of the extracts, and in vitro inhibition test the extracts on xanthine oxidase enzyme, relative to allopurinol (a comersial anti gout drug). Extraction methode for simplicia (maseration and concentration) and in vitro inhibition test reffered to Subandi et. al. (2016). The result had shown that all of the simplisia extracts have activity as xanthin oxidase inhibitor. The inhibition power were varies from 12.5% (steamed of *A. muricata* L) to 82.9% (sugar aple) at the concentration of 100 ppm. While at the same concentration the inhibition power of allopurinol was 48.4%.

Key words: xanthine oxidase inhibitor, antigout herbal, sugar apple

1. Introduction

Gout is a dissease cause by hyperuricemia, that is a condition where the uric acid in the blood plasma more than 6 mg / dLfor women and 7 mg/dL for men. Hyperuricemia, usually caused by a bad diet that exceeds purine, protein, alcohol, and carbohydrate intake [1-3]. In addition, there are various drugs that potentially dangerous for purine metabolism that also cause hyperuricemia, like Thiazides [3].

The prevalence of gout in western countries varies between 2.3 to 17.6% and increase every year. For eaxample in United States is 5%, in Scotland is 8%, while in the UK is around 6.6% [4]. In Indonesia, based on Basic Health Research Data 2013 was 11.9%[5], and also increases every year.

One common drug to treat gout is allopurinol, that works as competitive inhibitor of xanthine oxidase enzyme (EC 1.17.3.2), which plays an important role in the synthesis of uric acid. However, like the other synthetic drugs, for a long period of consumtion, it has such side effectsas diarrhea, nausea, redness of the skin, with or without itching [6]. Therefore, it still needs the other natural and saver drugswith the same efficacy to replace it.

According to some research results and experiences, herbal medicine is believed to be more secure to cure gout. For example, the flavonoids in the ethanol extract of celery was reported capable to inhibit xanthine oxidase as much as 74.01% [7]. Methanol extract of roselle (*Hibiscuss abdariffa* L.) was able to inhibit xanthine oxidase (IC50 of 0.64 ppm) and the most active fraction (water-methanol = 75:25) was able to inhibit the enzyme by 40.55%[8] . Besides that, the ethanol fraction of ciplukan herb (P*hysalis angulata* L) was able to inhibit xanthine oxidase with IC50 activity of 43.55 µg/ml [9] and also has been reported that the 100 ppm of methanol extract of *Switenia mahogany* seed inhibits xanthine oxidase as much as 47.2% [10].

Like allopurinol, which inhibit xanthin oxidase through competitive inhibition mechanism, the active compounds in herbal medicine were allegedly have the same mechanism, which means that it is based on the structural similarities between the compound and enzyme substrate, i.exanthin or hipoxanthin. Therefor the compounds of xanthon group that similar to xanthin structure, allegedly also able to inhibit xanthin oxidase (XO). In fact, some simplicia samples examined in this research also contained xanthon group [11 and 12]. For example, mangosteen peel, which contains identified xanthon compound , such as 1,3,6-trihidoksi-7-metoksi-2,8-bis(3-metil-2-butenil)-9H-xanten-9-on and 1,3,6,7-tetrahidroksi-2,8-bis(3-metil-2-butenil)-9H-xanten-9-on [13]. Both compounds are known as alpha-mangostin and gamma-mangostin [14], so the the simplicia samples were predicted have bioactivity as XO inhibitor.

According to the local wisdom, some simplicia from various local plant have been believed have activity to cure gout and this study was done to prove it scientifically. So, that simplicias had been choised in this study as samples. They are: epicarp of melinjo (*Gnetumgnemon*), epicarp of mangoosten (*Garciniamangostana*), outer and inner peel of peanut (*Arachishypogaea L.*), mesocarp ofsoursop (*Annonamuricata*) fruit, mesocarp ofsugar apple (*Annonareticulata*) fruit, leaf of pegagan (*Centella asiatica*) and leaf of daun sendok (*Plantago mayor*).

The aims of this research are to find out the simplicia extract of the plants samples that have in vitro activity as xanthine oxidase inhibitor, relative to the gout medicine: allopurinol, and identifying the compounds or classes of phytochemical compounds in the simplicia extracts which active as xanthine oxidase inhibitor.

This research was carried out in some stages, that are: the production of fruit juice and extract of simplicia plants, phytochemical tests for alkaloid, flavonoid, saponin, tannin and

polyphenols of juices and simplicia extracts, and in vitro inhibition test of juices and extracts of simplicia on xanthine oxidase enzyme, relative to allopurinol. Extraction methode for simplicia (maseration and concentration) and in vitro inhibition test of the samples against xanthin oxidase reffered to studied before by Subandi et.al [15].

The results of this research was expected to becomes an early stage to find empirical evidence that the simplicia extracts of some plants can inhibit the xanthine oxidase enzyme, thereby, it can be use to decrease the levels of uric acid in blood plasma. In the next stage, from the simplicias can be produce herbal health drinks or anti-gout drug.This result also was expected to raise the awareness of the stakeholders in conserving biological resources, especially medicinal plants.

2. Material and Methods

This descriptively explorative research was conducted in chemical laboratories at State University of Malang. The materials used in this research were obtained from indigenous plants, that are: epicarp of melinjo (*Gnetumgnemon*), epicarp of mangoosten (*Garciniamangostana*), outer and inner peel of peanut (*Arachishypogaea L.*), mesocarp ofsoursop (*Annonamuricata*) fruit, mesocarp ofsugar apple (*Annonareticulata*) fruit, leaf of pegagan (*Centella asiatica*) and leaf of daun sendok (*Plantago mayor*), as can be seen in the Figure1. As substrate and enzyme has been used Xanthin and xanthin oxidase (Sigma product), also enzyme buffer (phosphat Buffer), and 70% ethanol as solvent for maceration.

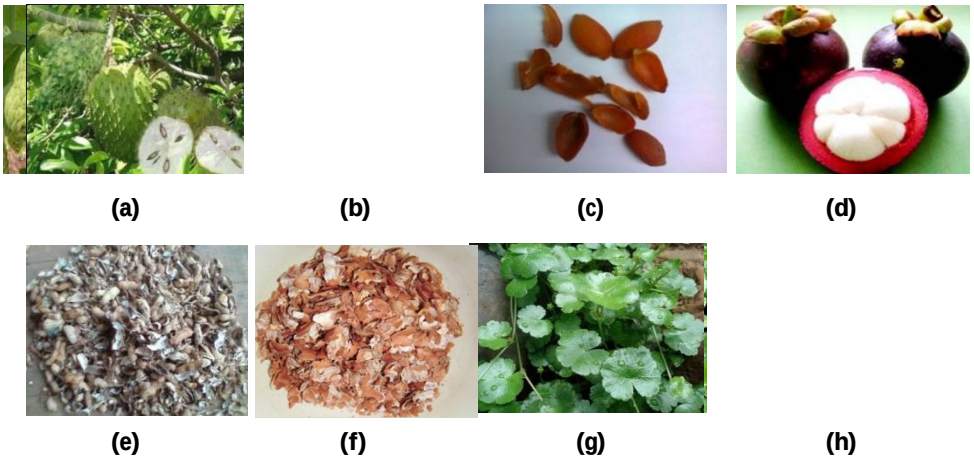


Figure 1. Samples of this study: (a) soursop (*Annona muricata* L.) fruit ; (b) sugar apple (*Annonasquamosa L.*) fruit; (c) "mlinjo" peel (*Gnetum gnemon* L.); (d) Mangosteen peel (*Garcinia mangostana* L.) ; (e) outer peel (shells) of peanuts and (f) inner peel of peanuts (*Arachishypogaea*L .); (g) leaf of of daun sendok (*Plantago major* L.); and (h) leaf of "pegagan" (*Centella asiatica* L.).

The equipment used were a 100 mL; 500 m and 1000 mLglass beaker, test tubes, pipette, magnetic stirrer, rotary vacuum evaporator, sentrifuge, oven, knives, pots, 100 ml; 250 mL; 500 mL; and 1000 mL of flask, glass funnel, measuring pipette 1 and 5 mL and burrete, analytical balance, filter paper, and UV-Vis spectrofotomer.

This research was carried out in some stages, that are: the production of fruit juice and extract of simplicia plants, phytochemical tests for alkaloid, flavonoid, saponin, tannin and polyphenols of juices and simplicia extracts, and in vitro inhibition test of juices and extracts of simplicia on xanthine oxidase enzyme, relative to allopurinol (a comersial anti gout drug). Extraction methode for simplicia (maseration and concentration) and in vitro inhibition test of the samples against xanthin oxidase reffered to Subandi et al , 2016 [15],

3. Results and Discussion

The results of the research include: the yield of fruit juice and extracts of simplicia; inhibition power of fruit juice and simplicia extracts to xanthine oxidase relative to allopurinol, and phytochemical test result of simplicia extracts.

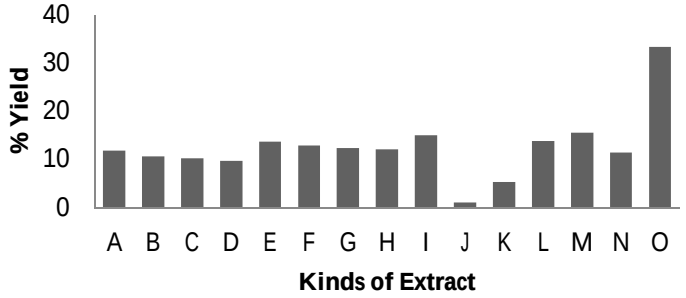
The yield of fruit simplicia extract

The yield of the production of simplicia extracts had been calculated by formula (1) and the result of all samples yield can bee seen in Figure 1.

$$Yield\ of\ X\ extract\ = \frac{\text{mass of X concentrated extract}}{mass\ of\ sample\ \text{X}} \text{X } 100\% \quad \ldots\ldots\ldots\ldots (1)$$

According to Figure 2, the smallest yield of the eaxtracts was the yield of outer peel of peanut (Figure 1. J) and the largest was the yield of mangosteen peel (Figure 2. O). This data understanable because most of peanut outer peel (sheells) are cellulose, while mangosteen peel contain many organic compounds (secondary metabolites) that are easily soluble in ethanol.

The production of simplicia extracts in this research had used maceration method, due to the methode was easy to do, and the equipments were quite simple. The maceration process was done by soaking the samples (dry powder) in solvents as well as being shaken to weaken the cell membrane and cell wall of plants. Maceration allowed the substances contained in the sample to dissolve. This was due to the differences in the concentration inside the cell and outside the cell. The solvent in the maceration process was ethanol 70%. That able to extract the polar and non-polar compounds. Ethanol 70% (as beeen used in this study) could extract polyphenols compounds and flavonoids compounds more than the pure ethanol [16].



A : NS m mes of *A. squamosa* L.
B : Sm mes of *A.squamosa* L.
C : NS m mes of *A. muricata* L.
D : NS mes *A. muricata* L
E : LE E of *Centellaasiatica* L
F : LWE *Centellaasiatica* L
G : LEE of *Plantago major* L
H : LWE of *Plantago major* L
I : Ip of *Arachishypogaea* L
J : Op of *Arachishypogaea* L
K : NS m ep of *G.gnemon* L.
L : S m ep of *G.gnemon* L.
M : NS im ep *G.gnemon* L.
N : S. im ep of *G.gnemon* L.
O : NS EEE of *G.mangostana* L

Note: NS = non steamed; S= steamed; mes= mesocarp; ep= epicarp ; m= mature; im= imature; Op= outer peel; Ip= inner peel; LEE= Leaf ethanol extract; LWE= Leaf water extract;

Figure 2. The Yield (%) of Simplicia Extracts

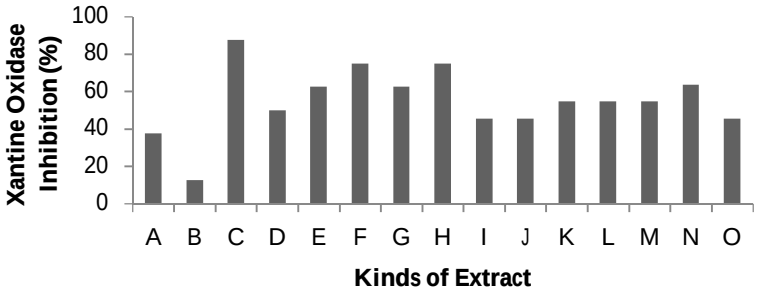
There was a special treatment in producing the extract of *A. muricata* L. and sugar apple . It did not use the maceration method, but it was immediately homogenized using a blender with the water solvent. A. *muricata* L.and sugar apple were divided into two treatments. One was initially cooked (steamed for 30 minute) and then blended, and another was directly blended. Steamed allegedly can disrupted the cell wall of the simplicia powder and increased the yield. As has reported before, the concentration of polyphenols in the *G. gnemon* L. peel extract increased after steamed treatment, that might be caused the damage of the tissue and the cell walls, so that many compounds came out and were easily extracted [17],. However,the decrease in levels of flavonoids was also possible because the flavonoid compounds in the extract could not stand the heat. In this study, the resulthas shown that steam treatment was not increase the yield (Figure 2 A to B and C to D), and also was not increase the bioactivity (power of inhibition) (Figure 3: A to B and C to D). This is possible, because steam treatment can damages and or reduce theactivity of the active compounds.

The Power of Inhibition of the simplisia extracts

The inhibition power of sample simplicia extracts (100 ppm each) can be seen in Figure 3.

Figure 3. has shown that, as be expected, all of the sample extracts have activity as xanthin oxidase inhibitor. The highest inhibition power was obtained for the water extract of non-steamed sugar apple fruit (82.9%) and the lowest inhibition power was water extract of steamed sour soup (12.5%), while the inhibition power of allopurinol 100 ppm was 48.4%.
Steamed (30 minutes) can decrease the bioactivity of the pine sugar and sour soup extract (Figure 3. A,B and C,D), while steamed treatment for melinjo peel (G gnemon L.) does not affect its bioactivity (Figure 2. K,L and M,N). Futhermore, extract of immature melinjo peel

was higher than the mature one. While for leaf extact of *Plantago major* and *Cantella asiatica*, the ethanol extracts were more active than water extract (Figure 3, F,G,H and I).



A : NS m mes of *A. muricata* L.
B :NS mes *A. muricata* L
C : NS m mes of *A. squamosa* L.
D : Sm mes of *A.squamosa* L.
E : LWE *Centellaasiatica* L
F : LEE of *Centellaasiatica* L
G : LWE of *Plantago major* L
H : LEE of *Plantago major* L
I : NS m ep of *G.gnemon* L.
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L : S. im ep of *G.gnemon* L.
M : Ip of *Arachishypogaea* L
N : Op of *Arachishypogaea* L
O : NS EEE of *G.mangostana* L

Note: NS = non steamed; S= steamed; mes= mesocarp; ep= epicarp ; m= mature; im= imature; Op= outer peel; Ip= inner peel; LEE= Leaf ethanol extract; LWE= Leaf water extract; EEE= ethanol extract of epicarp. While the inhibition power of allopurinol 100 ppm was 48.4%.

Figure 3. Inhibition Power of Simplicia Extracts (100 ppm each)

The difference in bioactivity of each extract due to the differences in treatment, has revealed the differences in concentration and composition of compounds in the extracts, as will be proven in the phytochemical test.

Compare to other studied before the higest xanthin oxidase (XO)-inhibitory activity of this study (pine aple extract = 82.9%) was still higher than ethanol extract of celery 74.01% [7],. methanol extract of roselle (*Hibiscussabdariffa* L.) (40.55%)[8], and the ethanol fraction of ciplukan herb (P*hysalisangulata* L) (70%) [9] and methanol extract of *Switenia mahogany* seed (47.2%)[10]. However, the highest result of this study is still lower than pure flavonoids and phenolics compound that are ferulic acid, p-coumaric acid, alkylgallate which has XO-inhibitory activity more than 95% respectively, at the same concentration [18]. So if various simplicia extracts in this study can be purified, it is estimated that the activity will be much higher. Besides that, it is also predicted that the bioactiv substances dominant in this extracts are flavonoids. This prediction is in line with previous findings that phenolics and flavonoids compound may acts as potent inhibitors against the metabolic enzymes such as cyclooxygenase,xanthine oxidase and lipooxygenase [19].

3.3 The Result of Phytochemical Test (Secondary Metabolites Composition)

Qualitative phytochemical testing was conducted to determine the content of secondary metabolites in the fruit juice and or extracts of simpliciasamples, that were assumed responsible for their bioactivity. Phytochemical testing has been donefor tannins, alkaloids, polyphenols, flavonoids and saponins. The result of phytochemical testing can bee seen in the Table 1.

Type of Sample	Flavo-noid	Sapo-nin	Tanin	Poly-phenol	Alkaloid
NS m mes of *A. squamosa* L.	++	-	-	-	++
S m mes of A. squamosa L.	+	-	-	-	+
NS m mes of *A. muricata* L.	+	-	-	+	-
NS mes *A. muricata* L	+	-	-	+	-
LEE of *Centella asiatica* L	+	+	-	+	-
LWE Centella asiatica L	+	+	-	+	-
LEE of *Plantago major* L	++	-	-	++	++
LWE of *Plantago major* L	+	-	-	+	+
Ip of *Arachis hypogaea* L	+	-	+	+	+
Op of *Arachis hypogaea* L	+	-	-	+	+
NS m ep of *G. gnemon* L.	+++	+	-	++	+
S ep of *G. gnemon* L.	++	+	-	+++	++
NS im ep G. gnemon L.	+++	+	-	++	+
S. im ep of G. gnemon L.	++	+++	-	+++	+++
NS EEE of *Garcinia mangostana* L	+	+	+	+	+

Table 1. Phytochemical Test Results of Simplicia Extracts

Note: NS = non steamed; S= steamed; mes= mesocarp; ep= epicarp ; m= mature; im= imature
LEE= Leaf ethanol extract; LWE= Leaf water extract; EEE= ethanol extract of epicarp
*) The more sign (+) the higher compoundconcentration in the sample

According the Table 1, all of the sample were contain flavonoid and most of them also contain polyphenol. This facts were in line with the bioactivity data (Figure 2), that all of the samples

have bioactivity as inhibitor against xanthin oksidase, meanwhile many kind of flavonoid and polyphenol class were active as inhibitor for that enzyme [19].

As the medicine of gout, allopurinol act as competitive inhibitor, due to its structure is similar to the substrate (Xanthin or hypoxanthin) [20], as can be seen in Figure 4. This causes the competition between substrates and inhibitors in binding to the enzyme active site.

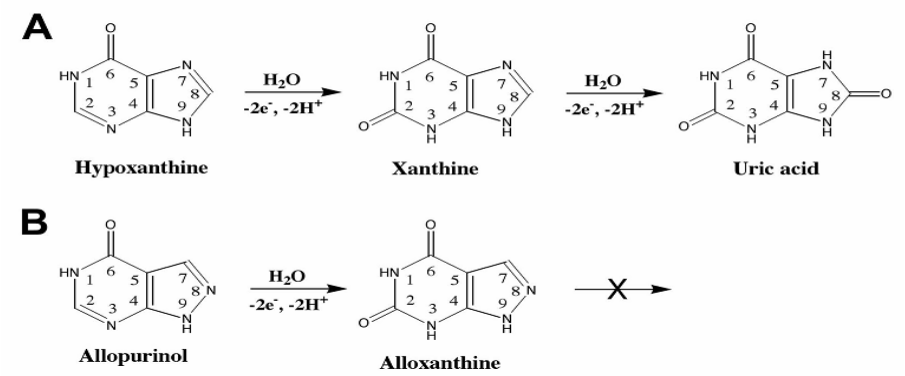

Figure 4.The Reaction of Xanthin Oxidase on substrat (A) and inhibitor (B) [20].

In this study, the one of the most possibility of the secondary metabolites in the extract of simplicia as a potential inhibitor of xanthine oxidase, was flavonoids. The compound had structural similarities with xanthine. This was caused by the presence of two aromatic rings which had a hydroxyl group as an electron acceptor of xanthine oxidase [21].

Lin C,M [22] states that the structure of this flavonoids caused the group of this compounds potential as a competitive inhibitor against xanthine oxidase. However, not all f lavonoid compounds can act as inhibitors of the xanthine oxidase enzyme. Some of the flavonoid compounds have high inhibition activity. The levels of inhibition depend on the position of the hydroxyl group in the basic frameworkThe flavonoid compounds that have a double bond at the C2 and C3 atoms tend to have the ability to act as an inhibitor. In addition, the presence of hydroxyl groups on the C5 and C7, as well as the carbonyl group C4 can form hydrogen bonds and play a role in the inhibitor interaction with the active site of xanthine oxidase enzyme. The basic structure of flavonoid can be seen in Figure 5.

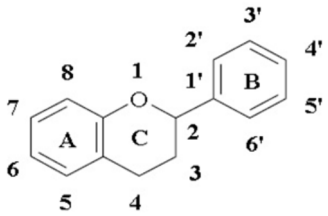

Figure 5. Basic Structure of Flavonoids [22]

The results of photochemical test showed that fruit juice and simplicia extracts also contained alkaloids, polyphenols, tannins and saponins, according to those compounds also have ability as a xanthine oxidase inhibitor [23]. According to Cos et. Al [21], alkaloids has a hydroxyl group as an electron acceptor of xanthine oxidase.

A. muricata L.plant is very potential for alternative inhibitors of the xanthine oxidase enzyme [24]. A research on A. muricata L.leaves had been conducted, and the results showed that A. muricata L. leaves could be used as an inhibitor of the xanthine oxidase enzyme. Inside the extract of A. muricata L.leaves, there are compound 2,3 dihidrobenzofuran; 3 - ethoxy - 1, 4, 4a, 5, 6, 7, 8, 8a-oktahidroisoquinolin; and 3-oxo-1-butenyl. These compounds can actively inhibit the formation of uric acid.

A. muricata L. fruit, in this research, is also able to inhibit xanthine oxidase. We predicted that the secondary metabolites that potential having the inhibition power are alkaloids and flavonoids, because these have a structure that is almost similar to the xanthine substrate. More research should be conducted, however, to ensure the secondary metabolites inside *C. asiatica* L. which capable to inhibit the xanthine oxidase enzyme.

4. Conclusions

Based on the description and the discussion of the results above, the conclution of this research are as follows. All of the simplisia extract samples have activity as xanthin oxidase inhibitor The inhibition power against xantin oxidase of the extracts from the highest to the lowest respectively are the fresh sugar apple extract (82.9%), raw old *G. gnemon* L. peel extract (45.46%), boiled old *G. gnemon* L. peel extract (45.46%) and mangosteen peel ethanol extract 45.45%, and steamed of *A. muricata* L. extract (12.5%). 3). The results of Phytochemical test has shown that the secondary metabolites contained in *A. muricata* L. fruit and sugar apple are flavonoid and alkaloid; in *C. asiatica* L. leaves, and P. *major* L. leaves are flavonoids, saponins, polyphenols and alkaloids; while in the extract of outer and inner peel of peanut, *G. gnemon* L. peel, and mangosteen peel are flavonoids, saponins, tannins, polyphenols and alkaloids.

Based on the research findings, the highest inhibition power against xanthin oxidase is the sugar apple fruit (buah srikaya), which rich in flavonoid.

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