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Review Article

Inhibitory Capacity of Xanthine Oxidase in Antigout Therapy by Indonesian Medicinal Plants

Rut Novalia Rahmawati Sianipar[1], Komar Sutriah[1,*], Dyah Iswantini[1,2,*], Suminar Setiati Achmadi[1,2]

ABSTRACT

The traditional medicine has been used in Indonesia since the days of the Ancient Mataram Kingdom (about 12 centuries ago). Indonesia is rich in medicinal plants. For this reason, it is necessary to inform the broader community regarding medicinal plants in Indonesia that have the potential as antigout. The prevalence of gout in Indonesia is in the range of 1.6–13.6 per 100,000 people and will increase with age. There are 25 species of Indonesian plants that have more than 50% xanthine oxidase (XO) enzyme inhibitory activity. XO is responsible for catalyzing hypoxanthine to xanthine then producing uric acid, accompanied by the formation of reactive oxygen species (ROS) during catalysis. The magnitude of the inhibitory power to XO ranged from 50.00±1.16% to 97.53%. The lowest inhibitory power of 50.00±1.16% was in *Phaleria macrocarpa*, while *Orthosiphon aristatus* had the highest inhibitory power of 97.53%. The major compounds that inhibit xanthine oxidase are flavonoids. The structural similarity of flavonoids in rings A and C with xanthine as a substrate causes hydrophobic interactions, hydrogen bonds, and van der Waals forces between flavonoids and XO. It means that flavonoids bind to the XO active site, thereby preventing the formation of uric acid. The type of inhibitory kinetics that occurs between flavonoids and XO is competitive inhibition. Five plants with competitive inhibition kinetics against XO are *Sida rhombifolia*, *Syzygium polyanthum*, *Cyperus rotundus*, *Ruellia tuberosa* and *Phaleria macrocarpa*.

Key words: Competitive inhibition kinetics, Flavonoid, Gout, Indonesia, Xanthine oxidase.

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INTRODUCTION

During the COVID-19 (Corona Virus Disease 2019) pandemic, gout is one of the comorbid diseases that increases the risk of infection with the SARS-Cov-2 (severe acute respiratory syndrome-coronavirus-2) virus.[1] Gout is an inflammatory joint disease triggered when monosodium urate crystals are deposited in the periarticular tissues, joints, and bones.[2-3] Gout is also the result of hyperuricemia, which means that the serum uric acid concentration exceeds the normal limit, in men above 7.0 mg/dL and in women above 6.5 mg/dL.[4] Excess purines consumption derived from animal protein foods, alcoholic beverages and diuretic-type drugs can increase serum uric acid levels. Without effective treatment, this condition can develop into chronic gout and even impair kidney function, coronary heart disease and stroke.[5]

Gout is one of the non-communicable degenerative diseases that arise due to the decline in body cell function with age and is not caused by infection with microorganisms such as protozoa, bacteria, fungi or viruses. This type of disease is responsible for at least 70% of deaths globally.[6] Gout attacks 1-2% of the world's population.[5] In Indonesia, gout is the second most common joint disease after osteoarthritis.[7] The prevalence of gout in Indonesia is in the range of 1.6-13.6 per 100.000 people.[8] The results of the Basic Health Research in 2007, 2013 and 2018 showed an increasing the prevalence of non-communicable diseases for joint diseases.[6]

A therapeutic approach to reduce uric acid is to inhibit the action of the enzyme xanthine oxidase (XO). This enzyme acts as a catalyst in the oxidation reaction of hypoxanthine to xanthine and also the oxidation of xanthine to uric acid.[9-10] Currently, the synthetic drug used as an inhibitor of the XO enzyme is allopurinol, approved by the US Food and Drug Administration since 1966 to treat gout. However, the administration of this drug brings side effects such as gastrointestinal disturbances, skin rashes, fever and kidney problems.[2] Therefore, to overcome the side effects of these treatments, people currently prefer traditional medicine. Herbal medicines from plant extracts are safer and more effective when used according to regulations.[11-12] Furthermore, WHO (World Health Organization) notes that 88% of the world's population has turned to traditional medicine.[13]

Indonesia is the largest archipelagic country in the world, with a potential of around 17,499 islands, of which 13,466 islands have been verified and registered with The United Nations Convention on the Law of the Sea (UNCLOS). Indonesia also has the third-largest tropical forest in the world after Brazil and D.R. Congo.[14] In addition, Indonesia occupies the third-largest position in the world that has the most tree species.[15] Based on the 2015-2020 Indonesian Biodiversity Strategy and Action Plan (IBSAP) data compiled by the Ministry of National Development Planning, the Ministry of Environment and Forestry, and The Indonesian Institute of Sciences, up to 15.5% of the total flora in the world is in Indonesia.[16] This country also has the second largest number of native medicinal plants, after the Amazon rainforest.[17] This is certainly a passion for Indonesia to find natural compounds as candidates for herbal medicines, referring that Indonesia is the country with the second-highest mega biodiversity after Brazil.[18]

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The traditional pathways of biodiversity, such as traditional medicine, represent the oldest forms of knowledge. This pathway is used as the starting point to build modern pathways of biodiversity, such as in the fields of research, biotechnology, pharmacy and bioeconomics.[17] Indonesia has more than 20,000 plant species, but only 1,180 species have been recorded and only about 300 species have been used for traditional medicine.[19] This article reviews the inhibitory capacity of XO in antigout therapy by Indonesian medicinal plants and proceed with major compound of XO inhibitor and competitive inhibition kinetics.

METHODS

Literature review studies were obtained from searches in the Science Direct database, Scopus, Google Scholar, Wiley Online Library and Pubmed. The keywords used in the database search were "*in vitro* xanthine oxidase inhibition", "Indonesian medicinal plants as xanthine oxidase inhibitors", "flavonoids", "competitive inhibition kinetics" and "Lineweaver-Burk plot."

GOUT

Gout comes from the Latin word gutta which means drip; in the 13th century it was believed that poison droplets fell on the joints and caused gout (uric acid).[20] Gout is an inflammatory joint disease caused by the accumulation of monosodium urate crystals in the periarticular tissue, joints and bones.[2-3] Serum uric acid concentrations that exceed normal limits (hyperuricemia) also cause gout for men above 7.0 mg/dL and women above 6.5 mg/dL.[4,21]

Uric acid, $C_5H_4N_4O_3$ (7,9-dihydro-1H-purine-2,6,8(3H)-trione), a heterocyclic organic compound with a molecular weight of 168 Da, is the end product of purine catabolism in the body. Uric acid at physiological pH is a weak acid with pKα 5.8. Most of the uric acid is uric acid/uric acid salts. When the concentration of urate increases in the blood, uric acid crystals form. The uric acid solubility in water is low and in humans, the average blood concentration of uric acid approaches the solubility limit (6.8 mg/dL). When uric acid levels exceed 6.8 mg/dL, uric acid crystals form as monosodium urate (MSU). Uric acid will be released into the body's circulation, which the kidneys will then excrete with urine. However, the kidneys cannot regulate purine secretion in excess purine condition, resulting in excess uric acid crystals and accumulation in joints and periarticular tissues. This high uric acid level can cause an inflammatory reaction, so-called gout.[22]

There are two types of gout, namely primary and secondary gout. Primary gout is hereditary and occurs due to a genetic defect that results in loss of control of purine synthesis, while secondary gout is temporary and will disappear if the cause is stopped.[23] There are four principles of gout treatment management, namely treatment of repeated attacks of gout, uric acid-lowering therapy, anti-inflammatory treatment and screening and management of comorbid diseases (comorbidities) in patients with gout such as heart disease, obesity, diabetes, impaired kidney function and hypertension.[24]

XANTHINE OXIDASE

The xanthine oxidase was originally called the "Schardinger enzyme" because Schardinger in 1902 reported that in milk there is an enzyme that oxidizes aldehydes to acids accompanied by a reduction of methylene blue. Then, in 1922, Morgan *et al.* revealed that milk contains an enzyme capable of oxidizing xanthine and hypoxanthine, together with the reduction of O_2 to H_2O_2 and this enzyme is called xanthine oxidase (XO). Hass & Hill and Hass & Lee reported that milk contains a substance, which they called "itate", capable of oxidizing nitrite to nitrate in the presence of an aldehyde and O_2. Under other conditions, the milk can reduce nitrate to nitrite. Finally, in 1938, Booth presented strong evidence that the Schardinger enzyme is a XO.[25]

Structurally, XO is a dimeric protein with a molecular mass of 300 kDa and each monomeric unit has three main groups. The first group is the active site of iron-sulfur [2Fe-2S] with a molecular mass of 20 kDa. Second, intermediate flavin adenine dinucleotide (FAD) with a molecular mass of 40 kDa. The latter is a molybdenum-pterin (Mo-Pt) center with a molecular mass of 85 kDa.[26]

XO itself plays a role in the uric acid formation. XO catalyzes hypoxanthine to xanthine then to uric acid, accompanied by the production of superoxide anion, H_2O_2 and reactive oxygen species (ROS) during catalysis (Figure 1) as follows:[25-26]

$$\text{hypoxanthine} + O_2 + H_2O \longrightarrow \text{xanthine} + H_2O_2$$

$$\text{xanthine} + 2O_2 + H_2O \longrightarrow \text{uric acid} + 2O_2^{\cdot-} + 2H^+$$

$$\text{xanthine} + O_2 + H_2O \longrightarrow \text{uric acid} + H_2O_2$$

$$2O_2^{\cdot-} + 2H^+ \longrightarrow H_2O_2 + O_2$$

Indonesian medicinal plants as xanthine oxidase inhibitors

Medicinal plants contain active compounds in all plant parts: roots, stems, leaves, fruit, seeds, flowers and barks. These active compounds have direct or indirect therapeutic effects in their use as medicinal agents. Medicinal plants are, also known as herbal plants, practically used in traditional medicine.[27]

Meanwhile, traditional medicine is defined as ingredient or ingredients derived from plant, animal, and mineral materials, extract preparations (galenic), or a mixture of these materials that have been used for generations for treatment and can be administered by following applicable norms in Indonesia community.[28] Traditional medicine has been used since the Ancient Mataram Kingdom, approximately 12 centuries ago. Thus, traditional medicine is the nation's cultural heritage and is still used by the Indonesian people.[29]

The use of traditional medicine that has developed into herbal medicine impacts a global scale. WHO work priorities in the WHO 2009 Regional Meeting on the Use of Herbal Medicine in Primary Health Care, resulted in an agreement to provide information on the use of herbal medicines. The WHO program strengthens the National Program on the use of Herbal Medicine in Basic Health Services.[30] Coupled with the "back to nature" trend, herbal medicines are increasingly developing globally.[31]

Gout disease, which is also called "the disease of King", has long been treated with herbal medicine.[32] Table 1 summarizes Indonesian medicinal plants as xanthine oxidase inhibitors with a specification of more than 50% inhibitory activity.

There are 25 Indonesian medicinal plants that act as XO inhibitors with more than 50% inhibitory activity. The magnitude of the inhibitory power ranged from 50.00±1.16% to 97.53%. The least inhibitory power is *Phaleria macrocarpa*, while *Orthosiphon aristatus* gives the highest inhibitory power. The inhibition kinetics that has been investigated are *Sida rhombifolia*, *Syzygium polyanthum*, *Cyperus rotundus*, *Ruellia tuberosa* and *Phaleria macrocarpa*, with competitive inhibition kinetics for XO.

Flavonoids as major compound of XO inhibitor

Flavonoids are secondary metabolites in plants and are formed through the shikimate and phenylpropanoid pathways. These bioactive compounds accumulate in roots, rhizomes, wood, bark, stem bark, seeds, leaves and flowers. Flavonoids are commonly found in vacuoles of epidermal cells, guard cells and subepidermal cells of leaves, aerial regions of monocotyledonous and dicotyledonous plants, vascular parenchyma cells, flowers, cell walls and cortex parenchyma cells.[52]

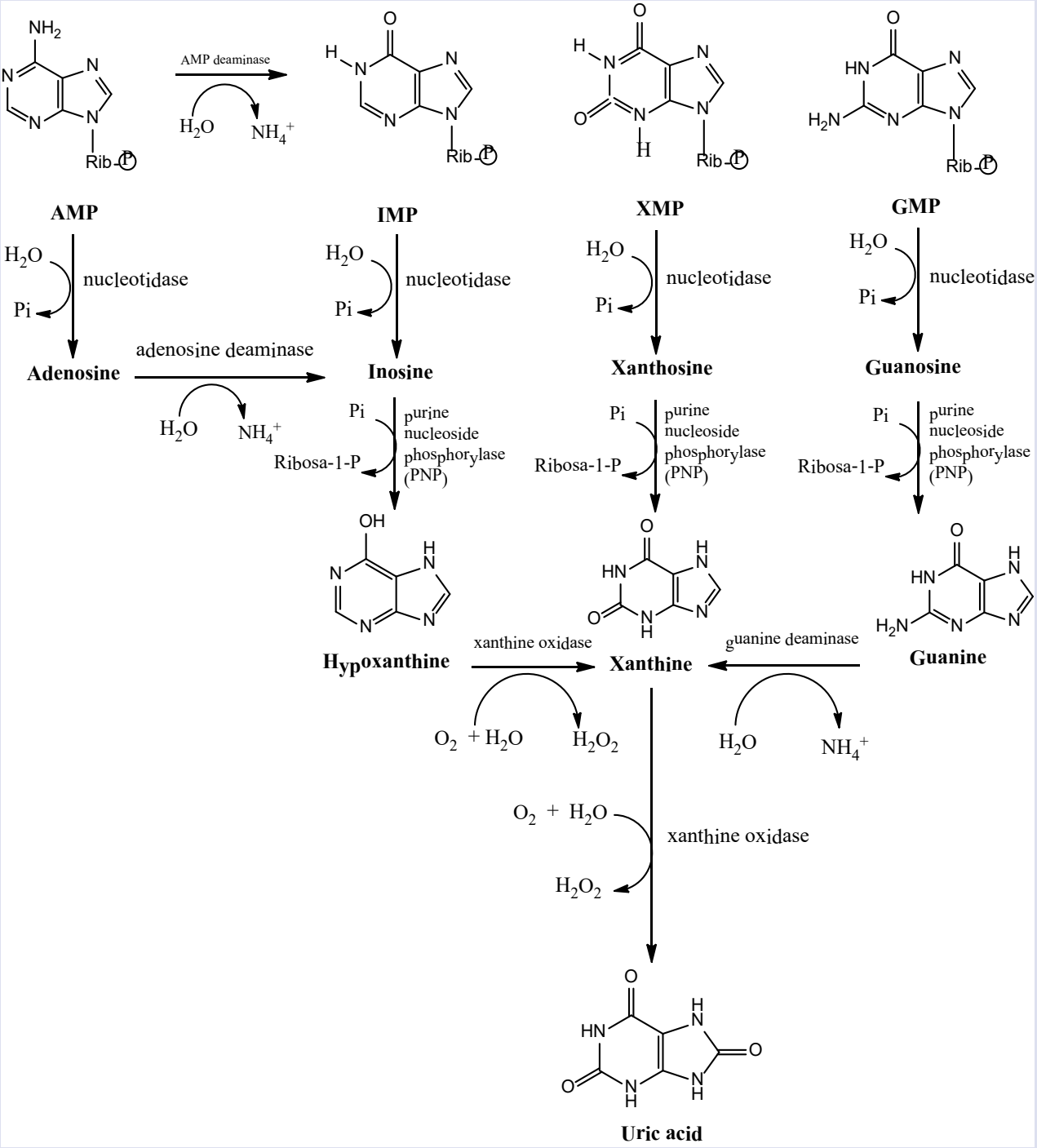


Figure 1: Mechanism of uric acid formation.[9]

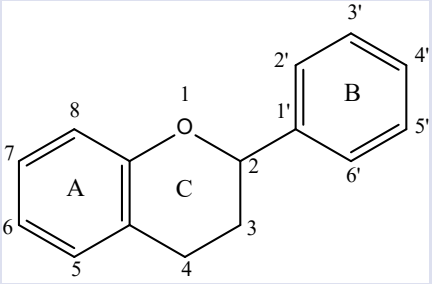


Figure 2: The flavonoids basic structure.

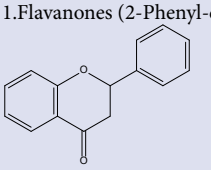
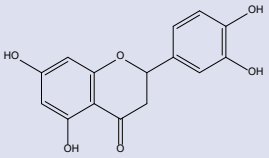
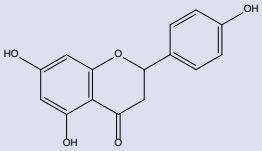
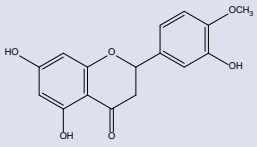
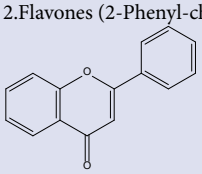
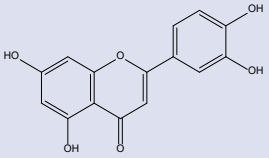
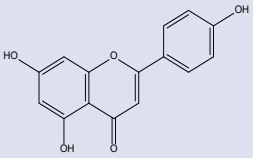
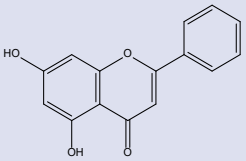
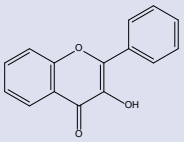
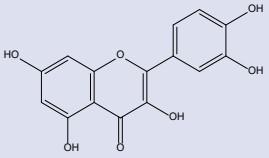
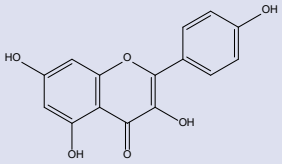
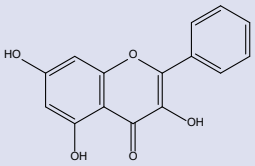
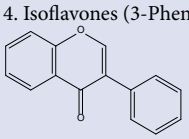
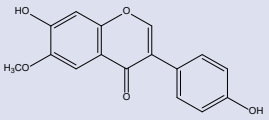
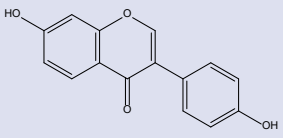
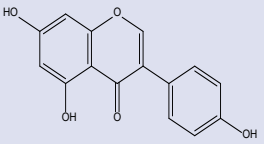
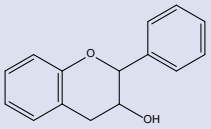
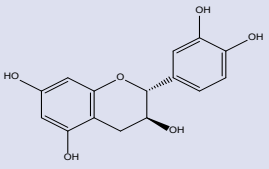
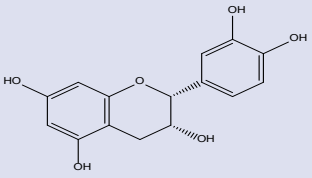
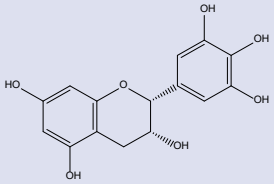
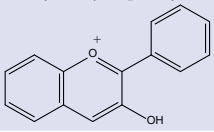
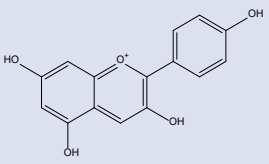
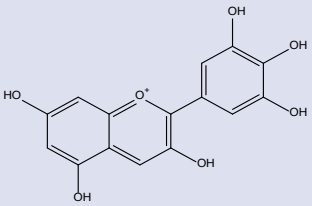
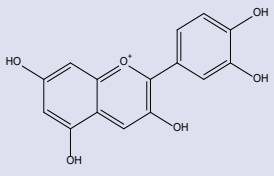
Table 1: List of Indonesian medicinal plants as *in vitro* xanthine oxidase inhibitors and their secondary metabolites.

No.	Scientific name	Local name	Plant parts	Extracting solvent	Inhibitory activity	Concentration	IC_{50}	Inhibition kinetics type	Major compound	Reference
1.	*Apium graveolens*	*Seledri*	Roots and herbs	Ethanolic chloroform: ethyl acetate (7:3)	88.62%	200 ppm	NA	NA	Flavonoids	33
2.	*Zanthoxylum acanthopodium*	*Andaliman*	Fruits	*n*-Butanol	69.9%	100 µg/mL	3.69 µg/mL	NA	Flavonoids, alkaloids, tannins, glycosides, anthraquinones, terpenoids	34
3.	*Alpinia galanga*	*Lengkuas*	Rhizomes	Ethanol	57.99±12.2%	100 µg/mL	65.36 µg/mL	NA	Flavonoids	35
4.	*Woodfordia floribunda*	*Sidawayah*	Flos	Ethanol	55.33±1.91%	100 µg/mL	94.79 µg/mL	NA	Flavonoids	35
5.	*Sida rhombifolia*	*Sidaguri*	Herbs	Ethanol	82.69%	200 ppm	91.15±5.74 mg/L	Competitive	Flavonoids	36
6.	*Myrmecodia tuberosa* (non Jack)	*Sarang semut*	Herbs	Ethanol 96%	64.54%	130 µg/mL	112.40 µg/mL	NA	Flavonoids	37
7.	*Peperomia peluucida*	*Ketumpangan air*	Herbs	Ethanol 96%	50.44%	12.5 ppm	19.5 µg/mL	NA	NA	38
8.	*Acalypha indica*	*Anting-anting*	Herbs	Ethanol 96%	60.75%	200 ppm	77.6 µg/mL	NA	NA	38
9.	*Momordica charantia*	*Pare*	Herbs	Ethanol 96%	63.56%	6.25 ppm	17.8 µg/mL	NA	NA	38
10.	*Sonchus arvensis*	*Tempuyung*	Leaves	Water continued with dichloromethane Ethanol 96%	77.41% 95.18±1.82%	500 µg/mL 200 µg/mL	119.02 ppm 4.39 µg/mL	NA	Flavonoids Quercetin (flavonoid)	39 40
11.	*Stelechocarpus burahol*	*Kepel*	Leaves	Ethanol 96% continued with ethyl acetate	63.79±4.28%	200 µg/mL	128.39±20.2 µg/mL	NA	Kaempferol-3-O-rhamnoside (flavonoid)	41
12.	*Persea americana*	*Alpukat*	Leaves	Ethanol 70%	77.54%	120 ppm	65.55 ppm	NA	Flavonoids	42
13.	*Ruellia tuberosa*	*Kencana ungu*	Flowers	Methanol continued with the *n*-butanol	NA	NA	0.21 µg/mL	Competitive	5-Hydroxy-3,7-dimethoxy-2-(4-((3*S*,4*S*,5*S*,6*R*)-4,5,6-trihydroxy-2-(hydroxyl methyl)-tetrahydro-2H-pyran-3-yloxy)phenyl)-4H-chromen-4-one (Flavonoids)	43
14.	*Pereskia bleo*	*Jarum tujuh bilah*	Leaves	Ethanol 96% continued with ethyl acetate	88.89%	100 ppm	NA	NA	Flavonoids	44
15.	*Annona muricata*	*Sirsak*	Leaves	Ethanol 96% and then purified	77.97%	11.8 ppm	0.02 ppm	NA	Quercetin 3-(2-galloylglucoside) (flavonoids)	45
16.	*Caesalpinia sappan*	*Sappan*	Stem	Ethanol 70%	59±1%	100 µg/mL	NA	NA	Polyphenols	46
17.	*Annona squamosa*	*Srikaya*	Stem	Ethanol and then purified	82.88%	100 ppm	NA	NA	Flavonoids	47
18.	*Syzygium polyanthum*	*Daun salam*	Leaves	Methanol continued with ethyl acetate	52.54±1.29%	80 µg/mL	18.43 µg/mL	Competitive	Flavonoids	48
19.	*Cyperus rotundus*	*Rumput teki*	Herbs	Methanol continued with ethyl acetate	51.01±0.95%	80 µg/mL	10.50 µg/mL	Competitive	Flavonoids	48
20.	*Phaleria macrocarpa*	*Mahkota dewa*	Fruits	Methanol continued with ethyl acetate	50.00±1.16%	80 µg/mL	19.23 µg/mL	Competitive	Flavonoids	48
21.	*Dillenia serrata*	*Songi*	Stem bark	Methanol	50.3%	100 µg/mL	NA	NA	Triterpenes	49
22.	*Beta vulgaris*	*Bit merah*	Roots	Ethanol 96%	55.11%	1,000 µg/mL	NA	NA	Flavonoids	50

23.	*Orthosiphon aristatus*	*Kumis kucing*	Herbs	Water; Ethanol 70%	97.53% 89.70%	450 µg/mL 500 µg/mL	84.78 µg/mL NA	NA NA	Flavonoids, tannins	51
24.	*Andrographis paniculate*	*Sambiloto*	Herbs	Water; Ethanol 70%	89.18% 79.05%	500 µg/mL 500 µg/mL	NA NA	NA NA	Flavonoids, tannins	51
25.	*Salacca zalacca*	*Salak*	Fruits	Water; Ethanol 70%	81.20% 77.72%	500 µg/mL 500 µg/mL	NA NA	NA NA	Flavonoids, tannins	51

NA: Not Available

Table 2: The main subclasses of flavonoid aglycones.[52,54]

Subclasses of flavonoids	Compounds		
1.Flavanones (2-Phenyl-chroman-4-one) 	 Eriodictyol	 Naringenin	 Hesperetin
2.Flavones (2-Phenyl-chromen-4-one) 	 Luteolin	 Apigenin	 Chrysin
3. Flavonols (3-Hydroxy-2-phenyl-chromen-4-one) 	 Quercetin	 Kaempferol	 Galangin
4. Isoflavones (3-Phenyl-chromen-4-one) 	 Glycitein	 Daidzein	 Genistein
5. Flavan-3-ols or Catechins (2-Phenyl-chromen-3-ol) 	 (+)-Catechin	 (-)-Epicatechin	 (-)-Epigallocatechin
6. Anthocyanidins or flavylium salt (3-Hydroxy-2-phenyl chromenylium) 	 Pelargonidin	 Delphinidin	 Cyanidin

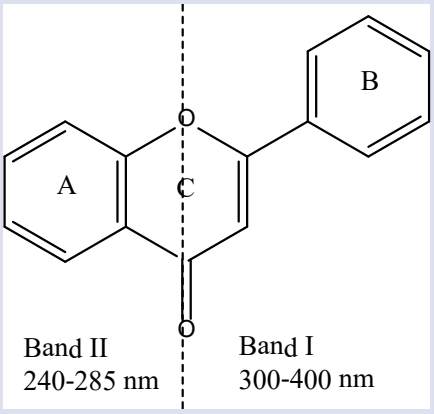


Figure 3: Band II absorption (originated from the A-ring benzoyl system) and band I (from the B-ring cinnamoyl system).[53]

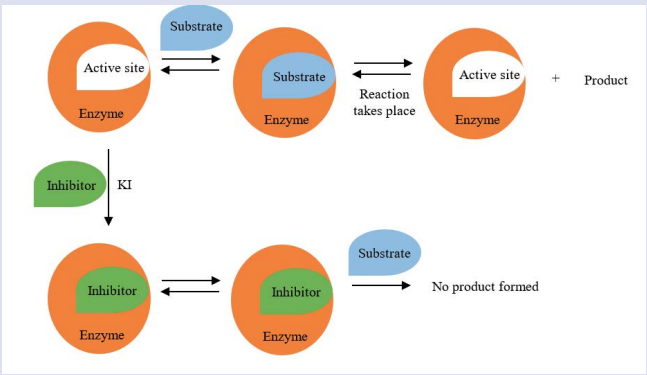


Figure 4: Mechanism of competitive inhibition.[9,62]

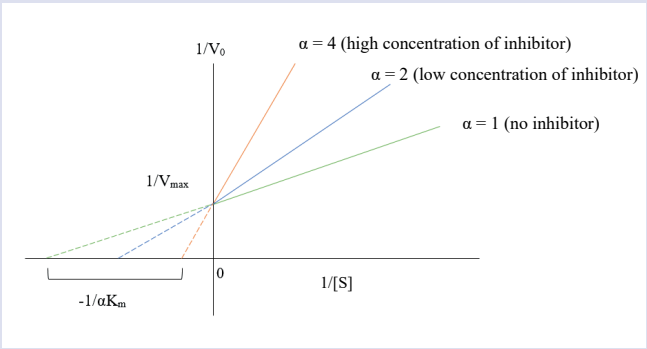


Figure 5: Lineweaver-Burk plot of competitive inhibition type.[8]

Approximately 10,000 flavonoids represent the third largest group of natural products, after alkaloids (12,000) and terpenoids (30,000).[53] Flavonoids belong to the phenolic compounds with the chemical structure of flavonoids depending on the C6-C3-C6 basic framework consisting of two benzene rings (A and B) linked through a heterocyclic pyran ring (C)[54] (Figure 1).

Flavonoids contain a conjugated aromatic system that can absorb UV-Vis light.[55] Band II (240–285 nm) corresponds to the benzoyl system of ring A, while band I (300–400 nm) represents the cinnamoyl system of ring B.[53] Flavonoids exist as aglycone derivatives, glycosides, methylated, acetylated and sulfate.[56] These compounds exhibit various biological activities such as antioxidant, anticarcinogenic and antimicrobial, antiallergic, anti-inflammatory, antiaging, anticancer and antiviral.[52,54,57]

Flavonoids are classified into 12 subclasses based on their chemical structure. From which, six of which play an essential role in the therapeutic activity are anthocyanidins, flavan-3-ols, flavonols, flavones, flavanones and isoflavones (Table 2).[58] The mechanism of phytochemical compounds in inhibiting XO has not been reported in detail.[10] Based on the structure of flavonoids in rings A and C, which is similar to xanthine as a substrate, there are hydrophobic interactions, hydrogen bonds and van der Waals forces between flavonoids and XO. It means that flavonoids bind to the active site of XO thereby preventing the formation of uric acid. The planar structure of flavonoids, the presence of double bonds between C2 and C3 and the content of hydroxyl groups at C5 and C7 played a crucial role in the XO inhibition. However, the presence of methylation in ring B; glycosylation at ring A and ring C; and hydrogenation of the C2=C3 double bond will decrease the binding affinity of XO.[10,32,59,60]

COMPETITIVE INHIBITION KINETICS

Determination of the type of inhibition kinetics can explain the mechanism of inhibition and affinity formed between XO enzymes, as the targets and the drug candidate compounds, whether they are temporary (competitive and uncompetitive inhibition) or permanent (non-competitive inhibition).[36,61] Bioavailability (easily absorbed in metabolic pathways), reduced toxicity effects and specific structure makes competitive inhibitors selected as initial candidates for medicinal compounds in the pharmacokinetic world.[61]

In competitive inhibition, the compound structure of the inhibitor resembles the substrate. This condition can be developed with the substrate to bind to the active site. The competitive inhibitor and the substrate have the same affinity for the active site of the enzyme. If the inhibitor concentration exceeds the substrate concentration, the active site of the enzyme will be occupied by the inhibitor (enzyme-inhibitor complex), which means no product will be formed as shown in Figure 4.[62]

The double-reciprocal plot or Lineweaver-Burk plot (Figure 5) indicates that the slope increases with increasing inhibitor concentration. At the same time, the *x*-intercept decreases, indicating that the presence of competitive inhibitors results in the value of K_m, the Michaelis-Menten constant, increase. On the other hand, the *y*-intercept has no effect, meaning that the competitive inhibitor does not change the maximum velocity (V_{max}). Thus, the Michaelis-Menten equation for competitive inhibition is [9,62,63]

$$V_0 = \frac{V_{max}[S]}{\alpha K_m + [S]}$$

with:

$$K_I = \frac{[E][I]}{[EI]}$$

where:

V_0 = initial velocity of reaction when substrate concentration increases (μM/min)

V_{max} = maximum velocity when the enzyme conditions are saturated with substrate (μM/min)

K_m = Michaelis-Menten constant; the substrate concentration when V_{max} (μM)

[S] = substrate concentration (μM)

α = a function of the inhibitor concentration

[I] = inhibitor concentration (μM)

[E] = enzyme concentration (μM)

[EI] = the enzyme-inhibitor complex concentration (μM)

KI = inhibition constant (μM)

Based on the kinetic parameters that have been reported in several studies, the subclasses of flavonoids that lead to competitive inhibitors of XO are flavones (luteolin, apigenin, chrysin),[64-65] flavonols (kaempferol, galangin, quercetin),[10,60] flavanols (teaflavins and catechins),[66-67] flavanones (eriodictyol)[64] and isoflavones (genistein).[68]

CONCLUSION

Natural wealth and knowledge of Indonesian traditional medicine combined with scientific advances may make Indonesia a superior country in developing herbal medicines. Research on 25 species of Indonesian plants with more than 50% XO enzyme inhibitory activity, flavonoids as major compound of XO inhibitor and competitive inhibition kinetics of XO can serve as a primary step in finding potential plants as antigout sources. However, the flavonoids content in these plants and the type of inhibition kinetics need to be further investigated.

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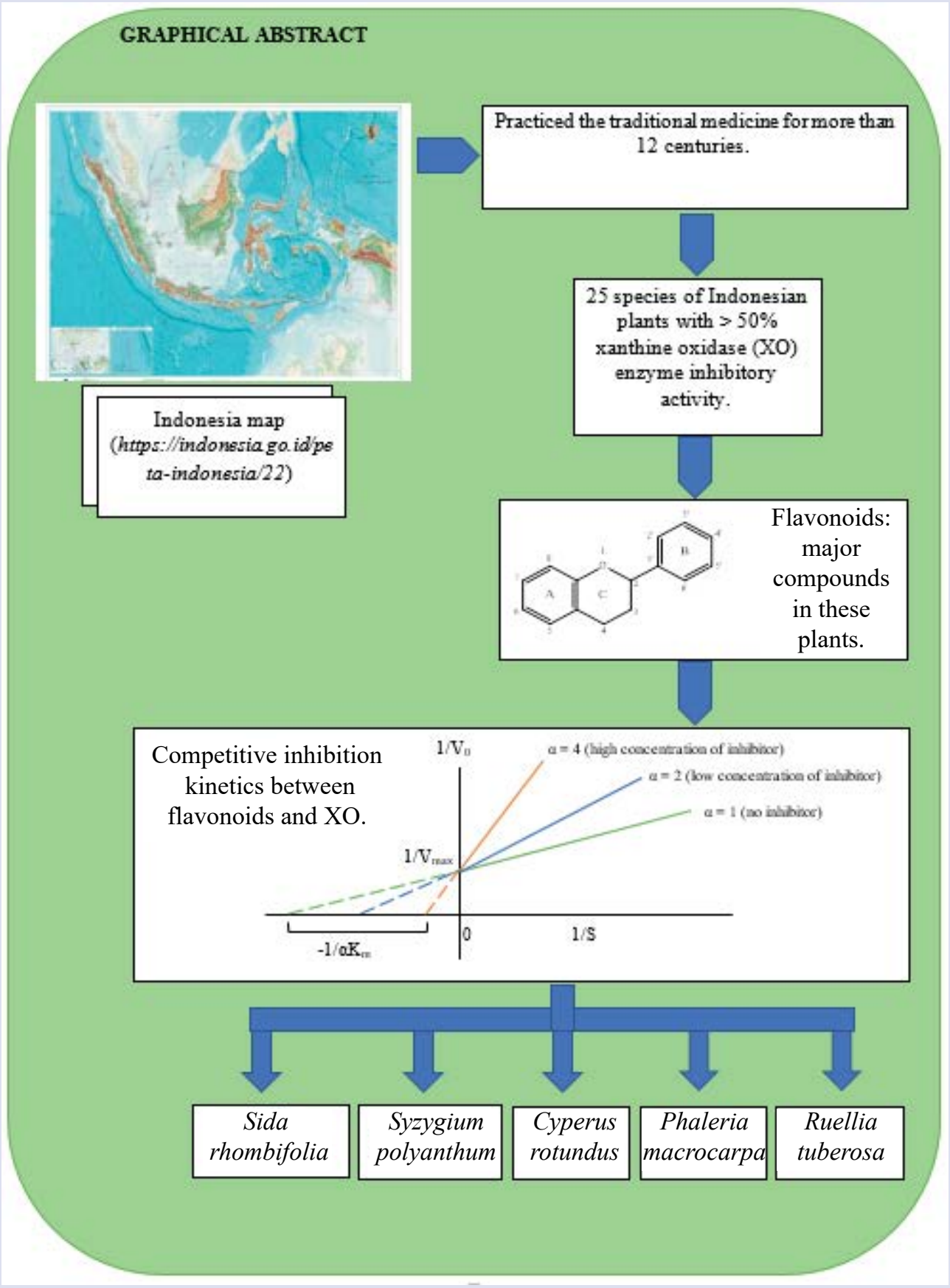
GRAPHICAL ABSTRACT



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