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

Rut Novalia Rahmawati Sianipar¹, Komar Sutria¹, Dyah Iswantini¹,², Suminar Setiati Achmadi¹,²

¹Department of Chemistry, Faculty of Mathematics and Natural Sciences, IPB University, Bogor 16680, INDONESIA.
²Tropical Biopharmaka Research Center, IPB University, Bogor 16128, INDONESIA.

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 97.83%. 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.

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. Gout is an inflammatory joint disease triggered when monosodium urate crystals are deposited in the periarticular tissues, joints, and bones. 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. 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.

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. Gout attacks 1-2% of the world’s population. In Indonesia, gout is the second most common joint disease after osteoarthritis. The prevalence of gout in Indonesia is in the range of 1.6-13.6 per 100,000 people. The results of the Basic Health Research in 2007, 2013 and 2018 showed an increasing prevalence of non-communicable diseases for joint diseases.

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. 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. 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. Furthermore, WHO (World Health Organization) notes that 88% of the world’s population has turned to traditional medicine.

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. In addition, Indonesia occupies the third-largest position in the world that has the most tree species. 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. This country also has the second largest number of native medicinal plants, after the Amazon rainforest. 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.
Gout comes from the Latin word *gutta* which means drip; in the 13th century it was believed that poisons dripped on the joints and caused gout (*uric acid*). Gout is an inflammatory joint disease caused by the accumulation of monosodium urate crystals in the periarticular tissue, joints and bones. 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.

Uric acid, \(\text{C}_5\text{H}_4\text{N}_4\text{O}_3\) (*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 pKa 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.

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. There are four principles of gout treatment management, namely treatment of repeated attacks and will disappear if the cause is stopped. There are four principles of gout treatment management, namely treatment of repeated attacks and will disappear if the cause is stopped.

**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**

**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 \(\text{O}_2\) to \(\text{H}_2\text{O}\) and this enzyme is called xanthine oxidase (XO). Hass & Hill and Hass & Lee reported that milk contains a substance, which they called "state", capable of oxidizing nitrite to nitrate in the presence of an aldehyde and \(\text{O}_2\). Under other conditions, the milk can reduce nitrate to nitrite. Finally, in 1938, Booth presented strong evidence that the Schardinger enzyme is a XO.

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. 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, \(\text{H}_2\text{O}_2\) and reactive oxygen species (ROS) during catalysis (Figure 1) as follows:

- \(\text{hypoxanthine} + \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{xanthine} + \text{H}_2\text{O}_2\)
- \(\text{xanthine} + 2\text{O}_2 + \text{H}_2\text{O} \rightarrow \text{uric acid} + 2\text{O}_2^- + 2\text{H}^+\)
- \(\text{hypoxanthine} + \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{xanthine} + \text{H}_2\text{O}_2\)
- \(\text{xanthine} + 2\text{O}_2 + \text{H}_2\text{O} \rightarrow \text{uric acid} + 2\text{O}_2^- + 2\text{H}^+\)
- \(2\text{O}_2^- + 2\text{H}^+ \rightarrow 2\text{H}_2\text{O}\)

**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.

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. 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.

The use of traditional medicine that has developed into herbal medicine impacts a global scale. The WHO program strengthens the National Program 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. Coupled with the "back to nature" trend, herbal medicines are increasingly developing globally.

Gout disease, which is also called "the disease of King", has long been treated with herbal medicine. 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*, *Syzgium polyamnium*, *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.
**Figure 1:** Mechanism of uric acid formation.

**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<sub>50</sub> | 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 acaanthopodium | 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 | 64.54% | 130 μg/mL | 112.40 μg/mL | NA | Flavonoids | 37 |
| 7.  | Peperomia pellucida | Ketumpanang air | Herbs | Ethanol | 50.44% | 12.5 ppm | 19.5 μg/mL | NA | NA | 38 |
| 8.  | Acalypha indica | Anting-anting | Herbs | Ethanol | 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. | Sanchothracus arvensis | Tempanyang | Leaves | Water continued with dichloromethane | Ethanol 96% | 77.41% | 95.18±1.82% | 119.02 ppm | Flavonoids | 39 |
| 11. | Stelechocarpus burahol | Kepel | Leaves | Ethanol 96% continued with ethyl acetate | Ethanol 96% | 63.79±4.28% | 200 μg/mL | 128.39±20.2 μg/mL | Flavonoids | 40 |
| 12. | Persea americana | Alpukat | Leaves | Ethanol 70% | Ethanol 70% | 77.54% | 120 ppm | 65.55 ppm | NA | 41 |
| 13. | Ruellea tuberosa | Kencana ungu | Flowers | Methanol continued with the n-butanol | Ethanol 96% continued with ethyl acetate | 88.89% | 100 ppm | 0.21 μg/mL | Competitive | 42 |
| 14. | Peresicka bleo | Jaran tujuh bilaah | Leaves | Ethanol 96% continued with ethyl acetate | Ethanol 96% continued with ethyl acetate | 88.89% | 100 ppm | 0.21 μg/mL | Competitive | 43 |
| 15. | Annona muricata | Sirsak | Leaves | Ethanol 96% and then purified | Ethanol 96% and then purified | 77.97% | 11.8 ppm | 0.02 ppm | Quercetin 3-(2-galloylglucoside) (flavonoids) | 44 |
| 16. | Caesalpinia sappan | Sappan | Stem | Ethanol 70% | Ethanol 70% | 59±1% | 100 μg/mL | NA | Polyphenols | 45 |
| 17. | Annona squamosa | Srikaya | Stem | Ethanol and then purified | Ethanol and then purified | 82.88% | 100 ppm | NA | Flavonoids | 46 |
| 18. | Syzygium polyanthum | Daun salam | Leaves | Methanol continued with ethyl acetate | Methanol continued with ethyl acetate | 52.54±1.29% | 80 μg/mL | 18.43 μg/mL | Competitive | 47 |
| 19. | Cypers rotundus | Rumpun teki | Herbs | Methanol continued with ethyl acetate | Methanol continued with ethyl acetate | 51.01±0.95% | 80 μg/mL | 10.50 μg/mL | Competitive | 48 |
| 20. | Phaleria macrocarpa | Mahkota dewa | Fruits | Methanol continued with ethyl acetate | Methanol continued with ethyl acetate | 50.00±1.16% | 80 μg/mL | 19.23 μg/mL | Competitive | 49 |
| 21. | Dillenia serrata | Songi | Stem bark | Methanol | Methanol | 50.3% | 100 μg/mL | NA | Triterpenes | 50 |
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)                                                | Glycritein           |
|                                                                                        | Daidzein             |
|                                                                                        | Genistein            |
| 5. Flavan-3-ols or Catechins (2-Phenyl-chromen-3-ol)                                    | (+)-Catechin         |
|                                                                                        | (-)-Epicatechin      |
|                                                                                        | (-)-Epigallocatechin |
| 6. Anthocyanidins or flavylum salt (3-Hydroxy-2-phenyl chromenylum)                     | Pelargonidin         |
|                                                                                        | Delphinidin          |
|                                                                                        | Cyanidin             |
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). The mechanism of phytochemical compounds in inhibiting XO has not been reported in detail. 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.

**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). 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.

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. 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:

$$V_0 = \frac{V_{max} \cdot [S]}{aK_m + [S]}$$

with:

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

where:

- $V_o$ = 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)
- $a$ = a function of the inhibitor concentration
- $[I]$ = inhibitor concentration (μM)
- $[E]$ = enzyme concentration (μM)
- $[EI]$ = the enzyme-inhibitor complex concentration (μM)
- $K_I$ = 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), flavonols (kaempferol, galangin, quercetin), flavanols (teatavins and catechins), flavanones (eriodictyol) and isoflavones (genistein).

**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.

**ACKNOWLEDGEMENTS**

The authors would like to thank the Ministry of Education and Culture, Research, and Technology of the Republic of Indonesia for granting this work through Penelitian Terapan Kompetitif Nasional scheme (year 2022).

**REFERENCES**

1. Ministry of Home Affairs Working Team for 2020 COVID-19 Task Force Support. Pedoman Umum Menghadapi Pandemi COVID-19 Bagi Pemerintah Daerah. Jakarta: Ministry of Home Affairs. 2020.

2. Pacher P, Nivorozhkin A, Szabo C. Therapeutic effects of xanthine oxidase inhibitors: Pharmacological Review. 2006;58(1):87-114.

3. Ayoub S, Rajamohanan AG, Acharya J, Gross J, Patel V. Chronic tophaceous gout causing lumbar spinal stenosis. Radiol Case Reports. 2021;16(2):237-240.

4. Kang D-H, Johnson RJ. Uric acid metabolism and the kidney. Chapter 43. Chronic Renal Disease, Second Edition. 2020;689-701.

5. Recommendations of the Indonesian Rheumatology Association. Pedoman Diagnosis dan Pengelolaan Gout. Jakarta: Indonesian Rheumatology Association. 2018.

6. Ministry of Health Republic of Indonesia. Profil Kesehatan Indonesia Tahun 2019. Jakarta: Ministry of Health Republic of Indonesia. 2020.

7. Simamora RH, Saragih E. Penyuluhan kesehatan masyarakat: Penatalaksanaan perawatan penderita asam urat menggunakan media audiovisual. Jurnal Pendidikan dan Pemberdayaan Masyarakat. 2019;6(1):24-31.

8. Ardhitama F, Rosita A, Lestariningih REM. Hubungan antara pengetahuan tentang gout arthritis terhadap perilaku pencegahan gout arthritis pada lansia. Global Health Science. ISSN 2503-5088. 2017;2(2):111-116.

9. Voet D, Voet JG. Biochemistry Fourth Edition. USA: John Wiley & Sons, Inc. 2010.

10. Ayyappan P, Nampoothiri SV. Bioactive natural products as potent inhibitors of xanthine oxidase. USA: Elsevier BV. 2020;64:391-416.

11. Ministry of Health Republic of Indonesia. Laporan Nasional Risikodas 2018. Jakarta: Ministry of Health Republic of Indonesia. 2018.

12. Jiang LL, Gong X, Ji MY, Wang CC, Wang JH, Li MH. Bioactive compounds from plant-based functional foods: A promising choice for the prevention and management of hyperuricemia. Foods. 2020;9(8):1-24.

13. World Health Organization. WHO Global Report on Traditional and Complementary Medicine 2019. 2019.

14. Brearley FQ, Adinugroho WC, Câmara-Leret R, Krisnawati H, Ledo A, Gie L, et al. Opportunities and challenges for an Indonesian forest monitoring network. Ann For Sci. 2019;76(54):1-12.

15. Food and Agriculture Organization of the United Nations and United Nations Environmental Programme. The State of the World’s Forests 2020. Forests, biodiversity and people. Rome: Food and Agriculture Organization of the United Nations. 2020.

16. Ministry of National Development Planning of Indonesia. Indonesian Biodiversity Strategy and Action Plan 2015-2020. Jakarta: Ministry of National Development Planning of Indonesia. 2016.

17. Rintelen KV, Arda E, Häuser C. A review of biodiversity-related issues and challenges in megadiverse Indonesia and other Southeast Asian countries. Research Ideas and Outcomes. 2017;3:e20860.

18. The Indonesian Institute of Sciences. Kekinian Keanekaragaman Hayati Indonesia. Jakarta: LIPI Press. 2014.

19. Fitriani, Sampepna E, Saputra SH. Karakteristik tanaman anak bajakah (Spaltholobus littoralis Hassk.) dari Loa Kubu Kabupaten Kutai Kartanegara. Jurnal Riset Teknologi Industri. 2020;14(2):365-376.

20. Suresh E. Diagnosis and management of gout: A rational approach. Postgrad Med J. 2005;81(959):572-579.

21. Bodofsky S, Merriman TR, Thomas TJ, Schlesinger N. Advances in our understanding of gout as an auto-inflammatory disease. Seminars in Arthritis Rheumatism. 2020;50(5):1089-1100.

22. Maioolo J, Oppedisano F, Gratteri S, Muscoli C, Mollace V. Regulation of uric acid metabolism and excretion. International Journal of Cardiology. 2015;213:8-14.

23. Dianati NA. Gout and hyperuricemia. J Majority. 2015;43(3):82-89.

24. Dalbeth N, Choi HK, Joosten LAB, Khanna PP, Matsuo H, Perez-Ruiz F, et al. Gout. Nature Reviews. 2019;51(1):1-7.

25. Kostic DA, Dimitrijevic DS, Stojanovic GS, Palic IR, Dordavse AS, Ickovski JD. Xanthine oxidase: Isolation, assays of activity and inhibition. Journal of Chemistry. 2015;1-8.

26. Singh JV, Bedi PMS, Singh H, Sharma S. Xanthine oxidase inhibitors: patent landscape and clinical development (2015-2020). Expert Opinion on Therapeutic Patents. 2020;30(10):769-780.

27. Jamshidi-Kia F, Lorigooini Z, Amini-Khoei H. Medicinal plants: past history and future perspective. Journal of HerbMed Pharmacology. 2018;7(1):1-7.

28. The National Agency for Drug and Food Control of Indonesia Regulation Nomor 32 Tahun 2019. Persyaratan keamanan dan mutu obat tradisional. Jakarta: The National Agency for Drug and Food Control of Indonesia. 2019.

29. Indonesian Academy of Sciences. Sains untuk Biodiversitas Indonesia. Jakarta: LIPI Press. 2014.

30. Regulation of the Minister of Health of the Republic of Indonesia No. 6 Tahun 2016. Formulir Obat Herbal Asli Indonesia. Jakarta: Ministry of Health Republic of Indonesia. 2016.

31. Ministry of Health Republic of Indonesia. Perkembangan Obat Tradisional di Indonesia. Jakarta: Ministry of Health Republic of Indonesia. 2019.

32. Liu L, Zhang L, Ren L, Xie Y. Advances in structures required of 3D-structures of alldolimon A by optical NMR. J Pharmaceut Biomed Anal. 2017;17(1):22-33.

33. Liu L, Zhang L, Ren L, Xie Y. Advances in structures required of 3D-structures of alldolimon A by optical NMR. J Pharmaceut Biomed Anal. 2017;17(1):22-33.
35. Yumita A, Suganda AG, Sukandar EY. Xanthine oxidase inhibitory activity of some Indonesian medicinal plants and active fraction of selected plants. International Journal of Pharmacy and Pharmaceutical Sciences. 2013;2(2):293-296.

36. Iswantini D, Yulian M, Muljiani S, Trividalia. Inhibition kinetics of Sida rhombifolia L. extract toward xanthine oxidase by electrochemical method. Indonesian Journal of Chemistry. 2014;14(1):71-77.

37. Ernawati E, Susanti H. In vitro xanthine oxidase inhibition activity of the sarang semut (Myrmecodia tuberosa (non Jack) Bl.) ethanol extract. Pharmaciana. 2014;4(1):15-22.

38. Paravansah P, Nuralifah N, Alam G, Natzir R. Inhibition of xanthine oxidase activity by ethanolic extract of Pteropus pellucidos L., Acalypha indica L. and Momordica charantia L. The Indonesian Biomedical Journal. 2016;8(3):161-166.

39. Trividalia, Oktaviani I, Iswantini D. Inhibition of tempyung (Sonchus arvensis) water extract fractions against xanthine oxidase by electrochemical method. AIP Conference Proceedings. 2020;2243:1-8.

40. Hendriani R, Nursamsiari, Tjiptaresmi A. In vitro and in silico evaluation of xanthine oxidase inhibitory activity of quercetin contained in Sonchus arvensis leaf extract. Asian Journal of Pharmaceutical and Clinical Research. 2017;10:50-53.

41. Sunarni T, Fidrianny I, Iwo MI, Wirasutisna KR. Constituent and antiherpetic activity of Stelechocarpus burahol leaves subfractions. Asian Journal of Pharmaceutical and Clinical Research. 2017;10(4):435-439.

42. Suwandi DW, Perdana F. Inhibition activity of xanthine oxidase of ethanol extract of avocado leaves with in vitro method. Jurnal Ilmiah Farmako Bahari. ISSN:2087-0337. 2017;8(2):40-45.

43. Ahmad AR, Elya B, Mun’im A. Antioxidant activity and isolation of xanthine oxidase inhibitor from Ruellia tuberosa L. leaves. Pharmacognosy Journal. 2017;9(5):607-610.

44. Sari PS, Sitorus S, Gunawan R. Inhibisi xantin oxidase oleh fraksi etil asetat dari daun jarum tuju bilah (Peresokia bloeo (Kunth) D.C) sebagai antihiperursemia. Jurnal Atomik. 2018;32(2):116-121.

45. Slamet, Setyahadi S, Simanjuntak P. Isolasi dan identifikasi senyawa aktif dari daun jambu biji (Averrhoa bilimbi (L) Roxb) sebagai penghambatan xantin oksidase. ISSN: 976-978-3812-42-7. The 5th Urecol Proceeding. 2017;6(2):139-144.

46. Ningish S, Churiyah. Evaluasi aktivitas inhibisi xantin oksidase dan kandungan senyawa polifenol dari ekstrak sirsak. Jurnal Bioteknologi & Biosains Indonesia. 2018;5(2):157-167.

47. Alvinota M, Oktavia I, Subandi S, Muntholib. Bioactivity of flavonoid in ethanol extract of Annona squamosa L. fruit as xanthine oxidase inhibitor. IOP Conf. Series: Materials Science Engineering. 2019;546(6):1-10.

48. Sakti AS, Widyastanto H, Maulidi G, Natsir R. Inhibition of xanthine oxidase activity of methanol extract fractions of various Indonesian ethnopharmacological plants. International Journal of Applied Pharmacosciences. 2020;12:43-46.

49. Sabandar CW, Jail J, Ahmad K, Aladdin N-A, Kamaruddin HS, Wahyuningrum R. Aktivitas antioksidan dan penghambatan xantin oksidase kutil batang songi (Dillenia aerata Thunb.). Galenicajournal of Pharmacy. 2020;6(1):151-159.

50. Putri G, Mahendra AN, Jawi IM. In vitro study of red beetroot ethanol extract (Beta vulgaris L.) as xanthine oxidase inhibitor. Intisai Sains Medis. 2021;12(1):414-419.

51. Trividalia, Julian AL, Tiarani SI, Sa’diah S, Iswantini D. Inhibition against xanthine oxidase enzyme by Andrographis paniculata, Orthosiphon aristatus and Salacca zalacca fruit water and ethanolic extracts as antioxidant. Proceedings of the 2nd International Conference on Science, Technology and Modern Society (ICSTMS 2020). 2021;576:462-469.

52. Rasouli H, Hossein-Ghazvini SMB, Khodarahmi R. Therapeutic potentials of the most studied flavonoids: highlighting antibacterial and antidiabetic functionalities. Elsevier BV. 2018;60(3).

53. Santos EL, Maia BHLNS, Ferriani AP, Teixeira SD. Flavonoids: classification, biosynthesis and chemical ecology. Chapter 1. Flavonoids:From Biosynthesis to Human Health. 2017.

54. Jan S, Abbas N. Chemistry of Himalayan phytochemicals. Chapter 4. Himalayan Phytochemicals. 2018;121-166.

55. Louie KB, Kosina SM, Hu Y, Otani H, de Raad M, Kutfin AN, et al. Mass spectrometry for natural product discovery. In Comprehensive Natural Products III: Chemistry and Biology (3rd ed.). Elsevier Ltd. 2020.

56. Teles YCF, Souza MSR, Souza M de FVD. Sulphated flavonoids: biosynthesis, structures and biological activities. Molecules. 2018;23(2):1-11.

57. Badshah SL, Faisal S, Muhammad A, Poulsou BG, Emwas AH, Jaremk M. Antiviral activities of flavonoids. Biomedicine & Pharmacotherapy. 2021;140(2021):111196.

58. Harborne JB. Plant phenolics. In "Encyclopedia of plant physiology, New Series" (E.A. Bell and B.V. Charlwood, eds.). Berlin and New York: Springer-Verlag; 1980;8:329-402.

59. Lin S, Zhang G, Liao Y, Pan J, Gong D. Dietary flavonoids as xanthine oxidase inhibitors: structure-affinity and structure-activity Relationships. Journal of Agricultural and Food Chemistry. 2015;63(35):7784-7794.

60. Ou R, Lin L, Zhao M, Xie Z. Action mechanisms and interaction of two key xanthine oxidase inhibitors in galangal: Combination of in vitro and in silico molecular docking studies. International Journal of Biological Macromolecules. 2020;162:1526-1535.

61. Zhao J, Huang L, Sun C, Zhao D, Tang H. Studies on the structure-activity relationship and interaction mechanism of flavonoids and xanthine oxidase through enzyme kinetics, spectroscopy methods and molecular simulations. Food Chemistry. 2020;323(126807):1-11.

62. Mohan C, Long KD, Mutneja M. An introduction to inhibitors and their biological applications. EMD Millipore Corp. 2013;1-42.

63. Pathak K, Gogoi U, Das A. Enzyme inhibition. Pharmaceutical Sciences. 2020;3:1-33.

64. Nelson D, Cox M. Lehrer Principles of Biochemistry Fourth Edition. New York: WH. Freeman and Company. ISBN 0-7167-4339-6. 2005.

65. Peng A, Lin L, Zhao M. Screening of key flavonoids and monoterpenoids for xanthine oxidase inhibitory activity-oriented quality control of Chrysanthenum monfolium Ramat. ‘Boju’ based on spectrum-effect relationship coupled with UPLC-T0F-MS and HS-SPME-GC/MS. Food Research International. 2020;137(109448):1-12.

66. Ghallab DS, Mohyeldin MM, Shawky E, Metwally AM, Ibrahim RS. Chemical profiling of Egyptian propolis and determination of its xanthine oxidase inhibitory properties using UPLC-MS/MS and chemometrics. Lwt. 2021;136(1):110298.

67. Chen J, Li Q, Ye Y, Ran M, Ruan Z, Jin N. Inhibition of xanthine oxidase by theafavins: possible mechanism for anti-hyperuricaemia effect in mice. Process Biochemistry. 2020;97:11-18.

68. Hung-Chu Y, Chen CJ, Wu SH, Hsieh JF. Inhibition of xanthine oxidase by Rhodiola crenulata extracts and their phytochemicals. Journal of Agricultural and Food Chemistry. 2014;62(17):3742-3749.

69. Kim JY, Wang Y, Li ZP, Baiseitova A, Ban YJ, Park KH. Xanthine oxidase inhibition and anti-LDL oxidation by prenylated isoflavones from Flemingia philippinensis root. Molecules. 2020;25(13):1-15.
Competitive inhibition kinetics between flavonoids and XO.
ABOUT AUTHORS

Rut Novalia Rahmawati Sianipar is a master student at Department of Chemistry, Faculty of Mathematics and Natural Sciences, IPB University, Indonesia. Currently, she is conducting thesis research on inhibition kinetics of traditional medicinal plant toward xanthine oxidase as antigout therapy.

Komar Sutriah is an Assistant Professor at Department of Chemistry, Faculty of Mathematics and Natural Sciences, IPB University, Indonesia. He has been working on thermodynamic and kinetics study of antioxidant additives.

Dyah Iswantini is a Professor at Department of Chemistry, Faculty of Mathematics and Natural Sciences, IPB University, Indonesia. She has been working on secondary metabolites for antiobesity and antigout from kinetics and thermodynamic point of views.

Suminar Setiati Achmadi is a Professor at Department of Chemistry, Faculty of Mathematics and Natural Sciences, IPB University. She has been working on natural product chemistry, especially focused on the secondary metabolites from Indonesian archipelago.

Cite this article: Sianipar RNR, Sutriah K, Iswantini D, Achmadi SS. Inhibitory Capacity of Xanthine Oxidase in Antigout Therapy by Indonesian Medicinal Plants. Pharmacogn J. 2022;14(2): 470-479.