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Research Article

A review of the *Phyllanthus* genus plants: Their phytochemistry, traditional uses, and potential inhibition of xanthine oxidase

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Abstract

Hyperuricemia is a risk factor for gout and other cardiovascular diseases. One of the therapies used is Allopurinol. Unfortunately, it has unwanted side effects. These conditions made researchers continue to seek and develop alternative treatments from natural products. One of which is from plants of the *Phyllanthus* genus. One of their contents was polyphenols, especially flavonoids. It is an alternative treatment for hyperuricemia because of its minimal side effects. The flavonoids in this genus were reported to have xanthine oxidase inhibitory: quercetin, kaempferol, rutin, apigenin, luteolin, myricetin, catechin, epicatechin, and epigallocatechin with IC_{50} values from 0.44 M to > 100μM. The presence of π-π interactions between planar rings A and C on flavones with phe 1009 and phe 914 and the addition of hydroxyl groups on flavonoid compounds plays a crucial role in inhibiting xanthine oxidase.

Keywords

flavonoids, hyperuricemia, IC_{50}, *phyllanthus*, xanthine oxidase

Introduction

The genus *Phyllanthus* is a plant group of the *Phyllanthaceae* family that consists of 1,301 species and is distributed widely in tropical and subtropical areas of Asia, Africa, America, and Australia (GBIF 2021). In Asia, these plants are ethnopharmacologically used in ayurvedic medicine, astringent, abdominal pain, diuretic, febrifuge, deobstruent, antiseptic, and used for the treatment of digestion, genitourinary, respiratory, skin diseases, hepatopathy, jaundice, and renal calculus (Kiran et al. 2021). In South America, as a traditional use to treat excess uric acid (Murugaiyah and Chan 2009). Several plants of this genus have been used to treat constipation, hypertension, fever, respiratory inflammation, muscle aches, diarrhea, gallbladder disease, urinary tract disorders, sexually transmitted diseases, diabetes, wounds, rheumatism, and arthritis (Ongchai 2019).

Increased activity of one of the prominent pro-oxidant enzymes: xanthine oxidase (XO), is involved in the pathogenesis of several diseases such as gout, inflammation, heart failure, stroke, atherosclerosis, diabetes, hypertension, colitis, inflammatory bowel disease, and rheumatoid arthritis. XO inhibition is the most widely accepted and effective treatment strategy for gout, hyperuricemia, and associated renal dysfunction (Ayyapan and Nampoothiri 2020). Allopurinol can provide inhibitory activity against the xanthine oxidase enzyme to reduce uric acid in gout. When administered in appropriate doses, its wide availability, low cost, and efficacy make allopurinol the recom-

mended first-line therapy for lowering uric acid levels. Allopurinol also has serious potential and life-threatening side effects. There were hypersensitivity, stevens-johnson syndrome, skin eruption, fever, involvement of internal organs, agranulocytosis, anemia, thrombocytopenia, leukopenia, and toxic epidermal necrolysis (which was marked by necrosis and peeling of the epidermis > 30% of the body surface area accompanied by a burning sensation), sick and can cause death (Mari et al. 2011; Stamp et al. 2020), so it's necessary to develop alternative treatments that can reduce uric acid levels.

This plant of the *Phyllanthus* genus contains alkaloids and terpenoids, polyphenolic compounds such as flavonoids, phenolic acids, stilbenes, anthocyanins, coumarins, and lignins (Kiran et al. 2021). Unlike antihyperuricemic drugs, polyphenol intake from food has few or no side effects, so it is likely that as an alternative treatment for hyperuricemia (Zhu et al. 2021).

The present review article will summarize and provide a comprehensive update on the last 10th years regarding the xanthine oxidase inhibitory activity of polyphenolic compounds in plants of the *Phyllanthus* genus. This review covers the literature from the previous 10th years using the keywords: *Phyllanthus*, traditional use, ethnobotany, ethnopharmacology, pharmacology, chemical compounds, flavonoids, inflammation, antioxidants, and xanthine oxidase inhibitory activity in searches using the Google Scholar database, Science Direct, Pubmed, Springerlink and Scopus.

Ethnopharmacological uses

In Asia, *Phyllanthus emblica* L. (*P. emblica*) has been used as a medicinal plant to treat diseases. The fruit of *Phyllanthus emblica* Linn or *Emblica officinalis* Gaertn (*Phyllanthaceae*), commonly known as Indian gooseberry or Amla, is used in indigenous traditional medicine systems, including Ayurveda, to treat several ailments: colds, hay fever, cough, asthma, bronchitis, diabetes, cephalalgia, ophthalmopathy, dyspepsia, colic, flatulence, hyperacidity, peptic ulcer, erysipelas, skin disease, leprosy, hematogenesis, inflammation, anemia, emaciation, hepatopathy, jaundice, diarrhea, dysentery, bleeding, vaginal discharge, menorrhagia, heart disorders, and hair loss premature graying (Li et al. 2020; Saini et al. 2022). In India, Asia, the Caribbean, and America, *Phyllanthus acidus* (L.) Skeels has traditionally been used to treat various diseases such as inflammation, gastric problems, rheumatism, bronchitis, asthma, respiratory disorders, diabetes, and hepatitis (Hossen et al. 2015; Tan et al. 2020). In Indonesia, Thailand, and India, the young leaves of *Phyllanthus acidus* are commonly used as an edible vegetable, and its aqueous infusion is a diet aid for weight loss (Geng et al. 2021).

Traditionally, *Phyllanthus niruri* is used as an antiurolithiasis, antidiabetic, and antihyperuricemic agent (Murugaiyah and Chan 2009; Beidokhti et al. 2017; Kasote et al. 2017). In Asia, it is used in traditional medicine for liver protection and antihepatitis B (Liu et al. 2014). This plant is also traditionally used to treat kidney stones (Alves et al. 2021). *Phyllanthus amarus* Schum. & Thon was used traditionally in Indian ayurvedic medicine to treat various ailments: stomach problems, genitourinary system, liver, kidney and spleen, diuretic, febrifuge, antiseptic, gonorrhea, menorrhagia, gastropathy, diarrhea, dysentery, ophthalmopathy, scabies, ulcers and wound (Patel et al. 2011). *Phyllanthus orbicularis* (*P. orbicularis*) (*Phyllanthaceae*) is a tropical plant endemic to Cuba and is used in traditional medicine as an infusion. This plant extract has various pharmacological activities such as antimutagenic, antioxidant, and antiviral (Francioso et al. 2019). *Phyllanthus phillyreifolius* (*P. phillyreifolius*), a plant species native to Reunion Island, is used in traditional medicine to treat diarrhea and as a diuretic (Grauzdytė et al. 2018a).

Chemical constituents

Plants of the *Phyllanthus* genus contain metabolites in the form of alkaloids, terpenoids, and polyphenolic compounds such as flavonoids, phenolic acids, stilbenes, anthocyanins, coumarins, and lignins which can be seen in Table 1.

Table 1. Chemical compounds are found in the genus *Phyllanthus*.

No.	Species	Chemical Compound	Pharmacological Activity	References
1.	*P. acidus*	Quercetin 3′ -O-glucoside	*Phyllanthus acidus* was reported to have pharmacological activities: hepatoprotective and hypoglycemic *in vivo*, antioxidant, α-glucosidase inhibition, antinociceptive, antiinflammatory, and antimicrobial activity *in-vitro*.	Kiran et al. 2021
		Phyllanthin		Ghafar et al. 2020
		Delphinidin-3-O-β-D-glucoside		Tan et al. 2020
		Kaempferol-3-glucoside		Chakraborty et al. 2012
		Cyanidin 5-O-β-D-glucoside		
		Catechin-3′-methyl ether		
		Kaempferol-3-glucoside-7- rhamnoside		
		Epicatechin		
		Quercetin-3-O-rhamnoside (Quercitrin)		
		Kaempferol-3-rhamnoside-4′- xyloside		
		Quercetin		
		Kaempferol		
		Myricetin		
		Quercetin-3-6″-caffeylgalactoside		
		Quercetin-3-2″-p-coumarylglucoside		
		Myricetin-3-glucoside		
		Rutin		

No.	Species	Chemical Compound	Pharmacological Activity	References
1.	*P. acidus*	Kaempferol-3,7-di-glucoside		
		Quercetin-3-O-sulfate		
		Kaempferol-3-O-sulfate		
2.	*P. amarus*	Quercetin 3′ -O-glucoside	*Phyllanthus amarus* showed antibacterial activity against *Staphylococcus aureus* (gram-positive) with a minimum inhibitory concentration (MIC) value of 17.7 μg/ml.	Kiran et al. 2021
		(2R,3S,4S)-leucodelphinidin		Eldeen et al. 2011
		Delphinidin-3-O- β -D-glucoside		
		Kaempferol-3-glucoside		
		Cyanidin 5-O-β-D-glucoside		
		Ellagic Acid		
3.	*Phyllanthus debilis*	Quercetin 3′ -O-glucoside	*In vitro* glochidon has moderate dose-dependent inhibitory activity against α-glucosidase, α-amylase, DPP-4, and PPAR-γ. The docking and MD results also showed that glochidon had moderate activity to inhibit DPP-4 and PPAR-γ, compared with standard agents and a strong tendency to interact with the GLUT1 protein. Computational ADME profiling determined that glochidon has excellent characteristics in acting as a hypoglycemic compound. *In vivo*, experimental results showed that at a dose of 20 mg/kg, glochidon significantly increased body weight, plasma insulin, HDL levels, and other biochemical markers. In addition, it lowers blood glucose, total cholesterol, triglycerides, low-density lipoprotein, and very-low-density lipoprotein.	Kiran et al. 2021
		Betulinic acid		Verma et al. 2021
		Gallocatechin		
		(2R,3S,4S)-leucodelphinidin		
		Delphinidin-3-O- β -D-glucoside		
4.	*Phyllanthus emblica*	Quercetin 3′ -O-glucoside	This plant provides various pharmacological activities: antioxidant, anticancer, immunomodulatory, anti-inflammatory, cell protection, diabetes management, dyslipidemia, obesity, cancer, liver disorders, arthritis, gingivitis, and wound healing. In addition, *Phyllanthus emblica* also has anti-aging activities, such as antioxidants, antityrosinase, and antimelanogenesis, and can repair kidney damage caused by cisplatin.	Kiran et al. 2021
		Phyllanthin		Variya et al. 2016
		Delphinidin-3-O- β -D-glucoside		Yadav et al. 2017
		Kaempferol-3-glucoside		Chaikul et al. 2021
		Cyanidin 5-O-β-D-glucoside		Purena et al. 2018
		Quercetin		
		Rutin		
		Apigenin-7-O-(6'-butyryl-β-glucopyranoside)		
		Luteolin-4'-O-neohesperidoside		
5.	*Phyllanthus lawii*	Quercetin 3′ -O-glucoside	*Phyllanthus lawii* has sturdily analgesic activity. This plant exerts significant peripheral and central analgesic activity in acetic acid-induced writhing mice.	Kiran et al. 2021
		Phyllanthin		Chandrashekar 2011
		Betulinic acid		
		Niranthin		
		Delphinidin-3-O- β -D-glucoside		
		Kaempferol-3-glucoside		
		Cyanidin 5-O-β-D-glucoside		
		Rutin		
		Hypophyllanthin		
6.	*Phyllanthus myrtifolius*	Quercetin 3′ -O-glucoside	*Phyllanthus myrtifolius* inhibited the growth of *Pseudomonas stutzeri* (gram-negative) with a MIC value of 78 μg/ml. Intense inhibitory activity against HIV-RT with IC_{50} value = 12.7 μg/mL. *Phyllanthus myrtifolius* gave potent antioxidant activity against DPPH with IC_{50} value = 10.2 μg/mL.	Kiran et al. 2021
		Kaempferol-3-glucoside		Eldeen et al. 2011
		Cyanidin 5-O-β-D-glucoside		
		Hypophyllanthin		
7.	*Phyllanthus reticulatous*	Quercetin 3′ -O-glucoside	*Phyllanthus reticulatous* gave potent antioxidant activity to DPPH with IC_{50} value = 10.8 μg/mL.	Kiran et al. 2021
		Betulinic acid		Eldeen et al. 2011
		Niranthin		
		Gallocatechin		
		(2R,3S,4S)-leucodelphinidin		
		Delphinidin-3-O- β -D-glucoside		
		Kaempferol-3-glucoside		
		Rutin		
		Ellagic Acid		
		Quercetin		
8.	*Phyllanthus urinaria*	Quercetin 3′ -O-glucoside	*Phyllanthus urinaria* inhibited the growth of *Pseudomonas stutzeri* (gram-negative) bacteria with a MIC value of 117 μg/ml. Intense inhibitory activity against HIV-RT with IC_{50} value = 10.4 μg/mL. *Phyllanthus urinaria* gave potent antioxidant activity against DPPH with IC_{50} value = 17.4 μg/mL.	Kiran et al. 2021
		Phyllanthin		Eldeen et al. 2011
		Betulinic acid		
		Delphinidin-3-O- β -D-glucoside		
		Ellagic Acid		
		Hypophyllanthin		
9.	*Phyllanthus virgatus*	Quercetin 3′ -O-glucoside	The methanol extract of *Phyllanthus virgatus* showed intense antioxidant activity and protection against oxidative DNA damage. In addition, the methanol extract of *Phyllanthus virgatus* inhibited α-amylase (IC_{50} = 33.20 ± 0.556 μg/mL), noncompetitively, compared to acarbose (IC_{50} 76, 88 ± 0.277 μg/mL), which is competitive inhibition. Moreover, this extract triggered glucose uptake activity in 3T3-L1 cells and showed a good correlation between antioxidant activity and α-amylase. Molecular docking studies of the main bioactive compounds (9,12-octadecadienoic acid, asarone, 11-octadecenoic acid, and acrylic) via GC-MS analysis of these extracts indicate that the inhibitory properties might be due to the synergistic effect of these bioactive compounds.	Kiran et al. 2021
		Betulinic acid		Hashim et al. 2013
		Zeatin		
		Delphinidin-3-O- β -D-glucoside		
		Kaempferol-3-glucoside		
		Rutin		
		Ellagic Acid		
		Hypophyllanthin		
		Quercetin		

No.	Species	Chemical Compound	Pharmacological Activity	References
10.	*Phyllanthus niruri*	Quersetin 3-O-glukoside	This plant has bioactivity as antihyperuricemia, where the methanol extract of *Phyllanthus niruri* inhibits the xanthine oxidase enzyme with an IC_{50} value of 39.39 µg/mL. *Phyllanthus niruri* has antiinflammatory, antinociceptive, antiplasmodium, and antidiabetic pharmacological activity.	Kasote et al. 2017
		Catechin		Mediani et al. 2015
		Quersetin 3-O-α-rhamnoside		Hossain and Rahman 2019
		Epicatechin		Murugaiyah and Chan 2009
		Rutin		Beidokhti et al. 2017
		6-hydroxy-7,8,2',3',4'-pentamethoxyisoflavone		Couto et al. 2013
		5,7-dimethoxy-3,4'-dihydroxy-3',8-di-C-prenyl flavanonol		Porto et al. 2013
		5,3'-dihydroxy-6,7,4'-trimethoxyflavone		Ifeoma et al. 2013
		7-hydroxy-2',4',5'-trimethoxyisoflavone		
		7-hydroxy-4'-methoxyisoflavone		
11.	*Phyllanthus orbicularis*	Catechin	Fideloside is the main flavonoid that exerts antioxidant and antiinflammatory activity in human monocytes by showing an increased effect on the production of the antiinflammatory cytokine IL-10. The aqueous extract of *Phyllanthus orbicularis* used as a protector against DNA damage caused by exposure to sunlight (UV). Procyanidins B1 and B2 provided antiviral activity by inhibiting HSV-2 replication and DNA synthesis with EC(50) values = 32.8 µg/mL and 24.2 µg/mL, respectively.	Francioso et al. 2019
		Epicatechin		Vernhes et al. 2013
		Procyanidin B2		Tamayo et al. 2018
		Rutoside		Alvarez et al. 2012
		Nicotiflorin		
		Kaempferol		
		Procyanidin C1		
		Fideloside		
12.	*Phyllanthus phillyreifolius*	Rutin	*Phyllanthus phillyreifolius* extract has pharmacological activity as an anticancer, antiviral, antioxidant, and antibacterial.	Grauzdytė et al. 2018a
		Quercetin-3-glucuronide		Haddad et al. 2020
		Quercitrin		Grauzdytė et al. 2018b
				Mahomoodally et al. 2019

Xanthine oxidase

Lipoxygenases (LOX), cyclooxygenases (Coxs), and xanthine oxidase (XO) are metalloenzymes whose catalytic cycles involve ROS, such as lipid peroxyl radicals, superoxide, and hydrogen peroxide. LOX and Coxs will catalyze the prime steps of the leukotriene biosynthesis and prostaglandins from arachidonic acid. They are crucial cascades in the inflammatory response (Dangles and Dufour 2006). XO is a predominant enzyme in the metabolism of purines to uric acid, catalyzing the conversion of hypoxanthine to xanthine and xanthine to uric acid. The chief role of XO in this pathway makes it a therapeutic target for hyperuricemia management. Allopurinol, oxypurinol, and febuxostat can inhibit the xanthine oxidase in the purine nucleotide degradation pathway, decreasing uric acid production. In addition, allopurinol and oxypurinol inhibit the enzyme orotidine-5′ monophosphate decarboxylase (OMPDC). This condition leads to orotic acid accumulation. Allopurinol and oxypurinol can defeat amidotransferase and PNP activity, but febuxostat cannot inhibit PNP and OMPDC2. Flavonoids may exert their antioxidant and antiinflammatory activities by reserving the LOX, COX, and XO (Dangles and Dufour 2006).

Flavonoids as xanthine oxidase inhibitors

Flavonoids are a group of phenolic compounds with many properties, especially in counteracting free radicals in vitro and in vivo. Many studies have shown that flavonoids can inhibit the production of ROS enzymes, such as xanthine oxidase, nitric oxide synthase, and myeloperoxidase (Atmani et al. 2009). Several flavonoids that play a role in the inhibitory activity of the xanthine oxidase enzyme are summarized in Table 2.

Planar rings A and C of flavones have π-π interactions with phe 1009 and phe 914, required for xanthine oxidase inhibition. However, the planar flavone framework alone is not sufficient to induce xanthine oxidase inhibition. A hydroxyl group at position 7 plays a role in the xanthine oxidase inhibition by flavones. The addition of the 5-hydroxyl group and the 7-hydroxyl decreased the IC_{50} value significantly. The structural similarity between 5,7-dihydroxyflavone (ring A) and enol xanthine indicates the same binding site in the allosteric center of xanthine oxidase (Hoorn et al. 2002).

The hydroxyl groups at positions C-5 and C-7 with the C2-C3 double bond play a key role in XO inhibitory activity. Flavones are slightly better XO inhibitors than flavonols, and all derivatives of flavonoids, except for isorhamnetin, are less active than the original compound (Atmani et al. 2009). 7-Hydroxyflavones is one of the flavones with one hydroxyl group with xanthine oxidase inhibitory capacity (≤40 µM), and flavone with one hydroxyl moiety has an IC_{50} value much higher than 40 µM. There was a significant decrease in the IC_{50} value when a hydroxyl group was added to C3' or C4'. At 3'-hydroxyl,

Table 2. Inhibitory activity of xanthine oxidase from flavonoids of the *Phyllanthus* genus.

No.	Compound and Structure	IC_{50} value	Sources	References
1.	Quercetin	0,44 – 2,92 μM	*Phyllanthus acidus* *Phyllanthus emblica*	Zhang et al. 2018 Ayyapan and Nampoothiri 2020 Atmani et al. 2009 Hoorn et al. 2002 Nagao et al. 1999
2.	Kaempferol	0,67 – 2,5 μM	*Phyllanthus acidus* *Phyllanthus orbicularis*	Ayyapan and Nampoothiri 2020 Atmani et al. 2009 Hoorn et al. 2002 Nagao et al. 1999
3.	Rutin	<50 μM	*Phyllanthus acidus* *Phyllanthus emblica* *Phyllanthus niruri* *Phyllanthus orbicularis* *Phyllanthus phillyreifolius*	Ayyapan and Nampoothiri 2020; Nagao et al. 1999
4.	Myricetin	1,27 - 2,38 μM	*Phyllanthus acidus*	Ayyapan and Nampoothiri 2020 Atmani et al. 2009 Hoorn et al. 2002 Nagao et al. 1999
5.	Apigenin	0,44 – 1,0 μM	*Phyllanthus emblica*	Ayyapan and Nampoothiri 2020 Atmani et al. 2009 Hoorn et al. 2002
6.	Luteolin	0,55 – 2,38 μM	*Phyllanthus emblica*	Ayyapan and Nampoothiri 2020 Atmani et al. 2009 Hoorn et al. 2002 Nagao et al. 1999
7.	Cathechin	>100 μM	*Phyllanthus fraternus* *Phyllanthus niruri* *Phyllanthus orbicularis*	Atmani et al. 2009
8.	Epicathechin	>100 μM	*Phyllanthus acidus* *Phyllanthus niruri* *Phyllanthus orbicularis*	Atmani et al. 2009 Hoorn et al. 2002
9.	Epigalokatekin	>100 μM	*Phyllanthus reticulatous*	Atmani et al. 2009; Nagao et al. 1999

the IC_{50} value decreased from 1 to 0.75 μM when apigenin was compared with luteolin and changed from 2.5 to 1.5 μM when kaempferol was compared with quercetin. The presence of a 4'-hydroxyl group increased the inhibitory activity compared to a flavone group that did not have a 4'-hydroxyl group. Luteolin is a flavone compound with hydroxyl groups at 5,7,3' and 4' positions providing the best xanthine oxidase inhibitory activity with the lowest IC_{50} value compared to other flavonoid groups. Several hydroxyl groups were also present that showed decreased xanthine oxidase inhibitory activity (Hoorn et al. 2002).

The presence of a 2'-hydroxyl group can lower the inhibitory activity of xanthine oxidase, which can being see from the increase in the IC_{50} value of flavonoid compounds that have a 2'-hydroxyl group, for example, kaempferol (without 2'-hydroxyl group) compared to morin (the presence of a 2'-hydroxyl group), where the IC_{50} value increased from 2.5 to 40 μM, respectively. The presence of 3-hydroxyl can also attenuate the inhibition of xanthine oxidase, luteolin compared with quercetin (the presence of a 3-hydroxyl group) showed an increase in IC_{50} from 0.75 to 1.5 μM, respectively. The addition of the 8-hydroxyl group increased the IC_{50} value from 4 to 10 μM when 7.3',4'-trihydroxyflavone compared with 7,8,3',4'-tetrahydroxyflavone. The 5'-hydroxyl group doesn't influence the inhibitory activity of xanthine oxidase (Hoorn et al. 2002).

The presence of hydroxyl groups at positions C-3 and C-3' plays a role in superoxide scavenging activity (Atmani et al. 2009). Flavonoids can act as enzyme inhibitors via the formation of enzyme-inhibitor complexes and prevent the formation of superoxide and uric acid. Or by superoxide scavenging. These can be recorded independently using chemiluminescence colorimetric methods. Based on their activity, flavonoids can be grouped into superoxide inhibitors without inhibitory activity on XO, XO inhibitors without superoxide scavenging activity (IC_{50} for uric acid formation ≈ IC_{50} for superoxide inhibition), XO inhibitors with superoxide scavenging activity (IC_{50} for uric acid formation > IC_{50} for superoxide scavenging), XO inhibitors with added pro-oxidant effect in superoxide formation (IC_{50} for uric acid formation < IC_{50} for superoxide scavenging), weak XO inhibitors with pro-oxidant effect on superoxide formation, and flavonoids without inhibitory effects on XO and superoxide. The planar C ring (flavones, flavonols) plays a role in XO inhibition (IC_{50} 0.5 to 10 μM except for 3-Hydroxyflavones, which do not interact with XO) (Dangles and Dufour 2006).

Catechins have superoxide scavenging activity and do not interact with XO. Only flavonols with a catechol group in ring B (quercetin, myricetin, fisetin) showed additional superoxide activity. In contrast, some hydroxylated flavones (chrysin, apigenin, luteolin) exhibit underlying pro-oxidant activity. Interestingly, glycosidation of the flavonoid core generally abolishes XO inhibition. For example, the IC_{50} values of quercetin for XO inhibition and superoxide scavenging were 2.6 and 1.6 μM, respectively. Quercetin-3-O rhamnoside (quercitrin) had an IC_{50} of 8.1 μM to ward off superoxide but could not inhibit XO (IC_{50} > 100 μM). Similarly, Q3GlcU and Q7GlcU were very poor inhibitors of XO (Kd 100 μM). However, Q3'GlcU (Kd 1.4 μM) and Q4'GlcU (Kd 0.25 μM) exerted intense inhibition activity against XO, which was as strong as quercetin. Therefore, while glucuronidation at the 3' or 4' position suppresses the free catechol moiety of ring B and thus has radical scavenging activity, XO affinity has been spared as if flavonol-XO binding occurs with marginal participation of ring B (Dangles and Dufour 2006).

Epicatechin and its oligomers have no inhibitory activity against XO. In contrast, an oligomer of epicatechin-3-O-gallate (4β-8 Interflavan linkage) are inhibitors whose potency increases with the number of monomer units (IC_{50} 7.2 to 4.4 μM from dimer to tetramer). Thus, the French maritime pine bark extract (pycnogenol) rich in procyanidins (75% by weight, DP 2 to 7) find to significantly reduce XO activity and inhibit protein electrophoretic mobility only under non-denaturation. In addition, the pure low molecular weight extract components had no effect. Therefore, it concludes that XO inhibition occurs by binding to XO of high DP procyanidins (DP > 3) (Dangles and Dufour 2006).

Conclusion

Nine flavonoids in the *Phyllanthus* genus are reported to have xanthine oxidase inhibitory activity. They were quercetin, kaempferol, rutin, apigenin, luteolin, myricetin, catechin, epicatechin, and epigallocatechin. Their IC_{50} was from 0.44 µM to > 100 µM. Quercetin and apigenin show the great activity of xanthine oxidase inhibitory compared to other flavonoids found in plants of the *Phyllanthus* genus with an IC_{50} value of 0.44 µM. The presence of – interactions between planar rings A and C on flavones with phe 1009 and phe 914, plays a chief role in inhibiting xanthine oxidase. Adding hydroxyl groups to flavonoid compounds can increase or decrease the inhibitory activity of xanthine oxidase. The hydroxyl groups at positions 5, 7, 3', and 4' can increase the inhibitory activity of xanthine oxidase, while the hydroxyl groups at 2', 3, and 8 will decrease the inhibitory activity against xanthine oxidase.

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