

PLANT PRODUCTS AND THEIR INHIBITORY ACTIVITY AGAINST XANTHINE OXIDASE

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Abstract

Plant phytochemicals are very extensive and varied sources for medicinal compounds. The need for finding alternative remedies for several illnesses that are linked to the products of an enzyme like xanthine oxidase, motivates researchers to find in nature searching for local new better remedies. Many plants' extracts are investigated, and many other sources like honey, food, and bacteria are being found to produce inhibitors of xanthine oxidase. Yet efforts are needed to find the perfect remedy with clinical effect and with no or little side effects. The aim of this review is to provide recent information on natural substances derived from plants that have the potential to inhibit xanthine oxidase, with attention to some medicinal plants from Jordan. As a consequence of the information presented in this review, several natural compounds have demonstrated to xanthine oxidase inhibitory effects which highlight them as potential candidates for application in the management of different chronic disorders.

Rezumat

Substanțele fitochimice din produsele vegetale sunt surse foarte variate de compuși cu efect terapeutic. Necesitatea de a găsi remedii alternative pentru mai multe afecțiuni care sunt determinate de producții unor reacții metabolice facilitate de prezența unor enzime, precum xantin oxidaza, motivează cercetătorii în căutarea unor noi remedii cu eficacitate crescută. Diferite extracte vegetale sunt investigate în momentul de față, și multe alte surse, cum ar fi mierea, alimentele și bacteriile, ca agenți inhibitori ai xantin oxidazei. Cu toate acestea, sunt necesare eforturi pentru a găsi remedii optime cu efecte clinice bune și fără sau cu puține efecte secundare. Scopul acestui articol este de a oferi informații recente despre substanțele naturale derivate din plante care au potențialul de a inhiba xantin oxidaza, cu accent asupra unor plante medicinale din Iordania. Ca o concluzie a informațiilor prezentate, mai mulți compuși naturali au dovedit efecte inhibitoare asupra xantin oxidazei, care îi evidențiază ca potențiali candidați pentru aplicare în managementul diferitelor patologii cronice.

Keywords: xanthine oxidase, gout, hyperuricemia, enzyme inhibition, plant extracts, natural compounds

Introduction

There are two functional forms of the enzyme xanthine oxidoreductase (XOR): xanthine dehydrogenase (XDH) and xanthine oxidase (XO). These forms are needed in the last steps in the purine's metabolism, catalysing the oxidation of hypoxanthine into xanthine, and the xanthine into uric acid. XDH passes in each oxidation step two electrons to one molecule of NAD⁺, and XO forms hydrogen peroxide or superoxide anion by passing the electrons to oxygen molecules.

Purines originate in the human body either from food ingestion or by the catabolism of its own DNA or RNA molecules, being the final product as uric acid, which is excreted mainly *via* renal filtration and to a lesser extent through the intestinal tract. Uric acid is excreted mainly *via* renal system through an anion exchanger (URAT1) and intestinally ABCG2 [1]. Uric

acid is mostly ionized at physiological pH. Having its pKa 5.4, it has very little solubility in water and in serum and reaches saturation at 6.8 mg/dL [2].

The term hyperuricemia (HUA) refers to the high uric acid concentration in serum and it reaches in some populations around 18%, and it leads mostly to gout by precipitation of uric acid crystals in many tissues and joints [3]. And it is also considered a cardiovascular risk factor [4]. Not all HUA cases develop gout since only 7% risk per year of presenting gout attack is present in high uric acid levels (more than 10 mg/dL) [5]. The necessity of prophylactic treatment to reduce HUA in asymptomatic patients in order to prevent gout attacks is under much debate [6]. The available methods for lowering uric acid in serum are [7]: lowering the uric acid production by use of XO inhibitors; conversion of uric acid into a more soluble and easily excreted form like allantoin, by

uricase, and increasing uric acid excretion with uricosuric agents.

The first approach is the most common for high uric acid therapy as it is the only one to reduce uric acid production from its precursors. As this method interferes with purine metabolism and prevents uric acid formation. This inhibition can be of three main types; mechanism-based, structure-based, and hybrid types. Mechanism-based is mediated by interaction with molybdenum (Mo) core, like with allopurinol. Structure-based near completely fill the canal of the XO enzyme leading to the Mo core, like with Febuxostat and Y-700. Hybrid type have both properties, interacting with Mo core and blocking the canal, like with FYX-051 [8].

XO in bovine milk is a 290 KDa homodimer and it was thought that the two subunits worked independently, until in 2004 Tai and Hwang proved that there is cooperative interaction between the two units for binding and catalysis [9].

The presence of mixed-type inhibition of XO in the literature is explained by this cooperative effect. Each subunit is composed of a molybdopterin unit in the C-terminal domain (85 KDa), and two iron-sulphur clusters in the N-terminal domain (20 KDa) and a FAD central domain (40 KDa) [10]. There is a 90% match between human XO and the bovine milk XO, for this analogy, it is used widely for *in vitro* testing of inhibitors.

XO contributes to the increase of free radicals level which is related to ageing, diabetes, atherosclerosis, cancer and stroke [11]. Oxidative stress can be caused by an excess of free radical levels above the quantity that can be neutralized by the antioxidant defence system. Oxidation of hypoxanthine to xanthine and further to uric acid, produces ROS, mainly as hydrogen peroxide (H₂O₂). Oxidative stress is defined as having high levels of ROS in the body. This can be a result of type 1 diabetes. Diabetic rats showed elevation in the XO activity in plasma and liver compared to non-diabetic rats, thus producing more free radicals resulting in oxidative stress [12]. Allopurinol, as XO inhibitor, counteracts oxidative stress, and by this effect prevents complications in type 1 diabetic patients [12].

In addition to that, XO is also targeted in some anti-cancer therapy types [13] and also in the hypertension treatment [14]. In the case of anti-cancer therapy, the objective is to minimize the inactivation of the drug 6-mercaptopurine by XO [15]. In hypertension, as mentioned before, XO is a major ROS producer in the body and is found overexpressed in cases of inflammatory conditions and ischemia. It is attracted by the generation of ROS mainly in the endothelium, where it decreases the nitric oxide (NO) bioavailability. Consequently, it reduces the NO-mediated lowering of blood pressure, adversely affecting hypertension. Regrettably, all the HUA drugs still have some side effects like rashes, hepatitis, fever, Stevens-Johnson syndrome, nephropathy, fatal liver necrosis and allergies

[16] for that, alternative drugs with fewer side effects are needed to treat xanthine oxidase associated metabolic disorders.

The main and most ancient source of food for animals are plants, and they are used for various medicinal values. Plants have been used as medicines before etiological agents of disease were known, and medicinal plants evolved as an experimental product. The results of those experiments helped to set a base from which advanced and useful medicines would rise. The use of plants medically dates back at least 5000 years to the Sumerian culture, who specified a well-established medicinal use for clay slab explaining twelve recipes for medicine preparation [17].

Today, natural alternative products are getting more important compared to synthetic drugs because of the wealth of various bioactive components. Usually, these components are categorized as secondary metabolites since they are not essential for the survival of plants, but they have supporting beneficial functions. Not less than 100,000 secondary metabolites have been purified and characterized, many of them have been set as active ingredients in common drugs or have provided a basis for the derivation of synthetic active ingredients that are used in medicines. Plants and natural drugs have the ability to perform beneficial functions with fewer side effects as compared to synthetic medicines [18].

In order to improve XO inhibition measurement, Mohammad *et al.* (2010) developed a new animal-model bioassay that is capable of detecting the XO inhibition in a very precise manner [19].

In this review, we will focus on the XO inhibitors, by describing the production origin of the inhibitors, classifying them into nine major chemical groups clarifying their metabolites and structure-activity relationship with XO.

Natural Inhibitors

A major class of secondary metabolites are polyphenols, around 100,000 to 200,000 secondary metabolites are in nature, and the phenylpropanoid pathway takes 20% of the carbon fixed by photosynthesis. This pathway synthesizes the majority of phenolics in nature, like flavonoids and stilbenes [20]. Polyphenols are categorized into 15 main classes and have minimum one phenol group [21]. The main types of polyphenols are phenolic acids, and it encompasses compounds (I) with a structure of hydroxycinnamic acid: C6-C3 (i.e. coumaric acid and caffeic acid), (II) with the structure of stilbenes: C6-C2-C6 (i.e. Resveratrol), (III) with the structure of flavonoids: C6-C3-C6 and (IV) with the lignans structure: C6-C4-C6 (i.e. Secoisolariciresinol) [22]. Various researchers reported that many polyphenolic compounds inhibit UA synthesis. Di Petrillo *et al.* reported uni-flowers honey from Thistle, Arbutus and Eucalyptus possessed the greatest

levels of phenols and hence the greatest anti-XO activities [23]. Karaçelik and Sahin claimed that the honey tested that had different inhibition concentrations of xanthine oxidase and other enzymes, are good for human use [24]. Quy and Xuan tested another natural source for anti-HUA, the medicinal fungus *Cordyceps militaris*, and found that it has a very strong inhibitory activity against XO [25].

Recently, He *et al.* (2019) investigated tuna protein hydrolysates, and after isolation, they identified the dipeptide FH as one of the best XO inhibitors, having an IC₅₀ of 25.7 µM [26].

Another source of XO inhibitory natural products was discovered by Xiong *et al.*, they tested Yaocha, which is composed of Tea flower powder, Bonito peptides powder and Rooibos powder in a ratio of 1:2.5:1. The Tea flowers provides saponins, and have various health benefits like antioxidant, anti-obesity and antiallergic. Rooibos also has various effects like relatively potent antioxidant, immune modulating, antimutagenic and chemo-preventive actions. Bonito peptides powder is manufactured from the deep-sea skipjack tuna and contains many carnosine and anserine that have hypouricemic activity and no undesirable side effects. They demonstrated that the natural complex health product Yaocha decreased UA level by XO inhibition [27]. Moreover, the marine bacterium, *Vibrio neocaledonicus*, was explored as a source of natural inhibitors, it was reported that it can produce 1H-indole-3-carboxaldehyde as a relatively strong XO inhibitor (IC₅₀ 13.36 ± 0.39 µM) [28]. In an *in vivo* experiment using a mouse model, the effect of *Morus alba* L dried twigs (*Mori ramulus*) extract on serum uric acid levels was investigated. The authors identified two coumarin glycosides (moriramulosid A and B) that act as hypouricemic agents [29].

Flavonoids

Flavonoids are polyphenols that are divided by their oxidation, into six subclasses: flavonols, flavones, flavanones, isoflavones, catechins and proanthocyanidins and anthocyanidins. Flavonoids are composed of two phenyl rings (A and B), hydroxyl groups (C-5 and C-7) on the A ring, a heterocyclic ring (ring C), and have powerful XO inhibitory activities [30].

XO inhibition with flavonoids *in vitro* has been largely studied by many researchers. Several techniques (kinetic analysis, spectroscopic, crystallographic, Fourier-transform infrared spectroscopy (FTIR), thermodynamic characteristics, and computational simulation) were experimented for uncovering the flavonoids mechanism of inhibition of XO [30, 31].

Factors affecting XO inhibition include binding mode, planar structure, longitudinal dipole moments, suitable pK_a and hydrophobicity [32, 33]. Glycosylation, hydroxylation, methylation and hydrogenation of C2=C3 double bond are essential for XO inhibition.

Hudaib *et al.* demonstrated that several Jordanian plants belonging to the family *Lamiaceae* (*Mentha spicata* L., *Majorama syriaca* (L.) Kostel, and *Rosmarinus officinalis* L.) had XO inhibitory activity due to the phenolics (flavonoids) content [34].

Flavonols

Wang *et al.* conducted a mechanistic experiment to reveal the molecular mechanism of XO inhibition by kaempferol. The results revealed that kaempferol inhibits XO in a competitive way (IC₅₀ (2.18 ± 0.02) × 10⁻⁶ mol/L), generation of XO-kaempferol complex through hydrophobic forces and kaempferol associated with some surrounding XO amino acid (Ser1075, Glu 802, Phe1013, Leu648, Leu1014 and Pro1076) and occupied the active site preventing further entrance of substrate (xanthine or hypoxanthine) toward the enzyme [35].

A natural flavonol, quercetin, have a strong XO inhibitory activity noted by many authors [36]. In another study, Pauff and Hille suggested that quercetin totally inhibits XO, converts to hydroxylate xanthine and generates superoxide (K_i: 1.2 µM, pH 7.4, 25°C) [36]. Cao *et al.* also clarified the mechanism of quercetin inhibition toward XO. They proposed that XO-quercetin complex crystal structure consists of a sole positioning with benzopyran moiety inserted among residues of amino acids (Phe 914, Phe 1009) and ring (B) in the direction of the solvent channel. Furthermore, this tight junction of quercetin to XO is due to van der Waals and steric complementarity interactions between conjugated tricyclic structure (quercetin) and specific active site amino acids residues (XO)[37].

Both flavonols (kaempferol and quercetin) were pointed by Hudaib *et al.* to be responsible of XO inhibition in the extract of *Ginkgo biloba* [34].

Several flavonols (quercetin, galangin, morin, kaempferol, myricetin, isorhamnetin, diosmetin, rutin and hyperin) were reported to negatively affect UA formation by *in vitro* XO assay. Yoon *et al.* argued that the flavonoid (quercetin) extracted from the plant *Corylopsis coreana* Uyeki *floss* can combat HUA [38]. Hadj Salem *et al.* isolated the flavonols quercetin, isoquercitrin, rutin, isorhamnetin-3-O-rutinoside, isorhamnetin and isorhamnetin-3-O-glucoside from the plant *Nitraria retusa* and tested its inhibition power for XO activity. Quercetin and isorhamnetin showed strong XO inhibitory activities (IC₅₀: 6.0 and 5.3 µM, respectively) close to reference drug allopurinol (IC₅₀: 2.4 µM). The inhibition rate decreased when the C3 hydroxyl group glycosylated. Yet, methoxyl at C3` on isorhamnetin B-ring does not influence XO inhibition. Additionally, they stated that isorhamnetin was more active than isorhamnetin-3-glucoside and isorhamnetin-3-O-Orutinoside, while isoquercitrin and rutin were less active than quercetin [39].

Qu *et al.* separated diosmetin, rutin, and diosmetin-7-O- β -D-glucopyranoside from the stem of the plant *Chrysanthemum morifolium* and tested for *in vitro* anti-HUA. Diosmetin (IC₅₀: 11.41 μ M) showed greater XO inhibitory activity than the other compounds; diosmetin-7-O- β -D-glucopyranoside (IC₅₀: > 100 μ M) and rutin (IC₅₀: >100 μ M) [40].

Nguyen ang Nguyen isolated seven flavonols: (2R,3S)-(-)-4-O-methyl-dihydroquercetin, (2R,3R)-(+)-4-O-methyl-dihydroquercetin, (2R,3R)-(+)-4,7-di-O-methyl-dihydroquercetin, (2R,3R)-(+)-7-O-methyl-dihydroquercetin, quercetin, quercetin-3,7,3-trimethyl ether and quercetin-3,3,4-trimethyl ether) from the aerial part of *Blumea balsamifera* L. and assessed it for XO inhibition. The compound (2R,3S)-(-)-4-O-methyl-dihydroquercetin had the strongest XO inhibitory activity among all separated compounds (IC₅₀: 0.23 \pm 0.01 μ M) [41]. A new compound (5,7,3,5-tetrahydroxy flavanone) extracted from the aerial part of *Blumea balsamifera* L was tested for XO inhibitory activity, but showed weak results (IC₅₀: >100 μ M) [41].

The fruit of *Stauntonia brachyanthera* produced isoquercitrin which showed higher XO inhibition activities (IC₅₀: 1.60 μ M) [42]. Likewise, Ding *et al.* discussed that quercetin-3-O- β -D-glucopyranoside, isorhamnetin-3-O- β -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside and quercetin from *Plumula nelumbinis* had XO inhibitory activity. Ammar *et al.* also stated that kaempferol 3-O-isorhamninoside from *Rhamnus alaternus* L inhibited XO (IC₅₀: 18 \pm 2 μ g/mL) [43].

Flavones

Lin *et al.* defined several flavones (apigenin, chrysin, baicalein and luteolin) showing strong XO inhibitory activities. Though, the inhibition of chrysin and apigenin was higher than other tested flavones [32, 44]. Additionally, molecular docking studies depict that hydrogen bonding (5C=OH and 4C=O) groups amino acid (Lys771 and Ser876) residue with chrysin whereas in case of apigenin hydrogen bond among (4C=O) and ether group with the side chain of (Glu802 and Lys771) amino acids residues make them potent XO inhibitors [32]. Another study for the mechanism of XO inhibition of chrysin was performed by Lin *et al.* They stated that chrysin interacts with the XO active centre (Mo) and weakens the bonding of XO to xanthine resulting in a change of XO structure [44]. XO is also competitively inhibited by luteolin (K_i: 2.38 \pm 0.05 \times 10⁻⁶ mol/L). The interaction of luteolin with XO is by hydrophobic linkage at the binding site [30].

Likewise, tetrahydroamentoflavone (THA) extracted from the seed of *Semecarpus anacardium* has hypouricaemic effects. The extracted compound, THA, inhibits potently XO, and its potency is similar to allopurinol (the drug of reference) (K_i 0.98 μ M) [45].

Both flavone compounds (lonicerin and acacetin-3-O- α -L-rhamnopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside) extracted from *Plumula nelumbinis* are potent XO inhibitors (IC₅₀: 37.4 and 28.2, respectively μ g/mL) [46]. Luteolin, 5-hydroxy-6,7,3',4'-tetramethoxyflavone and eupatilin extracted from an EtOH extract of *Gnaphalium affine* also demonstrated strong XO inhibitory activities. Eupatilin exhibited the most powerful inhibitory activity, higher than any other isolated compound (IC₅₀: 0.37 μ M) [47]. In 2012, De Marino *et al.* reported that four luteolin glycosides (luteolin 4'-O-glucoside, luteolin 7-O-glucoside, luteolin 7-O-neohesperidoside and luteolin 7-O-rutinoside) extracted from the plant *Teucrium polium* L. showed potential to lower uric acid in blood (EC₅₀: 23.7, 61.6, 13.4, 72.7 μ M, respectively) [48].

In the same way, many flavones that had XO inhibitory activities were extracted from the stems of *Chrysanthemum morifolium*: luteolin (IC₅₀: 33.31 μ M), luteolin-7-O- β -D-glucopyranoside (IC₅₀: 26.55 μ M), luteolin-7-O-rutinoside (IC₅₀: 71.17 μ M), acacetin (IC₅₀: 11.57), acacetin 7-O- β -D-glucopyranoside (IC₅₀: > 100 μ M), acacetin 7-O-(6''-O-acetyl)- β -D-glucopyranoside (IC₅₀: > 100 μ M), acacetin 7-O-rutinoside (IC₅₀: > 100 μ M), diosmetin (IC₅₀: > 100 μ M), diosmetin-7-O- β -D-glucopyranoside (IC₅₀: > 100 μ M), cirsilineol (IC₅₀: > 100 μ M), kaempferol-3-O- β -D-sophoroside (IC₅₀: > 100 μ M), rutin (IC₅₀: > 100 μ M), apigenin-6-C- α -L-arabinopyranosyl-8-C- β -D-glucopyranoside (IC₅₀: > 100 μ M), apigenin-6-C- α -L-arabinopyranosyl-8-C- β -D-xylopyranoside (IC₅₀: > 100 μ M), apigenin-6-C- β -D-xylopyranosyl-8-C- α -L-arabinopyranoside (IC₅₀: > 100 μ M) and apigenin-6,8-di-C- β -D-xylopyranoside (IC₅₀: > 100 μ M) [40]. Other study showed that extract of leaves of *Cynara scolymus* had strong XO inhibitory activity which resulted from luteolin-7-O-glucuronide (IC₅₀: 20.24 μ M), luteolin-7-O-glucoside (IC₅₀: 19.90 μ M) and luteolin (IC₅₀: 1.49 μ M). Notably, luteolin (IC₅₀: 1.49 μ M) was a stronger XO inhibitor than the drug of reference allopurinol (IC₅₀: 3.65 μ M) [49]. Tung and Chang reported that the extract of *Acacia confuse* had various flavones (7,3',4'-trihydroxy-3-methoxyflavone, transilitin, 3,7,8,3'-tetrahydroxy-4'-methoxyflavone, 7,8,3'-trihydroxy-3,4'-dimethoxyflavone, 7,3',4'-trihydroxyflavone, okanin and 40-tetrahydroxyflavone) that showed potent XO inhibition. This potent inhibition is caused by the 3'-4'-OH structure [50]. The compounds (selgin, luteolin, diosmetin, apigenin, eupafolin, chrysoeriol, acacetin, jaceidin, tricetin 3',4',5'-trimethyl ether, 5,7,3'-trihydroxy-6,4',5'-trimethoxyflavone and 4,5-O-di-caffeoylquinic acid methyl ester) extracted from the flower *Chrysanthemum sinense* had more potent XO inhibitory properties than allopurinol (IC₅₀: 1.24, 0.13, 0.36, 0.20, 0.19, 0.16, 0.51, 5.36, 28.5, 18.9 μ M, respectively) [51]. Also amentoflavone was found to inhibit XO (23.61 \pm 1.31 μ g/mL) [52].

Flavanones

The two flavanones extracted from the stems of *Chrysanthemum morifolium*, (2S)-hesperetin and (2S)-eriodictyol, inhibit XO, but their inhibition was weak (IC₅₀: > 100 µM) [40]. On the other hand, eriodictyol which was extracted from the flower part of *Chrysanthemum sinense* demonstrated powerful XO inhibition (IC₅₀: 43.8 µM) [51]. Another natural flavanone isolated from *Blumea balsamifera* L (5,7,3',5'-tetrahydroxy flavanone) had little inhibitory effect on XO (IC₅₀: > 100 µM) [41].

Anthocyanidins and proanthocyanidins

Proanthocyanidins are composed of flavan-3-ol units with some degree of polymerization from 3 to 11. Phuwapraisirisan *et al.* demonstrated that carallidin, a proanthocyanidin from *Carallia brachiata* (*Rhizophoraceae*), potently inhibits XO enzyme (IC₅₀: 12.9 µM). Carallidin is hardly found in nature and its ring D is substituted entirely. Carallidin has two modes of action in the XO system; it lowers uric acid production by inhibiting XO enzyme, and it sequesters superoxide radicals. Also, an added phenolic moiety is essential in affecting activity, probably by scavenging radicals and attaching as a complex with XO [53].

Other study found that the seed extract of *Hippophae rhamnoides* L which is rich in proanthocyanidins, showed strong inhibition on XO activity [54]. Anthocyanins are hydrophilic pigments, widely distributed in plants and possess a wide range of pharmacological effects. Many anthocyanins (peonidin-3-glucoside 8.7 ± 0.1 µM, pelargonidin-3-glucoside 7.0 ± 0.4 µM, cyanidin-3-glucoside 7.2 ± 0.1 µM) we found to inhibit XO activity in various *in vivo* and *in vitro* experiments [55].

Isoflavones

Genistein and daidzein were demonstrated to inhibit XO. Genistein inhibits XO in a competitive way (IC₅₀: 1.39 ± 0.11 µM), hydrophobic association of genistein-XO complex, alters the secondary structure conformation of XO and leads to inhibition of XO by impeding substrate (hypoxanthine or xanthine) linkage [44]. Kim *et al.* extracted three isoflavonoids from the tubers of *Apios americana* and tested them for XO inhibition. They found that the three compounds 2'-hydroxygenistein, 3'-methoxy-4',5,7-trihydroxyiso-flavone and lupinalbin A with medium inhibition power (IC₅₀: 21.8 ± 0.7, 31.6 ± 1.1, and 38.8 ± 3.5 µg/mL, respectively) [56].

Catechins

4'-hydroxyacetophenone and epicatechin-(4β,8)-epicatechin gallate were demonstrated to strongly inhibit XO in a mixed-type competitive mode [57]. In other experiment, Nguyen *et al.* extracted from the flower of *Chrysanthemum sinense* a catechin, jaceidin, which had potent inhibitory power on XO (IC₅₀: 1.15 µM

[51]. Epiphyllocoumarin-3-O-d-allopyranoside and 8-(2-pyrrolidinone-5-yl)-catechin-3-O- d-allopyranoside from *Davallia formosana* were also inhibitors of XO [57]. Chu *et al.* found in 2014 that a compound epicatechin-(4β,8)-epicatechin gallate (B2-3'-O-gallate) from the extract of *Rhodiola crenulata* inhibited XO (IC₅₀: 24.24 ± 1.80 µM) [57].

Hydroxycinnamic acids

Hydroxycinnamic acids are phenolic compounds with numerous health benefits that are present at elevated concentrations in many plants. Ferulic acid, cinnamic acid, caffeic acid and p-coumaric acid are hydroxycinnamic acid compounds [58]. Sarawek *et al.* reported in 2008 that extracts from *Cynara scolymus* contained chlorogenic acid, cynarin and dihydrocaffeic acid which slightly inhibited XO (IC₅₀: > 100 µM) [49]. In another study in 2015, Ding *et al.* argued that 4-hydroxycinnamic acid and 4-O-β-D-glucopyranosyl-trans-cinnamic acid from *Plumula nelumbinis* also showed weak XO inhibitory activity (IC₅₀: > 100 µM) [46].

Qu *et al.* extracted several hydroxycinnamic acids compounds from the stems of *Chrysanthemum morifolium*, namely (1,4-di-O-caffeoylquinic acid, 1,5-di-O-caffeoylquinic acid, methyl 3,4-di-O-caffeoylquinic acid, 4-O-caffeoylquinic acid, 3-O-caffeoylquinic acid, methyl 3,5-di-O-caffeoylquinic acid, chlorogenic acid methyl ester and 3,4-di-O-caffeoylquinic) that had weak to powerful XO inhibitory activities. 3-O-caffeoylquinic acid, 1,5-di-O-caffeoylquinic acid and methyl 3,5-di-O-caffeoylquinic acid demonstrated higher XO inhibitory activities (IC₅₀: 81.95, 55.50, 22.69 µM, respectively) in comparison to other compounds (IC₅₀: > 100 µM) [40]. In another investigation, Nguyen *et al.* purified some hydroxycinnamic acids from the flowering part of *Chrysanthemum sinense* and tested them for XO inhibition. The most powerful inhibition was achieved by 4,5-O-di-caffeoylquinic acid (IC₅₀: 2.31 µM), medium inhibition was found with 3,5-O-dicaffeoyl quinic acid (IC₅₀: 12.20 µM), methyl caffeate (IC₅₀: 12.3 µM), 4,5-O-dicaffeoylquinic acid (IC₅₀: 42.6 µM), caffeic acid (IC₅₀: 85.4 µM), and the least inhibition was depicted by 3-O-caffeoylquinic acid, 3,5-O-di-caffeoylquinic acid, 5-O-caffeoylquinic acid methyl ester and 5-O-caffeoylquinic acid. Furthermore, they described that carboxyl methylation and caffeoyl group position had a significant effect on XO inhibition. p-hydroxycinnamic acid (6.8 ± 0.2 µM) and ferulic acid (8.2 ± 0.3 µM) were also found to inhibit XO [51].

Tannins

Wu *et al.* isolated some ellagitannins (geraniin, corilagin and gallic acid) from *Geranium sibiricum* L. plant and assessed for XO inhibitory activities. Their results reported that corilagin (IC₅₀: 222.89 µM) and geraniin (IC₅₀: 129.88 µM) exhibited weak XO inhibitory activity

as compared to gallic acid (IC₅₀: 105.41 μM) [59]. An earlier investigation conducted by Unno *et al.* also demonstrated that leaf extracts from *Lagerstroemia speciosa* contained valoneic acid dilactone and ellagic acid also had potent XO inhibitory activities [60]. Protocatechuic acid (5.8 ± 0.4 μM) and 4,6-trihydroxy benzoic acid (6.5 ± 0.5 μM) from maize kernel strongly inhibit XO [55].

Chalcones

Chalcones are abundant compounds present in plants and possess prophylactic activities against several diseases, e.g., anti-inflammatory, anti-cancer, immunomodulatory, anti-HIV, inhibitor activity against glycosidase and XO. Tung and Chang showed that okanin, a chalcone from *Acacia confusa*, have greater inhibitory effect than other purified flavone compounds. Notably, okanin structure encompasses two rings joined by a linear chain and displays the strongest affinity toward XO. Furthermore, the type of inhibition is non-competitive [50]. Also, Kondo *et al.* extracted aspalathin from rooibos (*Aspalathus linearis*) and demonstrated that it possesses XO inhibitory properties (IC₅₀: 3.1 μM) [61]. Isosalipurposide purified from the EtOH extract of *Corylopsis coreana* Uyeki has XO inhibitory power [38].

In another study, Yang *et al.* extracted and purified from *Terminthia paniculata* six chalcone-flavonone heterodimers, among those, termipaniculatone A and termipaniculatone E (IC₅₀: 55.6 ± 1.7 and 89.5 ± 2.4 μM) [62].

Saponins

Lin *et al.* purified four saponins from the ethanolic (50 and 75%) roots extracts of *Ilex pubescens* (*Aquifoliaceae*) and assessed them for XO inhibitory activities. They purified ilexosaponin C, ilexosaponin B1, ilexosaponin B2 and prosapogenin compounds. The compound ilexosaponin C (IC₅₀: 9.18 ± 0.12 μM) and prosapogenin (IC₅₀: 5.25 ± 0.03 μM) demonstrated greater XO inhibitory activities when compared to other compounds (IC₅₀: ilexosaponin B1 17.18 ± 0.26 μM, ilexosaponin B2 26.46 ± 1.18 μM). The sugar part in their structure has an important role in XO inhibition since it's the only difference between ilexosaponin C and prosapogenin and the latter possessed higher XO inhibitory activities compared to other saponins [63]. Smilaxchinoside A and smilaxchinoside C from *Smilax riparia* also had powerful XO inhibitory activities [64]. In another investigation, a saponin glycoside (pallidifloside D) extracted from roots and rhizomes of *Smilax riparia*, showed potent inhibition toward XO [65]. Furthermore, they also purified riparoside B and timosaponin J from *Smilax riparia*. These two compounds showed XO inhibitory activities [66].

Terpenoids

Terpenoids are a vast group of plant compounds comprising more than 40,000 structures [67]. Many researchers noted that terpenoids from various plant species are efficient for treating HUA [42, 51, 68]. Three monoterpene glycosides (1-O-β-D-glucopyranosyl-8-O-benzoylpaeonisuffrone, paeoniflorin and albiflorin) were purified from *P. lactiflora* by means of a high-performance counter-current chromatography (HPLCC), medium-pressure liquid chromatography (MPLC) and tested for XO inhibitory activities. From the purified compounds albiflorin and paeoniflorin had XO inhibitory activities (IC₅₀: 110.96 ± 0.06 and 99.36 ± 0.08 μM, respectively). Nevertheless, the compound 1-O-β-D-glucopyranosyl-8-O-benzoylpaeonisuffrone did not have XO inhibitory activity because it lacks the hydroxyl part at C4 position in its structure which has an important role in XO inhibition [68].

Nor-oleanane triterpenoids extracted from the leaves and fruits of *Stauntonia brachyanthera* were described having the potential to inhibit XO [42, 69]. Liu *et al.* isolated in 2017 from the leaves of *Stauntonia brachyanthera* several nor-oleanane triterpenoids and assessed them for anti-HUA effect. Brachyantheraoside A3, brachyantheraoside A1, 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-30-norolean-12,20(29)-dien-28-oic acid, brachyantheraoside D1 and brachyantheraoside B4 demonstrated strong XO inhibitory activities. The inhibitory activities were: brachyantheraoside B4 (IC₅₀: 0.2 ± 0.16 μM), 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-30-norolean-12,20(29)-dien-28-oic acid (IC₅₀: 18.5 ± 0.29 μM), brachyantheraoside D1 (IC₅₀: 49.2 ± 0.33 μM), brachyantheraoside A1 (IC₅₀: 79.6 ± 2.11 μM) and brachyantheraoside A3 (IC₅₀: 91.3 ± 1.56 μM) [69].

Studying the structure composition of pure nor-oleanane triterpenoids, it revealed that after the substitution of C-28 by sugar moieties XO inhibition decline. As stated by Liu *et al.* the inhibition of XO is not present without a free carboxylic group at C-28. The compounds brachyantheraoside B₄ and 3-O-α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-30-norolean-12,20(29)-dien-28-oic acid demonstrated the highest XO inhibitory activity in comparison to (brachyantheraoside D1) since the hydroxyl group oxidation (brachyantheraoside B₄), or exocyclic double bond (L-rhamnopyranosyl-(1→2)-α-L-arabinopyranosyl-30-norolean-12,20(29)-dien-28-oic acid) at C-20 to the carboxylic group (brachyantheraoside D1) have a negatively effect. Consequently, oxygen substitution at C-23 may augment the inhibitory activities at some level [42].

Two cucurbitane-type triterpenoid glycosides (taiwacin A and momordicoside K) were purified from the stems of *Momordica charantia* [70]. The compound (cucurbita-1(10),5,22,24-tetraen-3α-ol) (IC₅₀: 36.8 ± 20.5 μM)

demonstrated stronger XO inhibition when compared to the (5 β ,19 β -epoxycucurbita-6,22,24-trien-3 α -ol) (IC₅₀: 124.9 \pm 8.3 μ M). Both compounds have a similar skeleton. This implies that in 5 β ,19 β -epoxycucurbita-6,22,24-trien-3 α -ol, the linkage of epoxy moiety between C-5 and C-19 weakened the XO inhibitory effect [71]. Lin *et al.* stated that taiwacin A and momordicoside K from the stems and fruits of *Momordica charantia* are strong XO inhibitors [70].

Still, the triterpenoids (garcinielliptones Q and S) from *Garcinia subelliptica* did not exhibit XO inhibition except phloroglucinol and garcinielliptones which were showing inhibition of XO in a dose-dependent manner (IC₅₀: 53.8 \pm 11.5 μ M) [72]. 24-methylenelanost-8-en-3 β -ol (IC₅₀: 16.96 \pm 0.69 μ M) showed XO inhibitory effect when measured to other triterpenoid compounds extracted from the stem bark of *Klainedoxa gabonensis* (betulinic acid, lupeol, 24-methylenelanost-8-en-3-one, 24Z-ethylidenelanost-8-en-3-one, 2-hydroxy-24-methylenelanostan-1,8-dien-3-one and 3-acetyl ursolic acid) [73]. β -amyrin from the *Garcinia subelliptica* (pericarp, heartwood and seed) and a diterpenoid tanshinone (IC₅₀: 11.71 \pm 0.89 μ g/ml) likely inhibited UA synthesis [72, 74]. Thymol, a monoterpenoid natural compound was reported inhibiting UA synthesis by attaching at the FAD domain of XO by hydrogen bonding (Glu 802) with the carbonyl group of the pyrimidine ring [75]. Thymol was also suspected by Hudaib *et al.* to be the responsible for the strong inhibition of XO by the extract of *Salvia spinosa* [34]. Another triterpenoid compound was extracted from *Tribulus arabicus*, purified and tested by Abu-Gharbieh *et al.* ursolic acid was found to have great XO inhibitory effect and was reported as a promising application as anti-HUA treatment [76].

Bustanji *et al.* attributed the anti-XO activity found in the extract of *Achillea fragrantissima* (Forssk.) (IC₅₀: 197.6 μ g/mL) to the plant content of monoterpenes, and flavone derivatives [77].

Stilbenes

Stilbenes are polyphenolic compounds (C₆-C₂-C₆) broadly distributed in plants. These compounds have a wide range of therapeutic activities: anti-inflammatory, anti-tumour, antioxidant etc. [78, 79]. Cajaninstilbene acid extracted from the leaves of *Cajanus cajan* (L.) Millsap demonstrated potent XO inhibitory effect (IC₅₀: 3.62 μ M) [80]. Remarkably, the compound cajaninstilbene acid showed stronger XO inhibitory activity when measured to standard drug allopurinol (IC₅₀: 8.95 μ M) and resveratrol (IC₅₀: 7.14 μ M) due to C₆ carboxyl group. Furthermore, molecular docking study results revealed that phenyl ring (C-C₆) of cajaninstilbene acid interact/sandwich with amino acids residues Phe (914 and 1009) and generates strong (π - π) effect with the two residues. Electrostatic interaction had a vital role in linking with XO enzyme. An oligostilbene

(vaticanol A) from the *Vatica mangachapoi* was also investigated for inhibition of XO (IC₅₀: 23.3 1.8 μ M) [81].

Recently, Tang *et al.* extracted and purified from *Gnetum parvifolium*, four stilbenoids: piceatannol, rhaponticin, resveratrol and isorhapontigenin. They tested these compounds for XO inhibition and found that they possess potent inhibitory effect (IC₅₀: 6.44, 5.997, 3.8,8 and 46.75 μ M, respectively) [82].

Phenylethanoid Glycosides

Phenylethanoid glycosides are water-soluble compounds present in various plant organisms and have numerous health benefits. Wan and Xie isolated, verbascoside from the seed of *Plantago asiatica* L. and assessed it for XO inhibition. Their investigation revealed that verbascoside strongly inhibits XO enzyme (IC₅₀ 81.11 mg/mL) [83]. In another study, Wan *et al.* reported that phenyl rings of verbascoside aid in the linking with XO *via* the MPT domain [84].

Alkaloids

Mohammad *et al.* proved that the aqueous extract of *Hyoscyamus reticulatus* had XO inhibitory activity (IC₅₀: 12.8 μ g/mL), and attributed this inhibition in the extract probably to the tropane alkaloids hyoscyamine and scopolamine, and also the secondary metabolites present like the chlorogenic acid, flavonoid (rutin), coumarin and tannins [85]. Later, Bustanji *et al.* confirmed the potent XO inhibitory effect demonstrated by the extract of *H. reticulatus* [77].

Ahmad *et al.* stated that oxindole alkaloids extracted from *Isatis costata* have a good probability to lower uric acid serum concentrations. They extracted and purified from *Isatis costata*, six oxindole alkaloids (isatinones A, costinones A, isatinones B, costinones B, trisindoline and indirubin). The compound costinones A (IC₅₀: 90.3 \pm 0.06 μ M) showed maximum XO inhibition among the other compounds (IC₅₀: costinones B 101.7 \pm 0.02 μ M, isatinones A 117.5 \pm 0.03 μ M, isatinones B 130.6 \pm 0.05 μ M, indirubin 170.5 \pm 0.01 μ M, and trisindoline 179.6 \pm 0.04 μ M).

Curiously, a red colour pigment (indirubin) which can be obtained from plants and microbes, has a wide range of pharmacological properties. It is used in traditional Chinese medicine as an active ingredient [86]. Scientists are experimenting engineering microbes in order to produce large quantities of indirubin [87]. Ahmad *et al.* studies lead to the usage of indirubin and other alkaloids for treating HUA [86]. Sang *et al.* also described other alkaloids extracted from *Nelumbinis folium* to have the ability to inhibit XO. The crude extract of *Nelumbinis folium* has a strong XO inhibitory activity (3.313 μ g/mL). Furthermore, there were also purified various alkaloids, e.g., nuciferine, O-nornuciferin, N-nornuciferin, roemerine, N-methyliso-

coclaurine, armapavine and asimilobine by using UHPLC-Q-TOF-MS [88].

Conclusions

HUA is a life-threatening disease, the prevalence rate is increasing significantly and evolving into pandemic occurrence. Facing HUA is an essential task that requires the cooperative efforts of scientists from various disciplines. For over 50 years the sole drug inhibitor of XO offered on the market was allopurinol. In the last 10 years, the hunt for new XO inhibitors increased because the enzyme products have been linked with gout and hyperuricemia and also with various other conditions, such as hypertension cardiovascular disease and diabetes.

According to studies, it is established that plants and their products may have the ability to manage HUA disorder via inhibiting the main key enzyme (XO). Inhibitory compounds enter XO active site, form XO-inhibitory compounds linking through hydrophobic bonds, attach with surrounding XO residues and occupy the active site which prevents further processing of the substrate and thus impedes the production of UA. Notably, a surplus of bioactive compounds in plants inhibits XO enzyme (luteolin, quercetin, isorhamnetin, galangin, chrysin, prosapogenin, cajanin stilbene acid, etc.) near or stronger than reference drug (allopurinol). Yet, there is still a need for clarifying the mechanism of how bioactive compounds link to XO. Finally, besides the cooperation of researchers worldwide, various innovative technological upgrading in natural bioactive compounds extraction, purification, method optimization, specification, identification, and assay for XO inhibitory activity (*in vitro* and *in vivo*) should be used to manage this disease.

Conflict of interest

The authors declare no conflict of interest.

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