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Research article

Molecular Docking of Bioactive Compounds from Thai Medicinal Plants Against Xanthine Oxidase for Gout Treatment

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Abstract: Xanthine oxidase (XO) is a crucial enzyme of the purine catabolism pathway, which catalyzes the reaction of hypoxanthine to xanthine and xanthine to uric acid. The high level of uric acid leads to gout, kidney disease, and several disorders. In this study, molecular docking was performed to investigate potential bioactive compounds from Thai medicinal plants that acted as XO inhibitors compared to commercial drugs. Among 30 bioactive compounds tested, 16 were classified as strong XO inhibitors. These compounds include asiatic acid, benzyl glucosinolate, beta-sitosterol, chlorogenic acid, curcumin, eupatorin, gamma-mangostin, hibiscitrin, lutein, nimbolide, piperine, quercetin, rosmarinic acid, rutin, sesamin, and vitexin. They exhibited binding affinity values ranging from -8.5 to -10.6 kcal/mol. Moreover, moderate XO inhibitors were identified with binding affinity of -6.2 to -8.0 kcal/mol, consisting of the 7 compounds of gallic acid, garcinia acid, gingerol, limonene, linalyl acetate, panduratin A, and scopoletin. As a result, Thai medicinal plants could serve as potential sources of bioactive compounds for further drug design for treating gout patients.

Keywords: Molecular docking; Xanthine oxidase; Gout; Thai medicinal plants

1. Introduction

Gout is an inflammatory arthritis that can affect anyone, regardless of gender and age. It has been reported to increase rapidly worldwide [1]. Gout is a prolonged presence of high uric acid levels (hyperuricemia) in the bloodstream. This condition leads to the formation of monosodium urate crystals, which can accumulate in synovial fluid or surrounding tissue, causing acute and chronic inflammation, tissue damage, and pain [2]. Moreover, gout patients are at risk of developing chronic conditions such as hypertension, chronic kidney disease, cardiovascular diseases, diabetes, or metabolic syndrome, which can impact their quality of life [3].

Gout is an inherited purine metabolism disorder involving xanthine oxidase (XO, EC 1.17.3.2), an enzyme responsible for breaking down purine nucleotides to uric acid. Excess uric acid can then crystallize and trigger the symptoms characteristic of gout. The XO-catalyzed reaction produces reactive oxygen species (ROS), causing oxidative damage to tissues and other diseases

linked to oxidative stress [4-5]. The XO inhibitors have been investigated as potential drugs to block the biosynthesis of uric acid and have shown promise in anti-cancer therapies [6]. Allopurinol is a common drug for gout treatment, mainly included as an XO inhibitor to reduce uric acid levels. However, it has been reported on side effects, such as hypersensitivity reactions, Stevens–Johnson syndrome, hepatotoxicity, and nephrotoxicity [7]. Febuxostat is a recommended drug for gout patients beyond allopurinol, with superior antioxidant and anti-inflammatory effects [8]. However, febuxostat has fewer effects on skin reactions than allopurinol but increases the risk of gout in patients with cardiovascular diseases [9]. Both drugs function as XO inhibitors by stably binding the XO active sites, preventing the conversion of xanthine to uric acid.

Many bioactive compounds were recently studied for their anti-oxidative, antibacterial, antiviral, and anti-mutagenic activities *in vitro* and *in vivo* experiments, including XO inhibitors from plants [10-11] and fungi [12]. To avoid these adverse side effects of drugs for gout treatment, bioactive compounds with fewer or no side effects are known to be investigated in medicinal plants. Traditional medicine in Asian countries showed several potential lead compounds for XO inhibition, such as cordauvarin A from Vietnamese *Uvaria cordata* [13], quercetin from Indonesian *Sonchus arvensis* [14], and quercetin, quinic acid and rutin from *Plumeria rubra* or Malaysian Red [10].

Molecular docking is a computational tool that predicts the interaction between a small molecule and a protein based on energy complementation at the atomic level. Widely used for identifying potential drug candidates, molecular docking predicts the binding affinity of small molecules to a protein or receptor of interest, offering a time- and cost-saving technique for screening interesting molecules from a large database [15]. Numerous studies have employed molecular docking for xanthine oxidase research [5, 16-19]. However, only a few studies have investigated XO activities *in vitro* in Thai medicinal plants [20-22]. Therefore, we aimed to screen the potential bioactive compounds as XO inhibitors using the molecular docking technique to investigate their function based on the interactions between proteins and ligands from Thai medicinal plants for gout treatment. The other identified XO inhibitors may serve as valuable candidates for further development into potential lead compounds.

2. Materials and Methods

2.1 Collection of structures

The three-dimensional structure of proteins was carried out from the Protein Data Bank (PDB) [23]. This study's PDB ID of xanthine oxidase (XO) was 1N5X. This structure was obtained through X-ray diffraction techniques with a resolution of 2.80 angstroms, R-value free of 0.275, and R-value work of 0.244 [24].

For the three-dimensional structure of ligands, 30 of 72 different biologically active compounds from Thai medicinal plants with potential XO binding affinity were selected. These ligands were sourced from the PubChem database and have associated PubChem Compound Identifiers (CIDs), as shown in Figure 1. Allopurinol and febuxostat were used as references for XO inhibitors. The 30 structures of bioactive compounds and 2 reference ligands of XO inhibitor were structurally drawn using the online tool chem-space.com (https://chem-space.com/search).

2.2 Molecular docking

The molecular docking was conducted between the mammalian XO protein (PDB ID: 1N5X) and ligands, as mentioned in Figure 1, using the CB-Dock2 web server, https://cadd.labshare.cn/cb-dock2/php/index.php, which executed based on the AutoDock Vina algorithm [25]. The docking process employs template-independent blind docking techniques to calculate the pockets and binding sites of the protein-ligand complexes with initial parameters with five possible coupling cavities. Subsequently, the best-performing cavity was selected and the binding affinity values for the interaction between protein and ligands were calculated. Docking scores were reported as binding energy to the Vina score (kcal/mol). The binding energy of ligands lower than their controls was chosen for further analysis to determine the positions of amino acids in the binding interaction with the XO. The protein-ligand interaction was visualized using BIOVIA Discovery Studio 2021 [26].

3. Results and Discussion

3.1 Molecular docking

Based on the results of protein-ligand interaction, 30 different bioactive compounds were assessed against XO using molecular docking methods, comparing them to two control ligands. It was observed that the binding affinity values for all ligands ranged from -1.5 to -10.6 kcal/mol (Figure 2). The binding affinities of both control ligands to the XO were consistent with previous studies, ranging from -5.6 to -7.1 for allopurinol [13-14] and -8.7 to -10.1 for febuxostat [27-28]. Variations in XO templates and analytical parameters may result in value discrepancies between studies.

From this calculated binding affinity, it can be inferred that two groups of bioactive compounds have different abilities to bind to XO protein. The first group exhibited a high potential for XO inhibitors, with binding affinity values ranging from -8.5 to -10.6 kcal/mol. This group includes 16 different compounds:rutin (-10.6 kcal/mol); quercetin and gamma-mangostin (-10.2 kcal/mol); rosmarinic acid (-10.1 kcal/mol); eupatorin (-10.0 kcal/mol); chlorogenic acid and lutein (-9.7 kcal/mol); sesamin and beta-sitosterol (-9.6 kcal/mol); curcumin, benzyl glucosinolate and nimbolide (-9.4 kcal/mol); vitexin and hibiscitrin (-9.3 kcal/mol); piperine (-9.2 kcal/mol); and asiatic acid (-8.5 kcal/mol). These compounds exhibited better binding affinities than control ligands, allopurinol, and febuxostat, which showed binding affinities of -6.1 and -8.1 kcal/mol, respectively. These results, according to the XO binding activity, were found in natural extracts from medicinal plants of *Thunbergia laurifolia* [29], *Carissa carandas* [30], Vietnamese *Uvaria cordata* [13], and *Garcinia mangostana* [31]. Rutin, quercetin, and gamma-mangostin emerged as this study's top XO-binding bioactive compounds. *In silico* and *in vitro* investigations of quercetin [14] and rutin [32] demonstrated that both compounds were more effective than allopurinol in binding to XO and inhibiting its activity. Furthermore, crude extracts containing quercetin and rutin from *Plumeria rubra* exhibited superior XO inhibition activity compared to allopurinol [10]. Additionally, quercetin supplementation effectively reduced uric acid levels in obese males with gout, with no observed side effects on kidney and liver function when consumed at a dose of 500 milligrams daily for 4 weeks [33]. Gamma-mangostin, extracted from *Garcinia mangostana*, demonstrated the potential to maintain blood uric acid levels in rats [31]. Molecular docking studies revealed that piperine exhibited slightly better binding affinity to the XO enzyme than allopurinol, as it does not directly bind to the enzyme's active site [34]. Moreover, the binding affinity of other compounds involved in XO inhibitors, such as cordauvarin A (-8.8 kcal/mol) [13], has been reported.

In addition, the second group of compounds had a lower binding affinity for XO than the first group, with binding affinity values ranging from -6.2 to -8.0 kcal/mol. These compounds also exhibited good binding capabilities compared to allopurinol (-6.1 kcal/mol) but not febuxostat. In this group, there were 7 compounds: pandurate A (-8.0 kcal/mol); gingerol (-7.8 kcal/mol); scopoletin (-7.7 kcal/mol); gallic acid (-6.7 kcal/mol); limonene (-6.5 kcal/mol); linalyl acetate (-6.3 kcal/mol); and garcinia acid (-6.2 kcal/mol). Shaik and colleagues reported the ability of gallic acid to inhibit the activity of the XO enzyme and prevent the occurrence of acute myocardial ischemia in experimental mice [35]. Based on the binding affinity score of allopurinol to XO of -6.1 kcal/mol (Figure 2), it could be pointed out that the 16 compounds in the first group have a higher potential for binding to XO and may be of greater interest for further investigation in the context of XO inhibitors compared to the compounds in the second group.

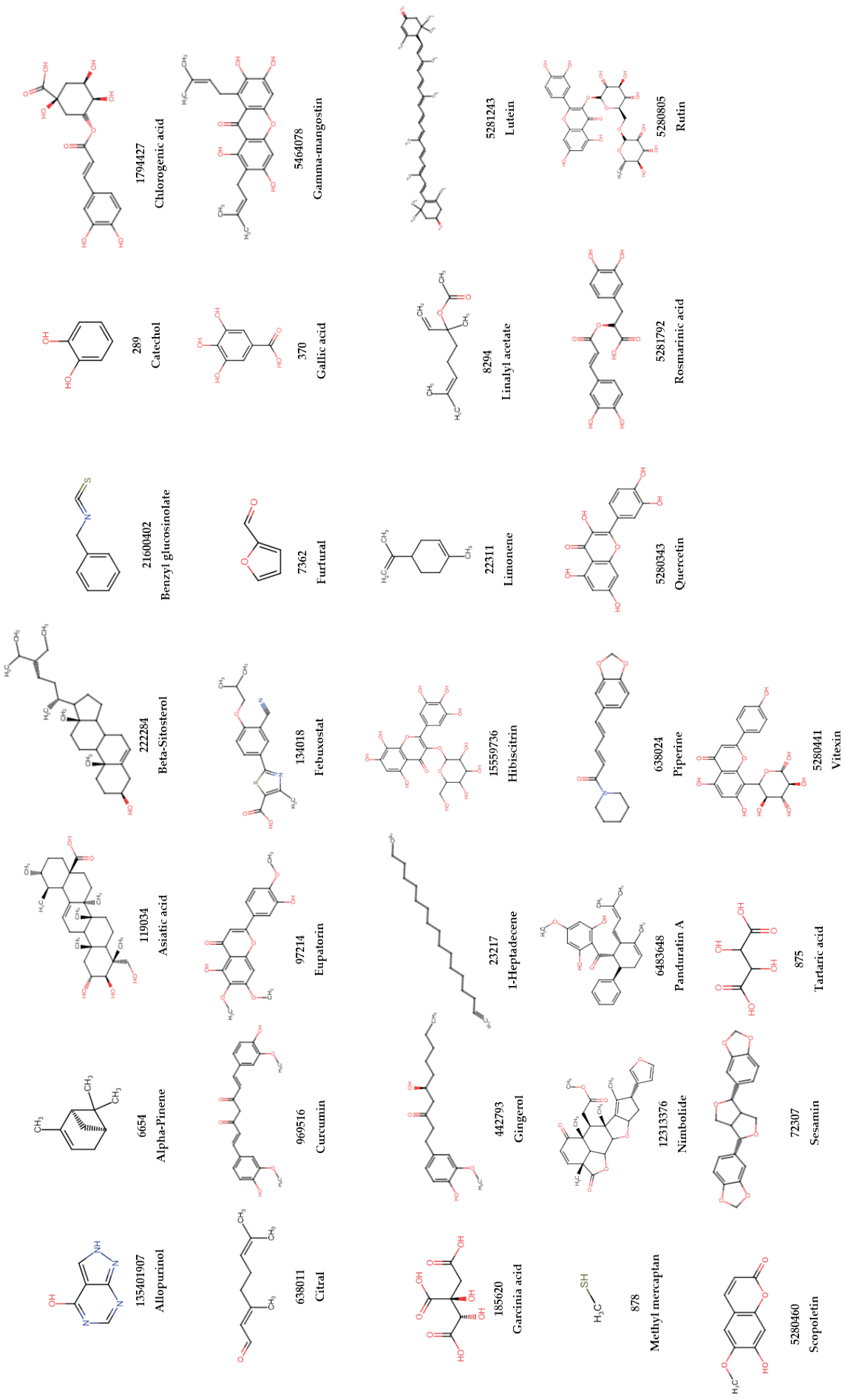

Figure 1. The structures of 30 bioactive compound ligands from Thai medical plants and (*) the two reference ligands of XO inhibitor. The PubChem CIDs are shown in number as mentioned.

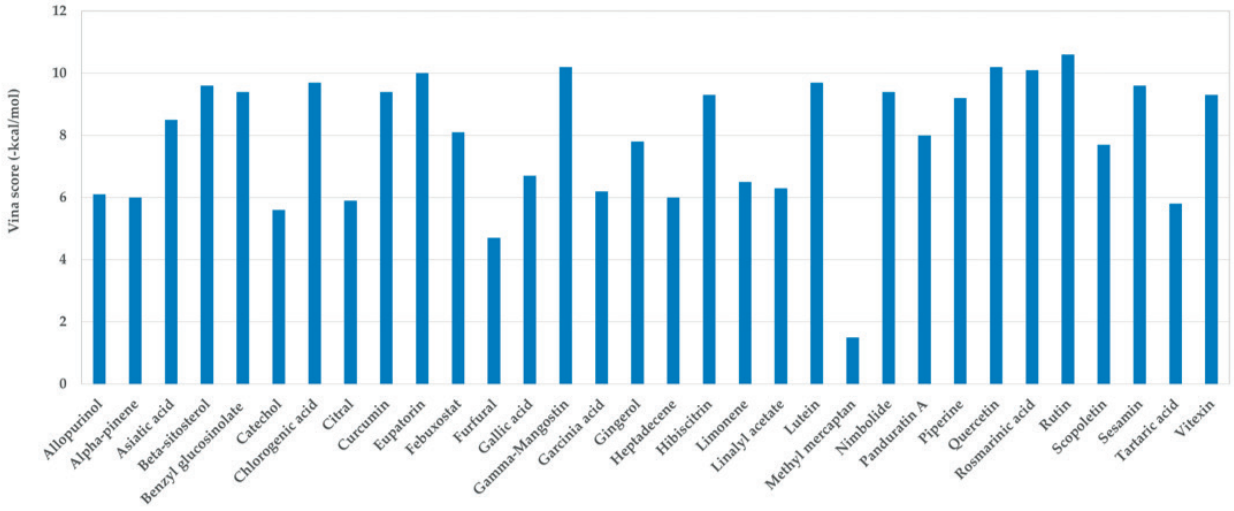

Figure 2. Binding affinities of various ligands found in Thai medicinal plants against XO.

Based on the 3D-structural interaction between ligands and XO, the amino acid residues involved in the binding interaction between XO and the 16 biologically active compounds from the first group (strong potential compounds as XO inhibitors) were further analyzed. The binding interaction between the ligands and the XO was found to involve amino acids from chains A and B of the protein. These interactions are mediated by hydrogen bonds, hydrophobic, ionic, and cation-π interactions (Figure 3 – 4). The specific amino acids involved in the binding interactions vary depending on the type of ligand. From this study, the bioactive compounds of rutin, chlorogenic acid, sesamin, beta-sitosterol, nimbolide, vitexin, hibiscitrin and asiatic acid were observed to interact with important amino acids Ala28, Arg32, Leu41, Cys73, Arg606, Pro675, Glu676, Glu679, and Asp828 in the XO structure. These amino acids also play a significant role similar to the binding interaction between febuxostat and the XO protein (Figure 3). Therefore, these compounds might inhibit the XO enzyme via a non-competitive inhibition mechanism such as febuxostat, a non-purine analog that acts as a non-competitive XO inhibitor [36]. The docking results of febuxostat with 1N5X protein showed interacted residues of Glu802, Thr1010, Arg880, Asn768, Leu873 and Leu648 [28]. For simulating an interaction between XO and ligands quercetin, gamma-mangostin, rosmarinic acid, eupatorin, curcumin, lutein, benzyl glucosinolate and piperine, it was found that the interaction of Glu332, Trp336, Lys422, Gln423, Arg427, Asp430, and Asp1170 was similar to the positions in the XO with allopurinol (Figure 4). From the virtual screening analysis of cordauvarin A from Vietnamese *Uvaria cordata*, the Glu802, Ala910, Gly913, and Phe914 were found as main amino acids that were responsible for the interaction at the active site of XO, whereas the Glu802, Thr1010, Arg880, Ala1079, Phe1009, and Phe914 of XO interacted with allopurinol [13]. These results suggested that the inhibition mechanism of these bioactive compounds might function similarly to allopurinol, a competitive inhibitor of XO [37].

According to XO sequences, bovine XO shares a 90% similarity with human XO in the overall sequence [38]. This conservation of amino acids should reveal a role of binding pattern and activity in both [24]. As a result, the predicted binding sites using molecular docking suggested that binding should correspond to configurations of the human XO.

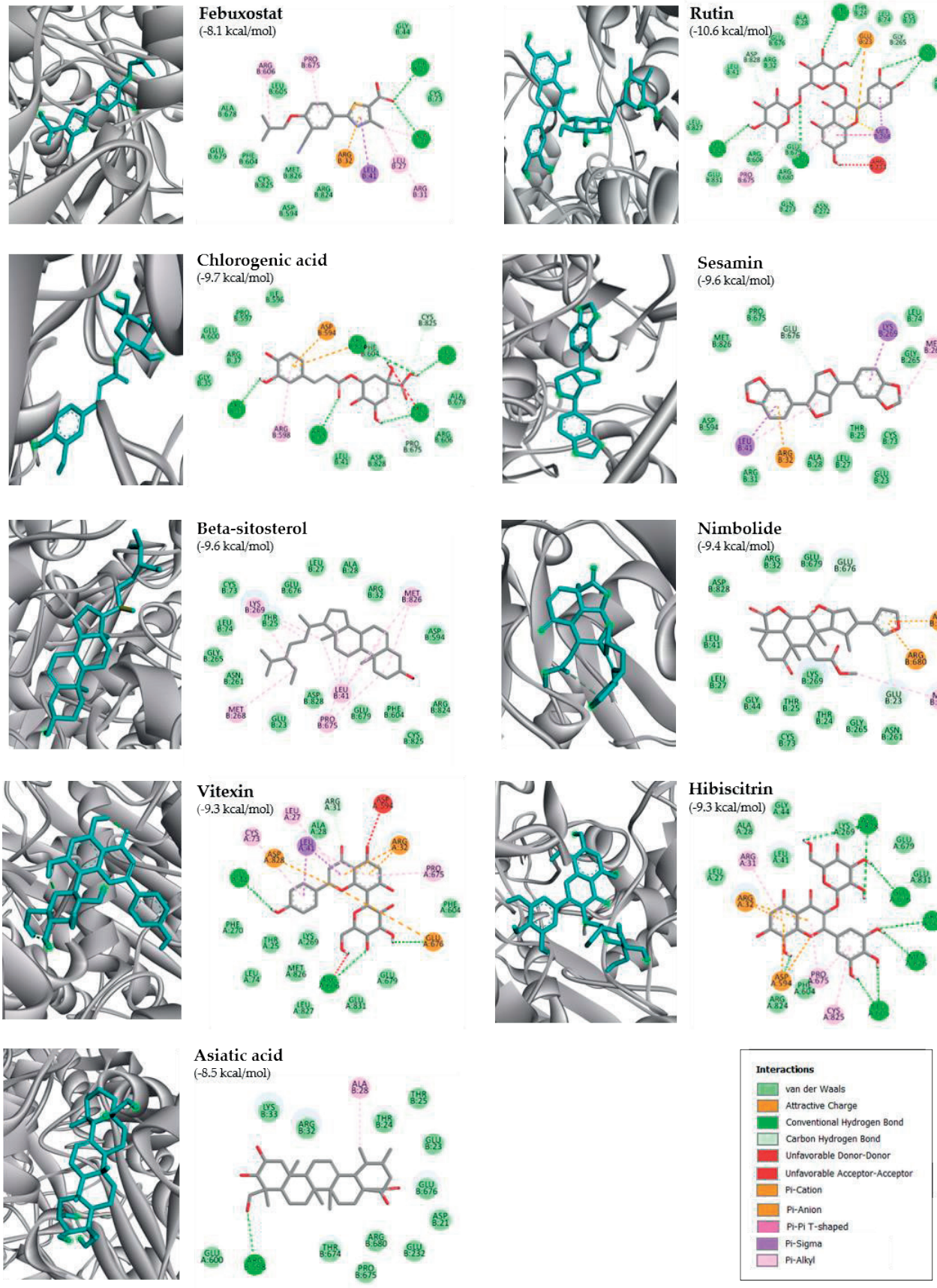

Figure 3. Visualization of XO with strong potential XO inhibitors, which interact with febuxostat.

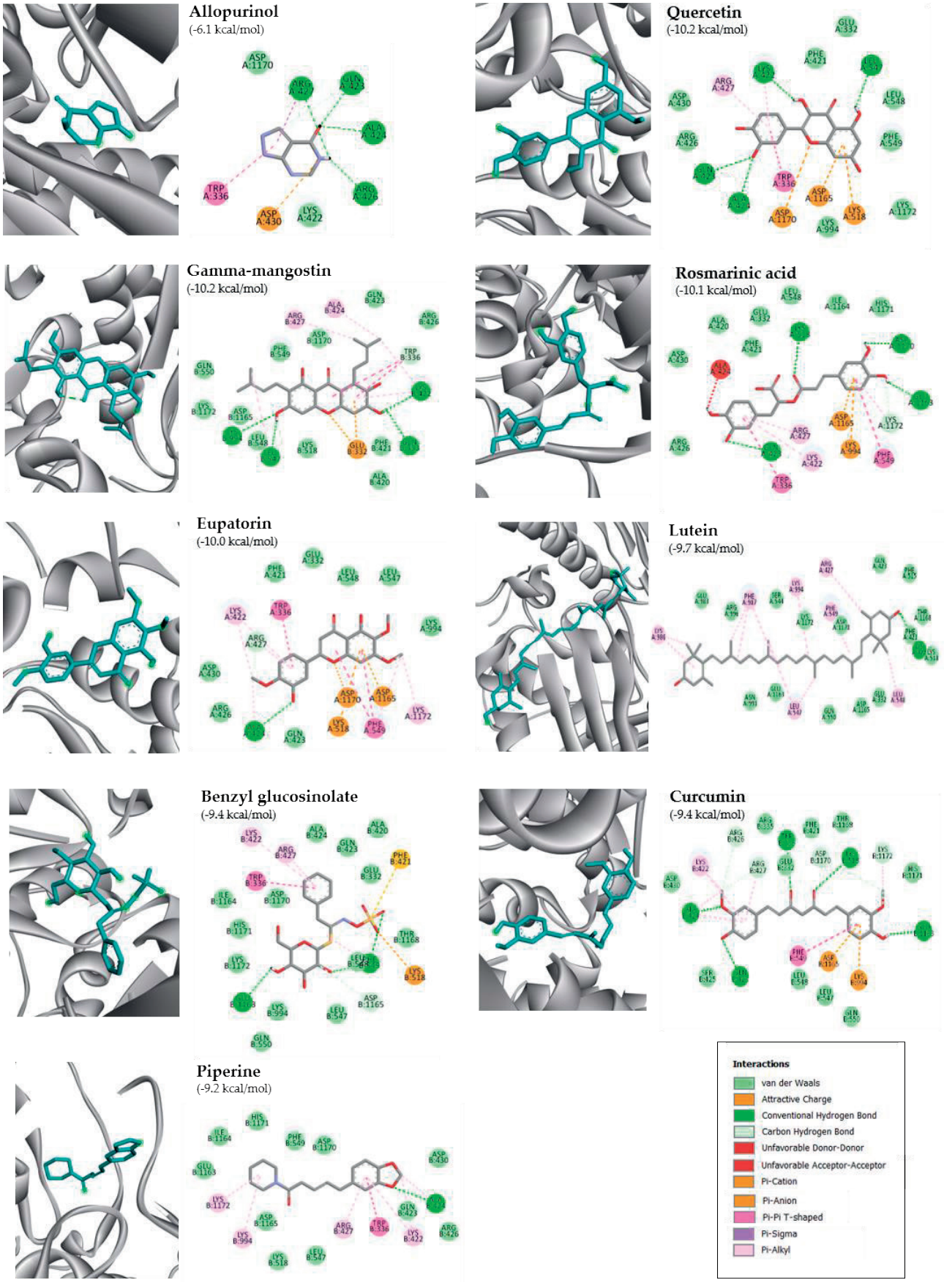

Figure 4. Visualization of XO with strong potential of XO inhibitors, which interacts with allopurinol.

Currently, gout treatment guidelines are to control uric acid levels in the blood using medication. Although allopurinol is often the first-line choice for reducing blood uric acid levels by inhibiting the XO activity, its use is limited by severe skin rashes. Therefore, febuxostat is preferred, especially in gout patients with impaired kidney function. However, it is not recommended to use febuxostat in patients with allergic reactions to allopurinol or in those with coronary artery disease [9]. Uric acid is primarily eliminated through the kidneys, and other treatment approaches involve using medications to enhance the excretion of uric acid through urine.

Table 1. Bioactive compounds and their sources from Thai medicinal plants

	Binding affinity (kcal/mol)	Bioactive compounds	Thai medicinal plants	References
Strong potential XO inhibitors	-10.6	Rutin	*Moringa stenopetala*	[39]
	-10.2	Gamma-mangostin	*Garcinia mangostana*	[31]
	-10.2	Quercetin	*Allium ascalonicum*	[40]
	-10.1	Rosmarinic acid	*Ocimum gratissimum*	[40]
	-10.0	Eupatorin	*Orthosiphon aristatus*	[41]
	-9.7	Chlorogenic acid	*Clitoria ternatea*	[40]
	-9.7	Lutein	*Tagetes erecta*	[42]
	-9.6	Sesamin	*Sesamum indicum*	[40]
	-9.6	Beta-sitosterol	*Leucaena leucocephala*	[40]
	-9.4	Curcumin	*Curcuma longa*	[40]
	-9.4	Benzyl glucosinolate	*Carica papaya*	[43]
	-9.4	Nimbolide	*Azadirachta indica*	[40]
	-9.3	Vitexin	*Garcinia cowa*	[44]
	-9.3	Hibiscitrin	*Hibiscus sabdariffa*	[45]
	-9.2	Piperine	*Piper nigrum*	[40]
	-8.5	Asiatic acid	*Centella asiatica*	[40]
Moderate potential XO inhibitors	-8.0	Panduratin A	*Boesenbergia rotunda*	[40]
	-7.8	Gingerol	*Zingiber officinale*	[40]
	-7.7	Scopoletin	*Syzygium cumini*	[40]
	-6.7	Gallic acid	*Azadirachta indica*	[40]
	-6.5	Limonene	*Myristica fragrans*	[40]
	-6.2	Garcinia acid	*Garcinia atroviridis*	[46]
	-6.3	Linalyl acetate	*Citrus aurantium*	[40]
Low potential XO inhibitors	-6.0	Heptadecene	*Cassia siamea*	[47]
	-6.0	Alpha-pinene	*Citrus hystrix*	[40]
	-5.9	Citral	*Citrus aurantifolia*	[40]
	-5.8	Tartaric acid	*Azadirachta indica*	[48]
	-5.6	Catechol	*Sauropus androgynus*	[40]
	-4.7	Furfural	*Cassia siamea*	[47]
	-1.5	Methyl mercaptan	*Paederia foetida*	[49]

Thailand has a large plant biodiversity with various bioactive compounds exhibiting biological and pharmacological activities of antioxidant, anti-inflammatory, and antimicrobial properties. Interestingly, from the molecular docking results, sources of strong and moderate potential bioactive compounds for XO inhibitors were found in many Thai medicinal plants mentioned in Table 1. These results were supported by using *Orthosiphon aristatus* for gout treatment by increasing diuresis [50]. The unripe fruit peels and leaves from *Carica papaya* showed high XO inhibitory activity [51]. As a result, Thai medicinal plants could be potential sources of bioactive compounds for further drug design for treating gout patients. Experimental investigations are imperative to assess the pharmacological efficacy of these compounds in treating gout.

4. Conclusions

In the present study, molecular docking is important in understanding the molecular interaction between bioactive compounds from Thai medicinal plants and XO enzymes. The 30 different bioactive compounds were screened for their potential as XO inhibitors compared to commercial drugs. The docking results showed that the 16 compounds were classified as strong XO inhibitors, including asiatic acid, benzyl glucosinolate, beta-sitosterol, chlorogenic acid, curcumin, eupatorin, gamma-mangostin, hibiscitrin, lutein, nimbolide, piperine, quercetin, rosmarinic acid, rutin, sesamin, and vitexin. The moderate XO inhibitors were identified with the 7 compounds of gallic acid, garcinia acid, gingerol, limonene, linalyl acetate, panduratin A, and scopoletin. The interaction of these ligands against XO protein could provide valuable insights for predicting potential bioactive compounds from Thai medicinal plants for the design and development of natural XO inhibitors. However, further experimental studies, including *in vitro*, *in vivo*, and preclinical tests, are required to evaluate the pharmacological potential of these compounds for gout treatment.

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