Journal of Research in Pharmacy

Research Article

www.jrespharm.com

Molecular docking approach to identify xanthine oxidase inhibitory effect of bioactive compounds in *Pogostemon cablin* (Blanco) Benth.

Tung BUI THANH [1] *, Thao TRINH PHUONG [1], Hang NGUYEN THU [1], Phuong TRINH MAI [1], Khanh DO THI HONG [1], Thuy NGUYEN THI [1]

[1] Pharmacology Department, VNU University of Medicine and Pharmacy, Vietnam National University Hanoi, Viet Nam

* Corresponding Author. E-mail: tungbt.ump@vnu.edu.vn (T.B.); Tel. +84-904429676.

Received: 30 Mar 2023 / Revised: 22 May 2023 / Accepted: 26 May 2023

ABSTRACT: Xanthine oxidase (XO) is a homodimeric enzyme found in many species, including humans responsible for catalyzing the synthesis of uric acid from hypoxanthine using oxygen as a substrate. *Pogostemon cablin* (Blanco) Benth. (*P. cablin*) has been widely used in traditional medicine for the treatment of gout. In this study, we used molecular docking method to find bioactive compounds from *Pogostemon cablin* (Blanco) Benth. for inhibiting the XO enzyme. 78 bioactive compounds were collected based on previous *Pogostemon cablin* (Blanco) Benth publications. and evaluated docking scores using Autodock vina software. Out of 78, four compounds had stronger inhibiting potential to XO target than positive controls. Lipinski's rule of five and ADMET property prediction analysis revealed positive results with two compounds, acacetin and apigenin. Therefore, *in vitro* and *in vivo* studies should be carried out to verify these compounds' XO inhibitory potential for future drug development.

KEYWORDS: Xanthine oxidase; *Pogostemon cablin* (Blanco) Benth; acacetin; apigenin; molecular docking.

1. INTRODUCTION

Hyperuricemia (HUA), is characterized by the concentration of serum uric acid above 7 mg/dL in men and above 6 mg/dL in women [1]. Increasing uric acid (UA) production, impairing renal UA excretion, or a combination of these two conditions leads to hyperuricemia occurs, resulting in the deposition of UA at the joints and kidneys [2]. High plasma UA is not only a prerequisite to gout but is also related to Metabolic Syndrome and risk factors for cardiovascular diseases [3].

Xanthine oxidase (XO) is a molybdoflavoprotein enzyme, involved in the metabolism of purines, plays an important role in HUA [4]. UA biosynthesis occurs in the last stage of purine metabolism [5]. UA is formed by xanthine and occurs via hypoxanthine by the action of XO [3]. Simultaneously, XO released H_2O_2 and O_2^-, which associated with various pathological events including inflammatory activation, metabolic disorders, endothelial dysfunction, and others [4, 6]. Therefore, XO has been recognized as a validated pharmacological target for the management of hyperuricemia and gout [7]. While being the most common medication for hyperuricemia treatment, febuxostat also comes with various side effects. Therefore, ample research must be performed to discover alternative compounds with similar XO-inhibitory effects in particular and hypouricemic action in general.

Pogostemon cablin (Blanco) Benth. (*P. cablin*) has been widely used in traditional medicine for the treatment of anti-inflammatories, colds, flu, headaches, fever, nausea, and diarrhea. Based on previous studies, *P. cablin* has been studied with many biological effects such as antioxidant, anti-microbial, anti-cancer cell proliferation and hepatoprotective mainly due to the presence of flavonoids [8-11]. Beside, *P. cablin* and its bioactive compounds were reported to prevent UA generation and showed potent XO inhibitory activity [12, 13]. In this study, we aimed to screen bioactive compounds from *Pogostemon cablin* with XO-inhibitory activity to find potential inhibitors of XO enzyme through the molecular docking method.

How to cite this article: Bui Thanh T, Trinh Phuong T, Nguyen Thu H, Trinh Mai P, Do Thi Hong K, Nguyen Thi T. Molecular docking approach to identify xanthine oxidase inhibitory effect of bioactive compounds in *Pogostemon cablin* (Blanco) Benth.. J Res Pharm. 2023; 27(6): 2452-2462.

ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.532

2. RESULTS

2.1. Evaluation of the docking model

Before screening compounds, co-crystalized ligand (QUE) was separated from the 3NVY (XO) complex to evaluate the docking model's accuracy and then redocked to the protein's active site. The root-mean-square deviation (RMSD) was determined by Chimera to evaluate the suitability of the docking parameters. The process was performed successfully if the RMSD value was less than or equal to 1,5 Å [14]. We received the RMSD of 3NVY was 0.824 Å – less than 1.5 Å, demonstrating that the result of molecular docking to the target is trustworthy.

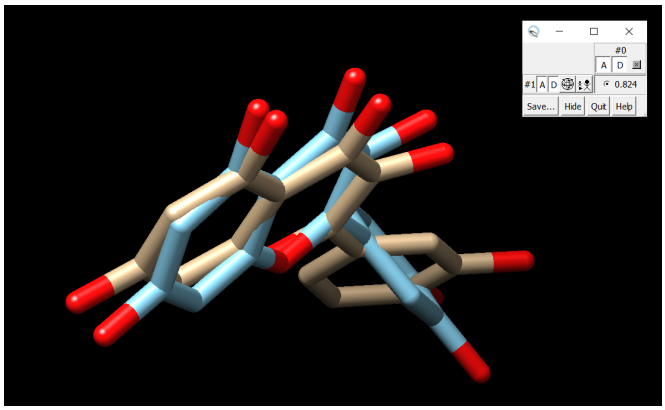


Figure 1. Co-crystallized ligand redock results of 3NVY

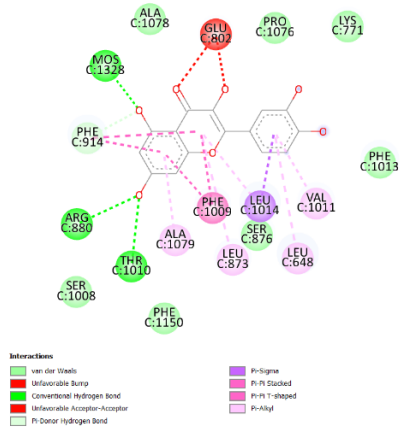


Figure 2. Interaction between QUE and 3NVY

2.2. Molecular docking results of compounds to the target protein

After preparing the proteins, to screen inhibitory activity potential against XO target, we docked 78 natural compounds from *P. cablin* [11, 15-17]. Previous research has identified GLU802, GLU1261, and ARG880 residues as essential players in catalyzing xanthine oxidation. Meanwhile, other residues located at the entrance, such as LEU648, LEU873, PHE649, PHE914, PHE1009, THR1010, VAL1011, PHE1013, and LEU1014, are involved in modulating the entry of small molecules, including substrates or inhibitors, into the catalytic center [18, 19].

Allopurinol and febuxostat are XO inhibitors, the only uricostatic drugs approved by the U.S. Food and Drug Administration (FDA) for the treatment of gout in 1966 and 2009 respectively [1, 20-22]. Allopurinol is an analog of hypoxanthine meanwhile febuxostat is a non-purine and selective inhibitor of XO [23, 24]. Therefore, in this study, we compared docking scores of potential compounds with two positive controls (Allopurinol and febuxostat) to evaluate their potential in inhibiting enzyme XO.

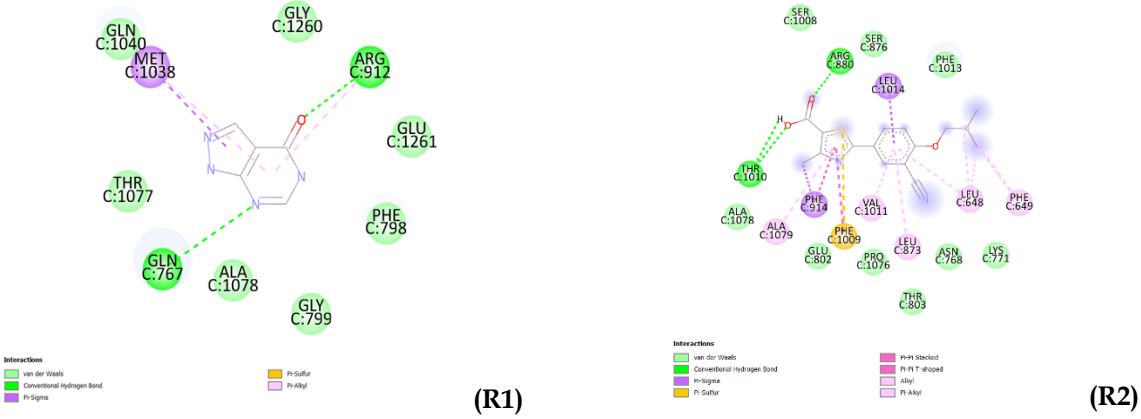


Figure 3. Interaction between allopurinol (R1) and febuxostat (R2) with XO

Allopurinol and febuxostat have a binding energy (BDE) of -6.1 (kCcal/mol) and -8.5 (kCal/mol) respectively to the XO target. The binding energy results of 78 natural compounds are shown in *Table 1*. *Figure 3* shows the interaction of Allopurinol and Febuxostat with the XO enzyme.

Table 1. The docking results of 78 compounds and reference compounds with XO.

No.	Bioactive compounds	Pubchem ID	Binding free energy (kcal/mol)	No.	Bioactive compounds	Pubchem ID	Binding free energy (kcal/mol)
1	Aciphyllene	565570	-7.7	40	Oleanolic acid	10494	-7.5
2	Alloaromadendrene	91354	-6.9	41	Patchouli alcohol	10955174	-6.4
3	β-Bourbonene	62566	-6.5	42	α-Patchoulene	521710	-5.8
4	α-Bulnesene	94275	-7.4	43	β-Patchoulene	101731	-6.3
5	β-Bulnesene	12302131	-7.2	44	β-Phellandrene	11142	-5.9
6	(+)-Camphene	92221	-5.7	45	α-Pinene	6654	-5.6
7	(−)-Camphor	444294	-5.9	46	β-Pinene	14896	-5.5
8	α-Caryophyllene	5281520	-6.8	47	Pogostol	532065	-7.5
9	β-Caryophyllene	5281522	-7.2	48	Pogostone	54695756	-7.3
10	trans-Caryophyllene	5281515	-6.4	49	α-Selinene	10856614	-6.9
11	Caryophyllene oxide	1742210	-6.6	50	β-Selinene	442393	-7.5
12	Copaene	12303902	-7.5	51	Seychellene	519743	-5.8
13	β-Cubebene	93081	-7.1	52	Spathulenol	92231	-7.0
14	β-Copaen-4-α-ol	91751357	-8.5	53	(−)-α-Terpineol	443162	-6.4
15	α-Elemene	80048	-6.3	54	Valencene	9855795	-7.0
16	β-Elemene	6918391	-7.2	55	**Acacetin**	**5280442**	**-8.8**
17	γ-Elemene	6432312	-6.9	56	**Apigenin**	**5280443**	**-8.8**
18	Elemol	92138	-6.9	57	Pachypodol	5281677	-7.5
19	Epiglobulol	11858788	-6.9	58	4′,5-Dihydroxy-3′,7-dimethoxyflavanone	321347	-8.2
20	Eucalyptol	2758	-5.8	59	5,4′-Dihydroxy-3,3′,7-trimethoxyflavanone	462696	-7.6
21	α-Elemenone	91748524	-6.5	60	Hesperetin 3'-methyl ether	14466294	-8.0
22	Epifriedelinol	119242	-7.6	61	Licochalcone A	5318998	-7.7
23	Friedelin	91472	-8.0	62	Ombuin	5320287	-8.4
24	cis-Farnesol	1549107	-7.0	63	Globulol	12304985	-6.9
25	Germacrene-A	5362634	-7.5	64	Retusine	5352005	-6.9
26	Germacrene-B	5281519	-6.9	65	5α-Stigmast-3,6-dione	13992092	-7.7
27	Germacrene-D	91723653	-7.4	66	Daucosterol	5742590	-8.0
28	α-Guaiene	5317844	-7.3	67	β-Sitosterol	222284	-6.7
29	β-Guaiene	15560252	-7.2	68	Stigmasterol	5280794	-7.5
30	γ-Guaiene	15560256	-7.9	69	Acteoside	5281800	-7.9
31	γ-Gurjunene	90805	-7.2	70	**Isocrenatoside**	**44559534**	**-8.7**
32	α-Gurjunene	15560276	-6.4	71	**3″-O-Methylcrenatoside**	**10326741**	**-8.8**
33	Heptanal	8130	-4.6	72	Tilianin	5321954	-7.9
34	Limonene	22311	-6.4	73	Rubusoside	24721373	-7.4
35	Myrtenol	10582	-5.9	74	Epifriedelinol	119242	-7.6
36	Nonanal	31289	-4.8	75	Methyl oleanolate	92900	-7.5
37	Norpatchoulenol	6451732	-6.5	76	Dibutyl phthalate	3026	-6.0
38	1-Octen-3-ol	18827	-4.7	77	Succinic acid	1110	-5.3
39	3-Octanol	11527	-4.6	78	Uracil	1174	-5.3

R1	**Allopurinol**	**135401907**	**-6.1**	R2	**Febuxostat**	**134018**	**-8.5**
	Quercetin	**5280343**	**-8.7**				

Based on Table 1, comparing to positive controls 4 compounds showed lower ΔG free energy: acacetin (-8.8 kCal/mol). apigenin (-8.8 kCal/mol). isocrenatoside (-8.7 kCal/mol) and 3″-O-methylcrenatoside (-8.8 kCal/mol).

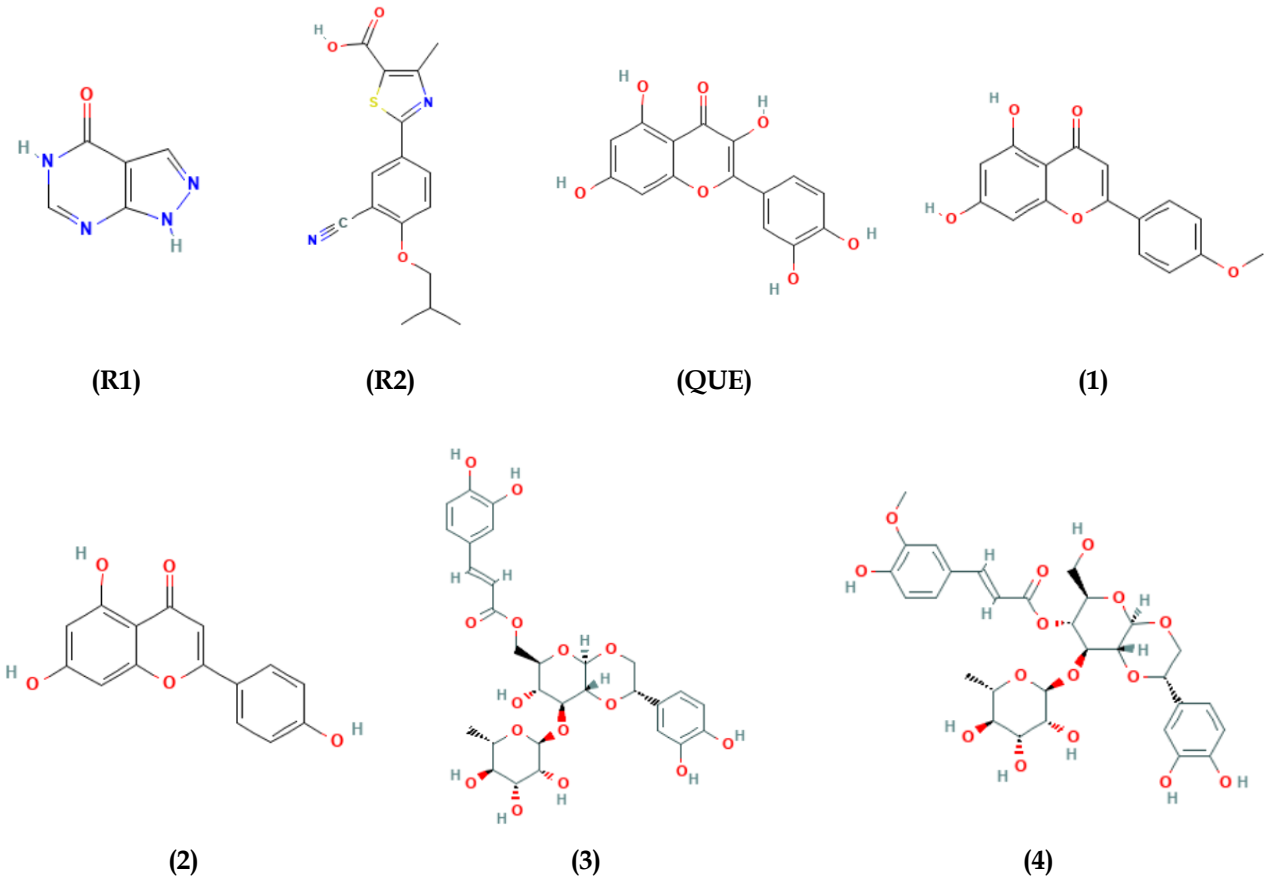


Figure 4. 2D structures of allopurinol (R1), febuxostat (R2), quercetin (QUE), acacetin, apigenin, isocrenatoside and 3″-O-methylcrenatoside (from 1-4)

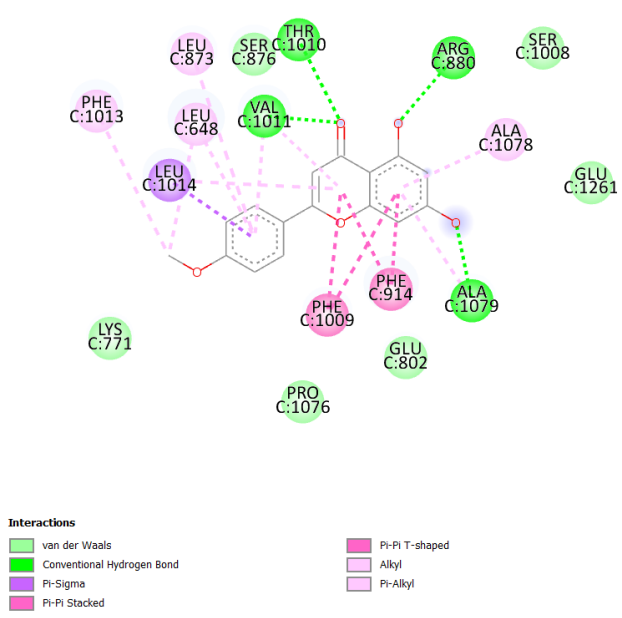
Table 2. Ligand-amino acid interactions of positive controls and 4 potential compounds against the XO enzyme.

No.	Name	Binding free energy (kcal/mol)	Involved amino acids
1	Acacetin	-8.8	LEU648, LEU873, ARG880, PHE914, PHE1009, THR1010, VAL1011, PHE1013, LEU1014, ALA1078, ALA1079
2	Apigenin	-8.8	LEU648, LEU873, ARG880, PHE914, PHE1009, THR1010, VAL1011, LEU1014, ALA1078, ALA1079
3	Isocrenatoside	-8.7	LEU648, LEU873, HIS875, SER876, PHE883, THR1010, VAL1011, PRO1012, LEU1014, TYR1140, PHE1142
4	3''-O-methylcrenatoside	-8.8	LEU648, PHE649, LYS771, ANA768, SER876, LYS771, VAL1011, PHE1013, LEU1014, PRO1076, TYR1121, GLN1122
R1	Allopurinol	-6.1	GLN767, ARG912, MET1038,

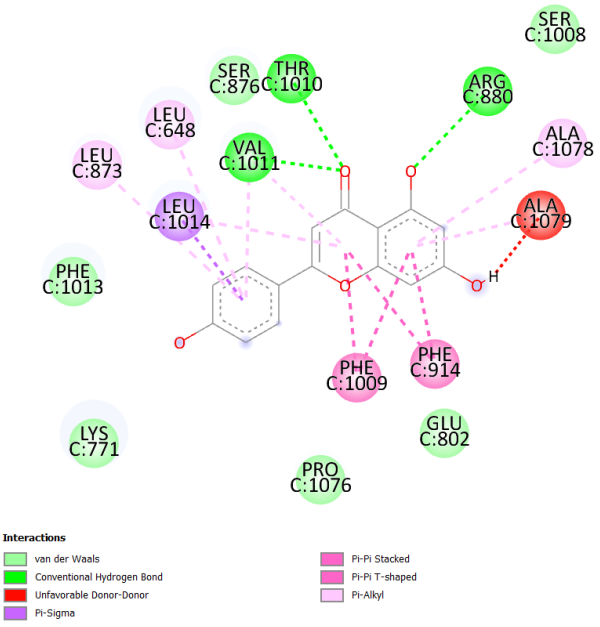
R2	Febuxostat	-8.5	LEU648, PHE649, LEU873, ARG880, PHE914, PHE1009, THR1010, VAL1011, LEU1014, ALA1079
QUE	Quercetin	-8.7	LEU648, LEU873, ARG880, SER876, GLU802, PHE914, PHE1009, THR1010, VAL1011, LEU1014, ALA1079, MOS1328

The ligand-amino acid interaction between acacetin, apigenin, isocrenatoside, and 3″-O-methylcrenatoside and XO target showed many interactions with similar amino acids in the active regions of the target compared to the positive control. Acacetin and Apigenin gave the same binding energy, both share many other similar interactions to Febuxostat such as π-σ bond with PHE914 and LEU1014, THR1010, alkyl bond with LEU648, LEU973, ALA1079. Out of 3 essential residues in oxidation catalyzing, both (1) and (2) have the interactions with ARG880 which also founded in all positive controls. Meanwhile, only QUE shows interaction with GLU802.

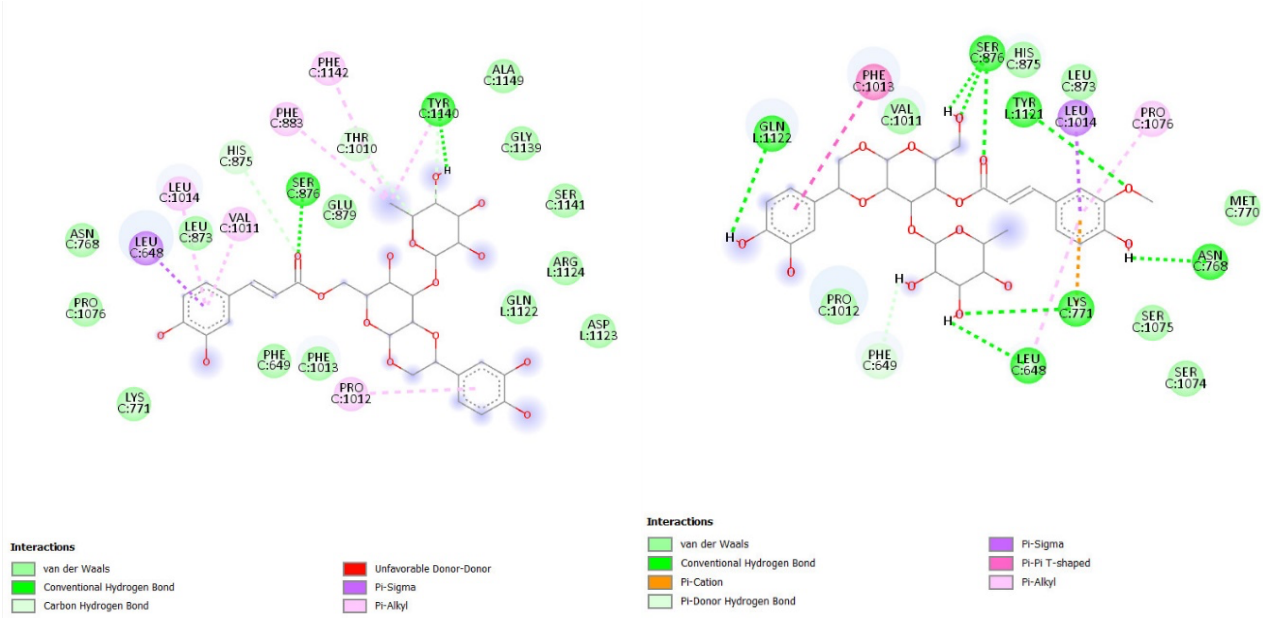
Isocrenatoside has less similar interactions but still interacts with many amino acids of XO target: LEU873, VAL1011, LEU1014…mostly through hydrogen and π-alkyl bond. Similarity, the interaction of 3″-O-methylcrenatoside with XO is hydrogen bonds, π-π, π-σ and π-alkyl bond to important amino acids: LEU648, LEU873, VAL1011. The receptor-ligand 2D interaction between 4 potential compounds and the protein target are shown in Figure 5.



(1)



(2)



(3) ***(4)***

Figure 5. Interactions between 4 compounds: acacetin (1), apigenin (2), isocrenatoside (3) and 3″-O-methylcrenatoside (4) with XO.

2.3. Lipinski's rule of five

Four compounds with lowest binding energy then evaluated by Lipinski's rule of five to identify whether they are drug-like or non-drug-like molecules. Compounds are considered to be "drug-like" when they have at least 2 of the 5 criteria of Lipinski's 5-criteria rule: MW below 500 Daltons; high lipophilicity (expressed as LogP less than 5); no more than 5 HBD; no more than 10 HBA1; and MR from 40 to 130 [25].

Table 3. The result of Lipinski's rule of five

No.	Name	Lipinski's rule of five					Drug-likeness
		MW	HBD	HBA1	LogP	MR	
1	Acacetin	284	2	5	2.722599	75.701080	Yes
2	Apigenin	270	3	5	2.419599	70.813881	Yes
3	Isocrenatoside	622	8	15	-0.479400	144.998444	No
4	3''-O-methylcrenatoside	636	7	15	-0.176400	149.885666	No

Among the above compounds. there are 2 out of 4 compounds that satisfy at least 2 criteria, namely acacetin and apigenin. Next, these compounds were further evaluated for their pharmacokinetic-toxicological properties including absorption, distribution, metabolism, excretion, and toxicity by predicting ADMET through pkCSM.

2.4. Prediction of absorption, distribution, metabolism, excretion, and toxicity (ADMET) profile

Table 4. Pharmacokinetic and toxicological prediction results.

Properties	Acacetin	Apigenin
Absorption		
Water solubility (log mol/l)	-3.284	-3.329
CaCO2 permeability (log Papp in 10^{-6} cm/s)	1.137	1.007
Intestinal absorption (human) (%)	94.318	93.25
Distribution		
VDss (human) (log L/kg)	0.346	0.822
BBB permeability (log BB)	-0.196	-0.734
Metabolism		
CYP2D6 substrate	No	No
CYP3A4 substrate	Yes	No
CYP2D6 inhibitor	No	No
CYP3A4 inhibitor	Yes	No
Excretion		
Total clearance (log ml/min/kg)	0.663	0.566
Toxicity		
AMES toxicity	No	No
Hepatotoxicity	No	No
Skin sensitisation	No	No

First. the absorption capacity of a substance is evaluated based on the following parameters: water solubility, Caco2 membrane permeability, and intestinal absorption. A substance is said to have good permeability when the Caco2 membrane permeability (log Papp in 10^{-6} cm/s) is greater than 0.9. The results from *Table 4* show that both compounds have values greater than 0.9, demonstrating good membrane permeability. Besides, the absorption capacity in the human intestinal absorption (HIA) of these compounds have high results, all over 90%.

Second, log BB values greater than 0.3 are thought to have good absorption across the blood-brain barrier. The results show that all 2 compounds give values less than 0.3. Therefore, they are not able to penetrate the blood-brain barrier.

In terms of metabolism, the cytochrome P450 system is an important enzyme system in drug metabolism in the liver with two important CYPs, CYP3A4 and CYP2D6. These are the two main isoforms of the CYP family involved in the metabolism of most drugs, especially CYP3A4. Here, acacetin is substrate of CYP3A4, also the CYP3A4 inhibitor. While apigenin is not a substrate of CYP3A4, so it is not metabolized in the liver.

In terms of elimination and toxicity, all compounds are capable of renal elimination. Additionally, according to the ADMET prediction results, all two compounds exhibit favorable predictive properties, including no DNA mutations, no liver toxicity, and no potential for skin irritation. Thus, these are two potential compounds in inhibiting XO enzymes due to their low binding energies, drug-like properties, and strongly predictive ADMET profiles.

3. DISCUSSION

In this study, we evaluated the ability to inhibit 3NVY protein of 78 compounds in *Pogostemon cablin* (Blanco) Benth. As a result, we obtained two compounds, acacetin and apigenin, which showed the ability to inhibit higher regimen than two positive controls, with drug-like properties and favorable pharmacokinetic-toxicological parameters.

Acacetin is a flavonoid compound found in *P. cablin*, also known as patchouli [26]. Many potential therapeutic effects of this active constituent has been studied including anti-inflammatory, anti-cancer, anti-

microbial and anti-obesity [27, 28]. Our data revealed that acacetin may inhibit XO enzyme with docking scores of -8.8 kCal/mol, indicating its potential as a natural XO inhibitor for treating gout and other hyperuricemia disorders. Recently, Heung Joo Yuk et al., showed *in vitro* that acacetin displayed greater inhibitory potency than allopurinol, a commonly used XO inhibitor, with an IC_{50} value of 0.58 µM compared to allopurinol's IC_{50} value of 4.2 µM [29]. Another study by Mai Thanh Thi Nguyen et al., also showed that Acacetin inhibit XO with IC_{50} values of 0.16 µM with competitive inhibition type [30].

Apigenin is a flavonoid that has also been identified in *P.cablin* [31]. It is a natural compound with a variety of potential health benefits, including antioxidant, anti-inflammatory, and anti-cancer properties [32-35]. This compound has also been studied for its potential role in the prevention and treatment of several diseases, including cardiovascular disease, neurodegenerative disorders, and metabolic syndrome [36, 37]. Apigenin give the binding energy of -8.8 kCal/mol, also share many other similar interactions with XO enzyme as Febuxostat. Jingqun Huang showed that the apigenin do not exhibited the XO inhibitory activity *in vitro* but significantly decreases in XO level *in vivo* [2] However. Li-Na Huo et al., revealed that apigenin has XO inhibitory activity with IC_{50} values of 0.44 with mixed inhibition type [38].Therefore, it needs to be confirmed by further studies.

4. CONCLUSION

In this study, we found that acacetin and apigenin are the two most potential XO inhibitors from 78 compounds of *Pogostemon cablin (Blanco)* Benth. These compounds simultaneously showed the most negative binding energy with XO enzyme, met the Lipinski's rule of five, and had suitable pharmacokinetic parameters and low toxicity. Therefore, more *in vitro* and *in vivo* research on XO enzyme inhibitory ability necessary to develop these potential compounds as hyperuricemia medication in the future.

5. MATERIALS AND METHODS

5.1. Model docking

Protein structure preparation: Based on previous publications, the 3D structures of the XO enzyme (PDB ID: 3NVY) was chosen and retrieved from the Protein Data Bank RCSB (https://www.rcsb.org/) [19, 39, 40]. Preparing protein for docking, we removed water molecules and co-crystallized ligands - QUE (3,5,7,3',4'-pentahydroxylflavone) by using Discovery Studio Visualizer 2021 software. Subsequently, hydrogen atoms were added, polar hydrogens were optimized, and Kollman charges were assigned using MGL Autodock Tools 1.5.6 software. The active site of Xanthine Oxidase was defined using grid boxes of size 40Å×40Å×40Å, with the grid center coordinates (x,y,z) set at (39.07, 21.90, 20.22). Finally, the processed protein structure was saved in PDBQT format in preparation for the docking program.

Ligands preparations: We collected 78 natural compounds from the plant *Pogostemon cablin* (Blanco) Benth based on previous publications [11, 15-17]. The ligand structures of these molecules and positive control (allopurinol, febuxostat) were retrieved in SDF format from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Subsequently, we converted them to PDB format using Chimera software. [41]. The optimization of the ligands was carried out utilizing Avogadro software, employing the MMFF94 force field and Conjugate Gradients method. Finally, the ligands were converted to PDBQT format using Autodock Tools 1.5.6. [42, 43].

Molecular docking study: The docking of ligands to the active site of Xanthine Oxidase (XO) targets was performed using Autodock Vina software [44]. The ligand-protein interaction energy is evaluated by the *scoring function* of Autodock vina. Molecular interactions between ligands with favorable free binding energies and molecular targets were visualized using Discovery Studio Visualizer 2021 software to analyze the results.

5.2. Evaluation of docking results

To evaluate the docking results, the co-crystalized ligand after being separated from the protein was redocked to the binding site of the target. The process was performed successfully if the root-mean-square deviation (RMSD) value was less than or equal to 1,5 Å. For molecules requiring docking, their binding potential was evaluated based on their interaction with amino acids, and their interaction energy was computed using Autodock Vina's scoring function.

5.3. Evaluation of Lipinski's rule of five

To evaluate the likelihood of a molecule to become a therapeutic drug, Lipinski's rule of five was utilized, which distinguishes between drug-like and non-drug-like molecules [25]. We utilized an online tool (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp) to apply the following criteria: molecular weight (MW), high lipophilicity (LogP), hydrogen bond donors (HBD), hydrogen bond acceptors (HBA1), and molar refractivity (MR) [45]. The chemical structures were obtained from the PubChem database in SDF format and were set at pH 7.0. Subsequently, we analyzed the pharmacokinetic and toxicological parameters of the drug-like compounds to obtain the final outcomes.

5.4. Evaluation of Lipinski's rule prediction of ADMET by computational analysis

To forecast the pharmacokinetic and toxicological characteristics of the compounds, we utilized pkCSM (http://biosig.unimelb.edu.au/pkcsm/prediction) and entered SMILES formulas retrieved from PubChem as input data [46]. The predictive outcomes of pharmacokinetic-toxicological (ADMET) parameters, such as absorption, distribution, metabolism, elimination, and toxicity, for promising compounds were evaluated.

Acknowledgements: This study was funded by University of Medicine and Pharmacy, Vietnam National University Hanoi, with grant number CS.22.01

Author contributions: Concept – B.T.T.; Design – B.T.T., T.P.T., N.T.H., T.M.P.; Supervision – B.T.T.; Resources – T.P.T., N.T.H., T.M.P., N.T.T.; Literature Search – T.P.T., N.T.H., T.M.P., D.T.H.K., N.T.T; Writing – T.P.T., N.T.H., T.M.P., N.T.T.; Critical Reviews – B.T.T;

Conflict of interest statement: The authors declared no conflict of interest

REFERENCES

[1] Afinogenova Y, Danve A, Neogi T. Update on gout management: what is old and what is new. Current opinion in rheumatology. 2022 Mar 1; 34(2): 118-24. https://doi.org/10.1097/BOR.0000000000000861

[2] Huang J, Wang S, Zhu M, Chen J, Zhu X. Effects of genistein, apigenin, quercetin, rutin and astilbin on serum uric acid levels and xanthine oxidase activities in normal and hyperuricemic mice. Food Chem Toxicol. 2011 Sep; 49(9): 1943-7. https://doi.org/10.1016/j.fct.2011.04.029

[3] de Oliveira EP, Burini RCJD, syndrome m. High plasma uric acid concentration: causes and consequences. Diabetol Metab Syndr. 2012; 4(1): 1-7. https://doi.org/10.1186/1758-5996-4-12

[4] Kumar R, Darpan, Sharma S, Singh R. Xanthine oxidase inhibitors: a patent survey. Expert Opin Ther Pat. 2011 Jul; 21(7): 1071-108. https://doi.org/10.1517/13543776.2011.577417

[5] Ajala OS, Ayeleso AO, Owolabi M, Akinleye MO, Ukpo G. Xanthine oxidase inhibitory potentials of flavonoid aglycones of Tribulus terrestris: in vivo, in silico and in vitro studies. Futur J Pharm Sci 2022; 8(1): 1-15. https://doi.org/10.1186/s43094-022-00448-y

[6] Doehner W, Landmesser U. Xanthine oxidase and uric acid in cardiovascular disease: clinical impact and therapeutic options. Seminars in nephrology. 2011 Sep; 31(5): 433-40. https://doi.org/10.1016/j.semnephrol.2011.08.007

[7] Ghallab DS, Shawky E, Metwally AM, Celik I, Ibrahim RS, Mohyeldin MMJR. Integrated in silico-in vitro strategy for the discovery of potential xanthine oxidase inhibitors from Egyptian propolis and their synergistic effect with allopurinol and febuxostat. RSC Adv. 2022; 12(5): 2843-72. https://doi.org/10.1039/D1RA08011C

[8] Dechayont B, Ruamdee P, Poonnaimuang S, Mokmued K, Chunthorng-Orn J. Antioxidant and Antimicrobial Activities of Pogostemon cablin (Blanco) Benth. Journal of Botany. 2017. https://doi.org/10.1155/2017/8310275

[9] Junren C, Xiaofang X, Mengting L, Qiuyun X, Gangmin L, Huiqiong Z, Guanru C, Xin X, Yanpeng Y, Fu P, Cheng P. Pharmacological activities and mechanisms of action of Pogostemon cablin Benth: a review. Chinese medicine. 2021; 16: 1-20. https://doi.org/10.1186/s13020-020-00413-y

[10] Peng X, Ang S, Zhang Y, Fan F, Wu M, Liang P, Wen Y, Gan L, Zhang K, Li D, Yue J. Chemical Constituents With Antiproliferative Activity From Pogostemon cablin (Blanco) Benth. Front Chem 2022; 10. https://doi.org/10.3389/fchem.2022.938851

[11] Swamy MK, Sinniah UR. A Comprehensive Review on the Phytochemical Constituents and Pharmacological Activities of Pogostemon cablin Benth.: An Aromatic Medicinal Plant of Industrial Importance. Molecules. 2015 May 12; 20(5): 8521-47. https://doi.org/10.3390/molecules20058521

[12] Liu F, Deng C, Cao W, Zeng G, Deng X, Zhou Y. Phytochemicals of Pogostemon cablin (Blanco) Benth. aqueous extract: Their xanthine oxidase inhibitory activities. Biomed Pharmacother. 2017; 89: 544-8. https://doi.org/10.1016/j.biopha.2017.01.040

[13] Mehmood A, Zhao L, Wang C, Nadeem M, Raza A, Ali N, Shah AA. Management of hyperuricemia through dietary polyphenols as a natural medicament: A comprehensive review. Critical Reviews in Food Science and Nutrition. 2019; 59(9): 1433-55. https://doi.org/10.1080/10408398.2017.1412939

[14] Gohlke H, Hendlich M, Klebe G. Knowledge-based scoring function to predict protein-ligand interactions. J Mol Biol. 2000; 295(2): 337-56. https://doi.org/10.1006/jmbi.1999.3371

[15] Wu J, Lu X, Tang W, Kong H, Zhou S, Xu G. Application of comprehensive two-dimensional gas chromatography-time-of-flight mass spectrometry in the analysis of volatile oil of traditional Chinese medicines. Journal of chromatography A. 2004 Apr 23; 1034(1-2): 199-205. https://doi.org/10.1016/j.chroma.2004.02.028

[16] Wang D, Yin Z, Zhang Q, Ye W, Zhang X, Zhang J. [Nonvolatile chemical constituents from Pogostemon cablin]. Zhongguo Zhong yao za zhi = Zhongguo zhongyao zazhi = China journal of Chinese materia medica. 2010 Oct; 35(20): 2704-7.

[17] Li K, Zhang H, Xie H, Liang Y, Wang X, Ito Y. Preparative Isolation and Purification of Five Flavonoids from Pogostemon Cablin Benth by High-Speed Countercurrent Chromatography and Preparative High-Performance Liquid Chromatography. Journal of liquid chromatography & related technologies. 2011 Jan 1; 34(15): 1617-29. https://doi.org/10.1080/10826076.2011.580486

[18] Lin S, Zhang G, Liao Y, Pan J, Gong D. Dietary Flavonoids as Xanthine Oxidase Inhibitors: Structure-Affinity and Structure-Activity Relationships. J Agric Food Chem. 2015 Sep 9; 63(35): 7784-94. https://doi.org/10.1021/acs.jafc.5b03386

[19] Cao H, Pauff JM, Hille R. X-ray crystal structure of a xanthine oxidase complex with the flavonoid inhibitor quercetin. Journal of natural products. 2014 Jul 25; 77(7): 1693-9. https://doi.org/10.1021/np500320g

[20] Grewal HK, Martinez JR, Espinoza LR. Febuxostat: drug review and update. Expert opinion on drug metabolism & toxicology. 2014 May; 10(5): 747-58. https://doi.org/10.1517/17425255.2014.904285

[21] Qurie A, Preuss CV, Musa R. Allopurinol. StatPearls. Treasure Island (FL) 2022.

[22] Bardin T, Richette P. The role of febuxostat in gout. Current opinion in rheumatology. 2019 Mar; 31(2): 152-8. https://doi.org/10.1097/BOR.0000000000000573

[23] Schumacher HR Jr. Febuxostat: a non-purine, selective inhibitor of xanthine oxidase for the management of hyperuricaemia in patients with gout. Expert opinion on investigational drugs. 2005 Jul; 14(7): 893-903. https://doi.org/10.1517/13543784.14.7.893

[24] Waller DG, Sampson AP. 31 - Hyperuricaemia and gout. In: Waller DG, Sampson AP, editors. Medical Pharmacology and Therapeutics (Fifth Edition): Elsevier; 2018. p. 385-9. https://doi.org/10.1016/B978-0-7020-7167-6.00031-2

[25] Lipinski CA. Lead-and drug-like compounds: the rule-of-five revolution. Drug discovery today: Technologies. 2004; 1(4): 337-41. https://doi.org/10.1016/j.ddtec.2004.11.007

[26] Tariq A, Mussarat S, Adnan M, AbdAllah EF, Hashem A, Alqarawi AA, Ullah R. Ethnomedicinal evaluation of medicinal plants used against gastrointestinal complaints. BioMed research international. 2015; 2015. https://doi.org/10.1155/2015/892947

[27] Singh S, Gupta P, Meena A, Luqman S. Acacetin, a flavone with diverse therapeutic potential in cancer, inflammation, infections and other metabolic disorders. Food Chem Toxicol. 2020 Nov; 145: 111708. https://doi.org/10.1016/j.fct.2020.111708

[28] Li S, Lv Q, Sun X, Tang T, Deng X, Yin Y, Li L. Acacetin inhibits Streptococcus pneumoniae virulence by targeting pneumolysin. The Journal of pharmacy and pharmacology. 2020 Aug; 72(8): 1092-100. https://doi.org/10.1111/jphp.13279

[29] Yuk HJ, Ryu HW, Kim DS. Potent Xanthine Oxidase Inhibitory Activity of Constituents of Agastache rugosa (Fisch. and C.A.Mey.) Kuntze. Foods. 2023 Jan 28; 12(3). https://doi.org/10.3390/foods12030573

[30] Nguyen MTT, Awale S, Tezuka Y, Ueda JY, Tran QL, Kadota S. Xanthine oxidase inhibitors from the flowers of Chrysanthemum sinense. Planta medica. 2006 Jan; 72(1): 46-51. https://doi.org/10.1055/s-2005-873181

[31] Li P, Yin ZQ, Li SL, Huang XJ, Ye WC, Zhang QW. Simultaneous determination of eight flavonoids and pogostone in Pogostemon cablin by high performance liquid chromatography. J Liq Chromatogr Relat Technol. 2014; 37(12): 1771-84. https://doi.org/10.1080/10826076.2013.809545

[32] Salehi B, Venditti A, Sharifi-Rad M, Kregiel D, Sharifi-Rad J, Durazzo A, Lucarini M, Santini A, Souto EB, Novellino E, Antolak H, Azzini E, Setzer WN, Martins N. The Therapeutic Potential of Apigenin. Int J Mol Sci. 2019 Mar 15; 20(6). https://doi.org/10.3390/ijms20061305

[33] Yoon JH, Kim MY, Cho JY. Apigenin: A Therapeutic Agent for Treatment of Skin Inflammatory Diseases and Cancer. Int J Mol Sci. 2023 Jan 12; 24(2). https://doi.org/10.3390/ijms24021498

[34] Imran M, Aslam Gondal T, Atif M, Shahbaz M, Batool Qaisarani T, Hanif Mughal M, Salehi B, Martorell M, Sharifi-Rad J. Apigenin as an anticancer agent. Phytotherapy research : PTR. 2020 Aug; 34(8): 1812-28. https://doi.org/10.1002/ptr.6647

[35] Li BS, Zhu RZ, Lim SH, Seo JH, Choi BM. Apigenin Alleviates Oxidative Stress-Induced Cellular Senescence via Modulation of the SIRT1-NAD[Formula: see text]-CD38 Axis. The American journal of Chinese medicine. 2021; 49(5): 1235-50. https://doi.org/10.1142/S0192415X21500592

[36] Middleton E, Kandaswami C, Theoharides TC. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacological reviews. 2000; 52(4): 673-751.

[37] Nabavi SF, Khan H, D'Onofrio G, Samec D, Shirooie S, Dehpour AR, Argüelles ES, Habtemariam S, Sobarzo-Sanchez E. Apigenin as neuroprotective agent: Of mice and men. Pharmacol Res. 2018 Feb; 128: 359-65. https://doi.org/10.1016/j.phrs.2017.10.008

[38] Huo LN, Wang W, Zhang CY, Shi HB, Liu Y, Liu XH, Guo BH, Zhao DM, Gao H. Bioassay-Guided Isolation and Identification of Xanthine Oxidase Inhibitory Constituents from the Leaves of Perilla frutescens. Molecules. 2015 Sep 25; 20(10): 17848-59. https://doi.org/10.3390/molecules201017848

[39] Yu Z, Cao Y, Kan R, Ji H, Zhao W, Wu S, Liu J, Shiuan D. Identification of egg protein-derived peptides as xanthine oxidase inhibitors: Virtual hydrolysis, molecular docking, and in vitro activity evaluation. Food Sci Hum Wellness. 2022; 11(6): 1591-7. https://doi.org/10.1016/j.fshw.2022.06.017

[40] Tan A. Novel 1, 2, 3-triazole compounds: Synthesis, In vitro xanthine oxidase inhibitory activity, and molecular docking studies. J Mol Struct. 2020; 1211: 128060. https://doi.org/10.1016/j.molstruc.2020.128060

[41] Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin T. UCSF Chimera-a visualization system for exploratory research and analysis. Journal of computational chemistry. 2004; 25(13): 1605-12. https://doi.org/10.1002/jcc.20084

[42] Hanwell MD, Curtis DE, Lonie DC, Vandermeersch T, Zurek E, Hutchison GR. Avogadro: an advanced semantic chemical editor, visualization, and analysis platform. J Cheminformatics. 2012; 4(1): 1-17. https://doi.org/10.1186/1758-2946-4-17

[43] Morris GM, Huey R, Lindstrom W, Sanner MF, Belew RK, Goodsell DS, Olson AJ. AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. Journal of computational chemistry. 2009; 30(16): 2785-91. https://doi.org/10.1002/jcc.21256

[44] Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. Journal of computational chemistry. 2010; 31(2): 455-61. https://doi.org/10.1002/jcc.21334

[45] Jayaram B, Singh T, Mukherjee G, Mathur A, Shekhar S, Shekhar V, editors. Sanjeevini: a freely accessible web-server for target directed lead molecule discovery. BMC bioinformatics; 2012: Springer. https://doi.org/10.1186/1471-2105-13-S17-S7

[46] Pires DEV, Blundell TL, Ascher DB. pkCSM: predicting small-molecule pharmacokinetic and toxicity properties using graph-based signatures. Journal of medicinal chemistry. 2015; 58(9): 4066-72. https://doi.org/10.1021/acs.jmedchem.5b00104