

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

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**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<sub>2</sub>O<sub>2</sub> and O<sub>2</sub><sup>-</sup>, 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.

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## 2. RESULTS

### 2.1. Evaluation of the docking model

Before screening compounds, co-crystallized 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.

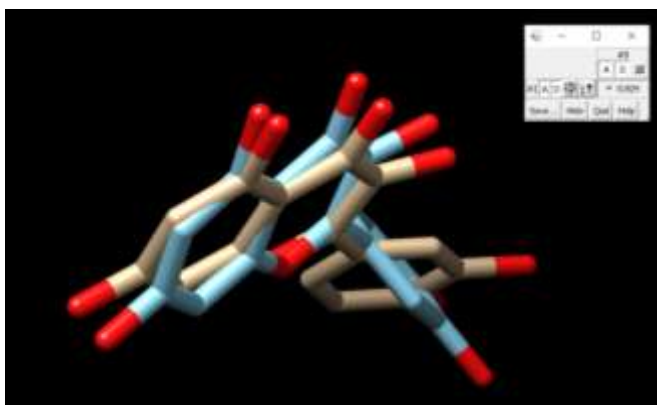


Figure 1. Co-crystallized ligand redock results of 3NVY

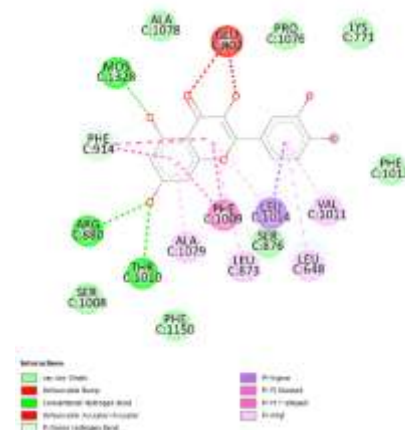
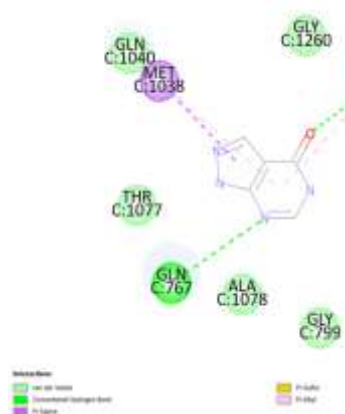


Figure 2. Interaction between QUE and 3NVY

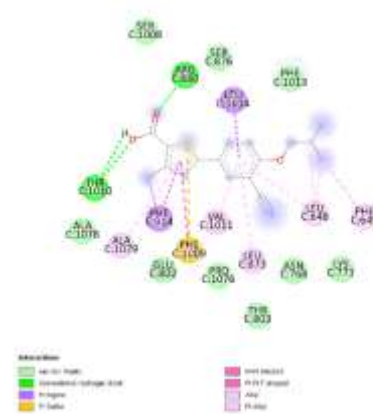
### 2.2. Molecular docking results of compounds to the target protein

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.



(R1)



(R2)

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

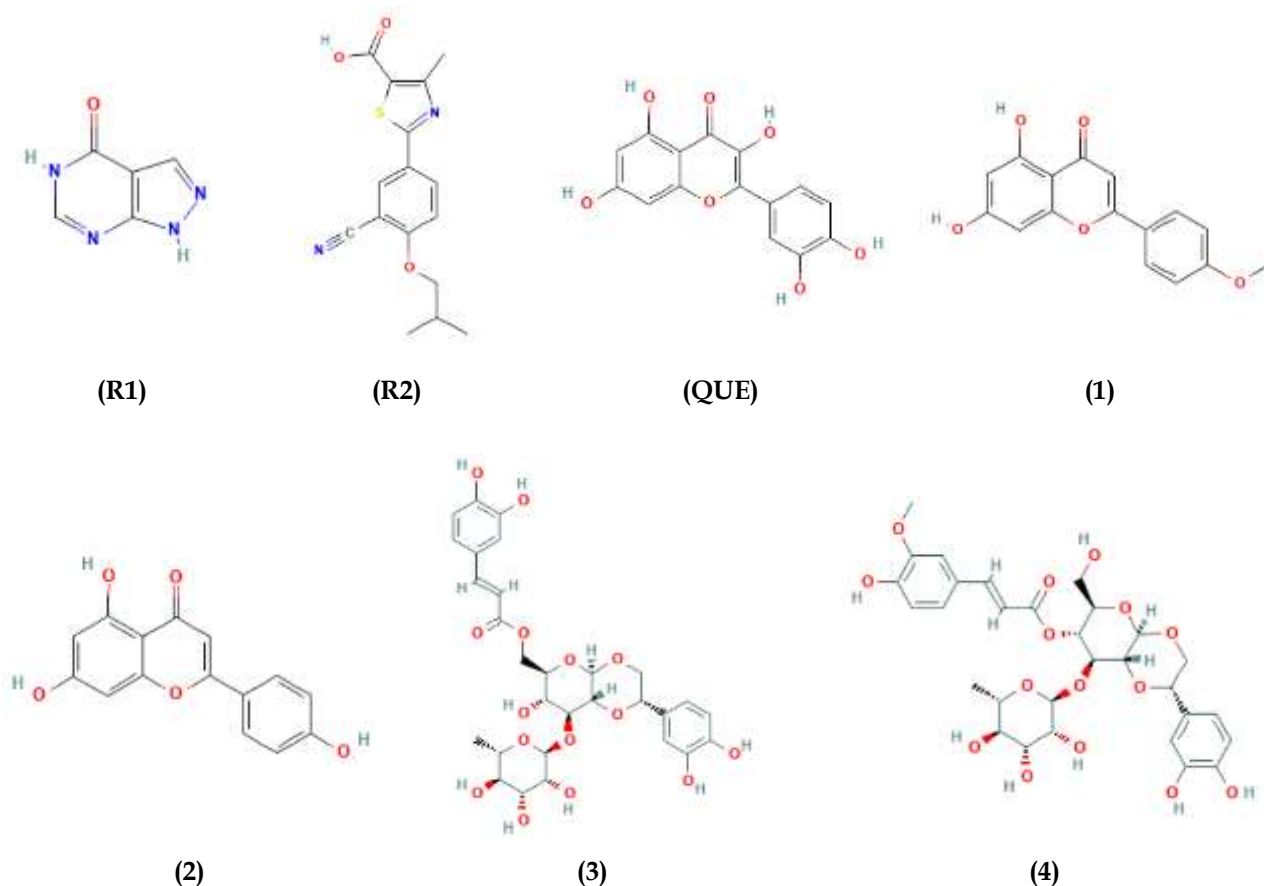
Allopurinol and febuxostat have a binding energy (BDE) of -6.1 (kCal/mol) and -8.5 (kCal/mol) respectively to the XO target. The binding energy results of 78 natural compounds are shown in Table 1. Figure 3 shows the interaction of Allopurinol and Febuxostat with the XO enzyme.

**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	$\beta$ -Bourbonene	62566	-6.5	42	$\alpha$ -Patchoulene	521710	-5.8
4	$\alpha$ -Bulnesene	94275	-7.4	43	$\beta$ -Patchoulene	101731	-6.3
5	$\beta$ -Bulnesene	12302131	-7.2	44	$\beta$ -Phellandrene	11142	-5.9
6	(+)-Camphene	92221	-5.7	45	$\alpha$ -Pinene	6654	-5.6
7	(-)-Camphor	444294	-5.9	46	$\beta$ -Pinene	14896	-5.5
8	$\alpha$ -Caryophyllene	5281520	-6.8	47	Pogostol	532065	-7.5
9	$\beta$ -Caryophyllene	5281522	-7.2	48	Pogostone	54695756	-7.3
10	trans-Caryophyllene	5281515	-6.4	49	$\alpha$ -Selinene	10856614	-6.9
11	Caryophyllene oxide	1742210	-6.6	50	$\beta$ -Selinene	442393	-7.5
12	Copaene	12303902	-7.5	51	Seychellene	519743	-5.8
13	$\beta$ -Cubebene	93081	-7.1	52	Spathulenol	92231	-7.0
14	$\beta$ -Copaen-4- $\alpha$ -ol	91751357	-8.5	53	(-)- $\alpha$ -Terpineol	443162	-6.4
15	$\alpha$ -Elemene	80048	-6.3	54	Valencene	9855795	-7.0
16	$\beta$ -Elemene	6918391	-7.2	55	<b>Acacetin</b>	<b>5280442</b>	<b>-8.8</b>
17	$\gamma$ -Elemene	6432312	-6.9	56	<b>Apigenin</b>	<b>5280443</b>	<b>-8.8</b>
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	$\alpha$ -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	5 $\alpha$ -Stigmast-3,6-dione	13992092	-7.7
27	Germacrene-D	91723653	-7.4	66	Daucosterol	5742590	-8.0
28	$\alpha$ -Guaiene	5317844	-7.3	67	$\beta$ -Sitosterol	222284	-6.7
29	$\beta$ -Guaiene	15560252	-7.2	68	Stigmasterol	5280794	-7.5
30	$\gamma$ -Guaiene	15560256	-7.9	69	Acteoside	5281800	-7.9
31	$\gamma$ -Gurjunene	90805	-7.2	70	<b>Isocrenatoside</b>	<b>44559534</b>	<b>-8.7</b>
32	$\alpha$ -Gurjunene	15560276	-6.4	71	<b>3"-O-Methylcrenatoside</b>	<b>10326741</b>	<b>-8.8</b>
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  $\Delta G$  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)

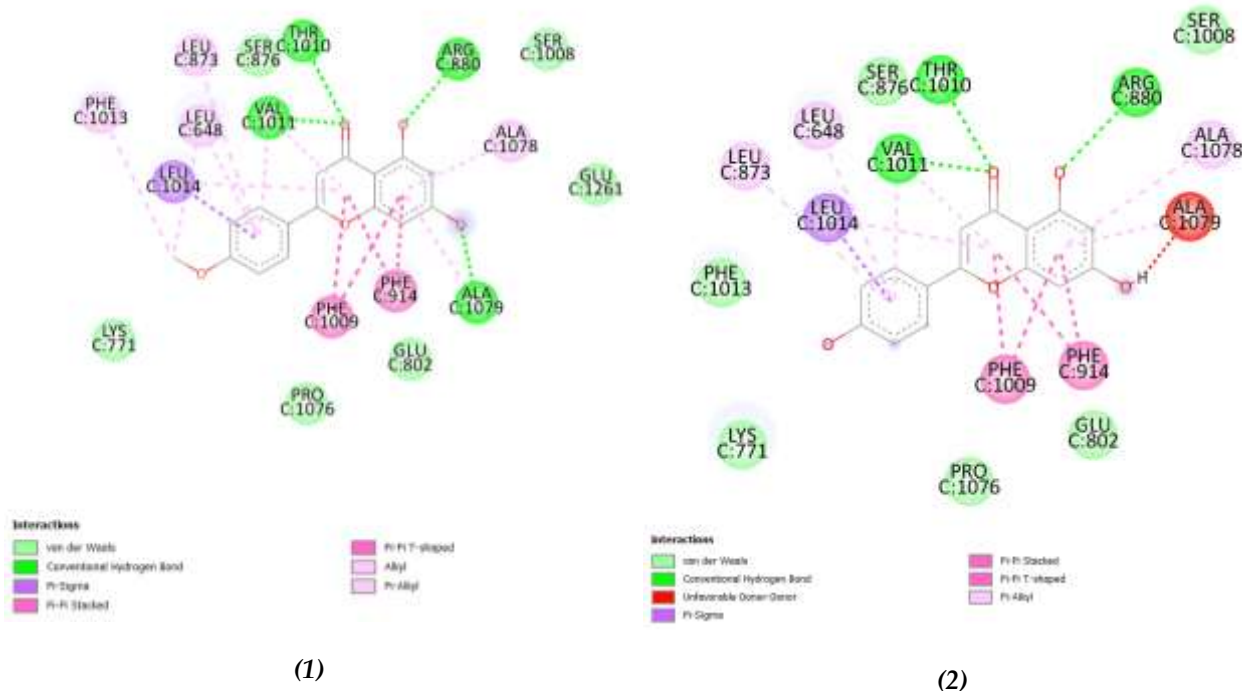
**Table 2.** Ligand-amino acid interactions of positive controls and 4 potential compounds against the XO enzyme.

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,

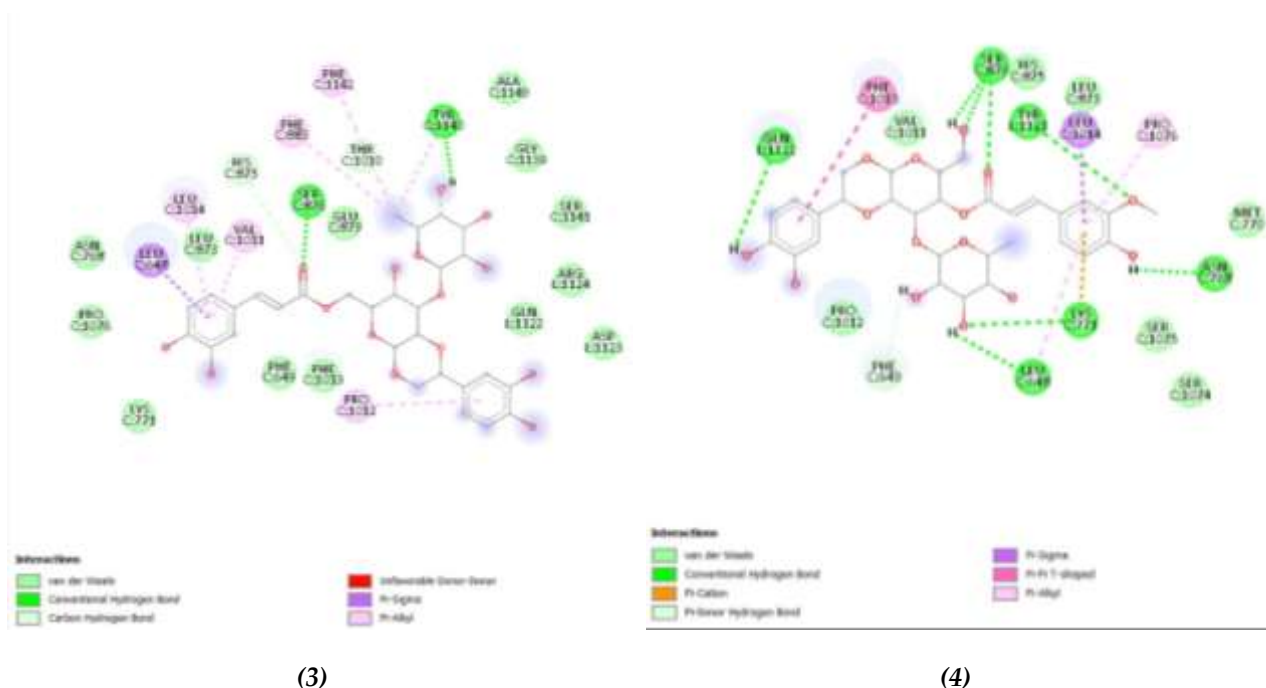
<b>R2</b>	Febuxostat	-8.5	LEU648, PHE649, LEU873, ARG880, PHE914, PHE1009, THR1010, VAL1011, LEU1014, ALA1079
<b>QUE</b>	Quercetin	-8.7	LEU648, LEU873, ARG880, SER876, GLU802, PHE914, PHE1009, THR1010, VAL1011, LEU1014, ALA1079, MOS1328

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  $\pi$ - $\sigma$  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  $\pi$ -alkyl bond. Similarity, the interaction of 3"-O-methylcrenatoside with XO is hydrogen bonds,  $\pi$ - $\pi$ ,  $\pi$ - $\sigma$  and  $\pi$ -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.







**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
<b>Absorption</b>		
Water solubility (log mol/l)	-3.284	-3.329
CaCO <sub>2</sub> permeability (log P <sub>app</sub> in 10 <sup>-6</sup> cm/s)	1.137	1.007
Intestinal absorption (human) (%)	94.318	93.25
<b>Distribution</b>		
VD <sub>ss</sub> (human) (log L/kg)	0.346	0.822
BBB permeability (log BB)	-0.196	-0.734
<b>Metabolism</b>		
CYP2D6 substrate	No	No
CYP3A4 substrate	Yes	No
CYP2D6 inhibitor	No	No
CYP3A4 inhibitor	Yes	No
<b>Excretion</b>		
Total clearance (log ml/min/kg)	0.663	0.566
<b>Toxicity</b>		
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 P<sub>app</sub> in 10<sup>-6</sup> 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<sub>50</sub> value of 0.58 µM compared to allopurinol's IC<sub>50</sub> value of 4.2 µM [29]. Another study by Mai Thanh Thi Nguyen et al., also showed that Acacetin inhibit XO with IC<sub>50</sub> 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<sub>50</sub> 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'-pentahydroxyflavone) 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-crystallized 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/pkcsml/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.

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

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