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An *in silico* Molecular Docking and ADME Analysis of Naturally Derived Biomolecules against Xanthine Oxidase: A Novel Lead for Antihyperuricemia Treatment

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Abstract: Xanthine oxidase (XO) is the significant target enzyme for treating hyperuricemia, gout, and other related illnesses. These clinical problems can be alleviated to some extent by inhibiting the function of xanthine oxidase. Molecules derived from nature can play a key role in this. This study used naturally derived compounds with anticancer action to investigate the binding affinity with XO. Naturally derived molecules retrieved from NPACT (Naturally occurring Plant-based Anticancerous Compound-Activity-Target) database. Molecular docking studies and ADME (Absorption, Distribution, Metabolism, and Excretion) were analyzed. The result of molecular docking studies showed that the selected naturally derived molecules have a better binding affinity with XOthan the standard drug allopurinol. Furthermore, all the selected molecules satisfy the ADME descriptors and have no violation of Lipinski's rule of five. Based on these findings, 18 compounds were chosen for further research. This research will aid in the search for new xanthine oxidase (XO) inhibitor alternatives. Detailed successful *in vitro* and *in vivo* studies are needed to propose new drug molecules for treating hyperuricemia and its associated diseases.

Keywords: ADME; docking studies; hyperuricemia; virtual screening; xanthine oxidase.

1. Introduction

In recent decades, the prevalence of hyperuricemia has increased [1]. Hyperuricemia is a metabolic disorder that raises the risk of major illnesses such as gout, diabetes, cardiovascular problems, hypertension, and renal failure [2,3]. It is one of the hallmarks of tumor lysis syndromes is hyperuricemia [4]. Hyperuricemia is caused by a lack of uric acid excretion or overproduction [5,6]. The primary enzyme in this metabolic cascade is xanthine oxidase, which produces uric acid as a byproduct of purine catabolism (XO). The oxidative reaction that transforms hypoxanthine to xanthine and eventually to uric acid is catalyzed by XO [7]. One of the most effective treatment strategies for hyperuricemia is to lower the level of uric acid in the body fluid. Therefore, XO is considered an important therapeutic target for treating hyperuricemia and its associated diseases, as it can be achieved by inhibiting the function of the XO [8,9]. Xanthine oxidase is a homodimer metallo-flavoprotein with a molecular weight of 290 kDa [10]. Allopurinol is an XO-inhibiting medication often used to treat hyperuricemia, and current research has shown that these synthetic medications can have substantial adverse

effects [11,12]. To address these issues, a novel medicine with fewer side effects and stronger anti-hyperuricemia activity is needed. Natural products have been proved in numerous investigations to have a wide range of capabilities, some of which are presently used as medications. The significance of naturally occurring compounds' anti-hyperuricemia capabilities must be investigated.

The scientific validation of the various capabilities of plant derivative molecules has been demonstrated through numerous studies but is still incomplete. The plant-derived molecule based *in silico* virtual screening is an efficient method currently used for drug development research. Using these methods, it is possible to find new lead molecules quickly at a moderate cost [13,14]. The current study examined these plant-derived molecules' ADME analysis and binding potential against XO. This work will help to propose new leads for further detailed *in vitro* and *in vivo* studies against the hyperuricemia condition.

2. Materials and Methods

2.1. Molecular Docking studies.

2.1.1. Ligand preparation.

A total of 1574 molecules were retrieved from NPACT (Naturally occurring Plant based Anticancerous Compound-Activity-Target) database, and the structure of the standard drug (allopurinol) was taken from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The Structural optimization of collected compounds and standard drugs was carried out with Lig prep module of the Schrodinger suit. All structures were minimized with the OPLS2005 force field and the ionization state generated by the Epik module at the physiological pH of 7.

2.1.2. Protein preparation and grid generation.

The crystal structure of the Xanthine oxidase (PDB 1FIQ) enzyme was retrieved from the protein data bank (PDB). The protein preparation wizard module of the Schrodinger suite was used for the protein preparation; the hydrogens were added to the polar atoms, water molecules were deleted, and the metals were treated properly. The prepared protein was minimized using the OPLS-2005 force field. Finally, the grid was set up at the well-known active site of the enzyme [15].

2.1.3. Molecular docking.

Molecular docking was performed with the glide dock module of the Schrodinger suite. The induced fit docking (IFD) of collected plant molecules and the standard drug with XO was carried out independently with the standard precision method. The best docking poses with the least glide g score were chosen for the atomic level of interaction studies. The Pymol software was used to visualize and interpret atomic-level interaction.

2.1.4. ADME analysis.

ADME analysis of molecules was performed using the Qik prop module of the Schrödinger suite. The descriptors include molecular weight, H bond acceptor, H bond donor, QPlogPo/w, QPlogS, QPPCaco, QPlogBB, PHOA, and Rule Of Five (RO5). The molecules which satisfy the recommended values were selected for further analysis [16,17].

3. Results and Discussion

Herbal medicines have been used globally for various health care needs since prehistoric times [18]. Numerous research has proved the scientific validity of plant-derived molecule's diverse capacities. However, they are yet incomplete. Traditional drug development research is an expensive and time-consuming process [19]. A novel drug molecule discovery is a complex sequential process, which includes target identification, finding of lead molecules, lead optimization, and preclinical *in vitro* and *in vivo* analysis [20,21]. As a result, computer-aided drug development research can help to save time, resources, and money. *In silico* pharmacology is a fast-expanding field that encompasses the development of tools for capturing, analyzing, and integrating biological and medical data from various sources using software. More precisely, it refers to the use of this data in developing computational models or simulations that can be used to generate predictions, propose hypotheses, and ultimately lead to medical and therapeutic breakthroughs [22]. Target-based virtual screening approaches rely on the presence of structural information about the target, which can be obtained either experimentally or computationally using homology modeling techniques. This approach has a proven track record of finding and producing new bioactive chemicals. These techniques can give a virtuous approximation of the protein cavity's ligand orientation and structural conformation. The binding affinity of bioactive compounds towards the xanthine oxidase can be estimated by using a binding score.

XO is accountable for uric acid production. Abnormal level of uric acid leads to hyperuricemia and associated diseases. Allopurinol is a XO inhibitory drug commonly used for anti-hyperuricemia treatment. Recent research over the past decade has shown that such synthetic drugs can have serious side effects [23, 24]. To alleviate such complications, a new drug with fewer side effects and higher anti-hyperuricemia activity is needed. Many studies have shown that natural products have a variety of capabilities, some of which are now used as medicines. The importance of the anti- hyperuricemia properties of naturally occurring substances needs to be studied. The virtual screening method is a time and cost-effective method to propose new leads. The current study screened better xanthine oxidase inhibitor leads from the 1574 herbal molecules in the NPACT database with anticancer activities. As mentioned earlier, the clinical condition of hyperuricemia is one of the aftereffects of chemotherapy treatments [25]. So, anti-hyperuricemia target-based studies on herbal molecules with anticancer activity are of great importance. Molecular docking studies are a significant method for evaluating protein-ligand interactions [26]. The induced fit docking studies were performed to identify the molecules with a better binding affinity towards the XO. Based on the induced fit docking analysis, 48 molecules with the lowest glide score than the standard drug allopurinol were screened out and taken for ADME analysis. About 60% of drugs fail to attain pharmacokinetic properties during drug development [27]. ADME analysis help in the earlier prediction of pharmacokinetic properties and would play a major role in reducing the economic burden in drug development research. ADME analysis analyses of the pharmacokinetic properties of selected molecules were carried out using the Schrödinger QikProp module.

The properties in ADME analysis included such as Molecular weight of the molecule (Range: 130.0 to 725.0), Number of hydrogen bond donors (Range: 0.0 to 6.0), and Number of hydrogen bond acceptors (Range: 2.0 to 20.0), Predicted octanol/water partition coefficient. (Range: -2.0 to 6.5), Predicted aqueous solubility (–6.5 – 0.5), Predicted apparent Caco-2 cell

permeability in nm/sec (<25 poor, >500 great), Predicted brain/blood partition coefficient (–3.0 – 1.2) Predicted human oral absorption on 0 to 100% scale (>80% is high <25% is poor), Number of violations of Lipinski's rule of five (maximum is 4), as per the rule the accepted range of properties were molecular weight < 500, QPlogPo/w < 5, donorHB ≤ 5, accptHB ≤ 10. The compounds which satisfy these rules are considered drug-likeness [16]. Of the 48 compounds, 18 satisfy the recommended range of ADME descriptors and have no violations of Lipinski's rule of five (Table 1). The details of 18 selected compounds showed in Table 1. (The detailed evaluation of the molecular level of interactions showed (Table 2) that the herbal molecules can bind with amino acid residues present in the enzyme's catalytic active site. The binding pose view of the selected molecules is depicted in Figure 1-Figure 3. The molecular level of interaction of standard drug allopurinol is represented in Figure 3C. The interaction studies of Curcumin [28], Gallic acid [29], Ellagic acid [30], Quercetin [31], Hispidulin [32], and Naringenin [33] with XO have been discussed in previous studies. Still, detailed studies are needed to understand the binding activity further. Interactions studies of other compounds with xanthine oxidase have not yet been reported. Based on the current study, more research could be done to develop new natural xanthine oxidase inhibitors that will greatly help treat hyperuricemia, gout, and other related diseases.

Table 1. ADME analysis of selected molecules. (**A**) The molecular weight of the molecule (Range: 130.0 to 725.0), (**B**) Number of hydrogen bond donors (Range: 0.0 to 6.0), (**C**) Number of hydrogen bond acceptors (Range: 2.0 to 20.0), (**D**) Predicted octanol/water partition coefficient. (Range: -2.0 to 6.5), (**E**) Predicted aqueous solubility (–6.5 – 0.5), (**F**) Predicted apparent Caco-2 cell permeability in nm/sec (<25 poor, >500 great), (**G**) Predicted brain/blood partition coefficient (–3.0 – 1.2) (**H**) Predicted human oral absorption on 0 to 100% scale (>80% is high <25% is poor), (**I**) Number of violations of Lipinski's rule of five (maximum is 4).

Compound ID	Mol wt (A)	DHB (B)	AHB (C)	QPlogP o/w (D)	QPlog S (E)	QPP Caco (F)	QPlogBB (G)	PHOA (H)	RO 5 (I)
Curcumin	368.385	2	4.75	3.743	-4.864	235.084	-2.061	91.3	0
D-malic acid	134.088	2	6.8	-1.881	-0.997	121.346	-1.689	53.233	0
Helichrysetin	286.284	2	4	1.523	-3.677	260.775	-1.914	79.111	0
Gallic acid	170.121	4	4.25	-0.569	-0.704	9.963	-1.667	41.486	0
Pinostilbene	242.274	2	2.25	1.843	-2.835	1225.467	-0.824	93.012	0
Ellagic acid	302.197	4	8	-1.338	-1.816	7.757	-2.333	35.033	0
2',4'-Dihydroxy dihydrochalcone	242.274	2	3.2	1.83	-3.97	1021.855	-0.738	91.525	0
Xenognosin	256.301	2	2.25	2.572	-4.142	1225.482	-0.808	100	0
Quercetin	302.24	4	5.25	0.388	-2.806	21.062	-2.31	52.907	0
Hesperetin	302.283	2	4.75	1.803	-3.795	132.068	-1.532	75.462	0
Hispidulin	300.267	3	5.75	-0.058	-1.785	320.588	-0.896	71.46	0
Kushenin	286.284	2	4.25	1.368	-2.917	1297.722	-0.547	90.673	0
8-hydroxynaringenin	288.256	4	5.45	-0.343	-1.524	149.509	-1.328	63.858	0
Harmol	198.224	2	3.5	0.853	-2.641	1551.939	-0.31	89.051	0
Canthin-6-one	220.23	0	3	1.008	-0.78	2656.594	0.116	94.135	0
Euxanthone	228.204	1	3	0.687	-0.976	880.545	-0.391	83.673	0
Eugenol	164.204	1	1.5	2.674	-2.417	3053.544	-0.131	100	0
Naringenin	272.257	3	4.7	0.348	-2.229	290.658	-1.151	73.071	0

Table 2. Details of molecular level interaction of selected herbal molecules with the XO.

Compound Name	H bond interaction residues	Other interaction residues	Glide g score Kcal/mol
Curcumin	Ser 876, Thr 1010	Phe 649, Phe 914, Phe 1009	-10.398
D-malic acid	Arg 880, Thr 1010, Val 1011 Ser 876	Arg 880	-9.130
Helichrysetin	Thr 1010, Arg 880, Lys 771	Phe 1009, Phe 914 and Phe 1013	-8.531
Gallic acid	Arg 880, Thr 1010, Ser 876	Phe 914 and Phe 1009	-8.442
Pinostilbene	Thr 1010 and Arg 880	Phe 914	-8.05
Ellagic acid	Ser 876, Glu 802, Asn 768, Lys 771	Phe 1013	-8.035

Compound Name	H bond interaction residues	Other interaction residues	Glide g score Kcal/mol
2',4'-Dihydroxy dihydrochalcone	Thr 1010, His 884, Ser 876	Glu 802, Mos 1334, Phe 914 and Phe 1009	-7.856
Xenognosin	Thr 1010	Glu 802, Phe 914, Phe 1009	-7.609
Quercetin	Ser 876, Lys 771, Glu 802	Phe 1013	-7.456
Hesperetin	Arg 880, Thr 1010	Phe 914	-7.277
Hispidulin	Lys 771	Glu 802, Phe 649, Phe 914, Phe 1009, Phe 1013	-6.836
Kushenin	Lys 771 and Ser 876	Glu 802, Phe 1013	-6.720
8-hydroxynaringenin	Lys 771, Glu 802	Glu 802, Phe 1013, Phe 649	-6.522
Harmol	Glu 802, Mos 1334	Phe 914, Glu 802	-6.328
Canthin-6-one	Ser 876	Glu 802, Phe 1013, Phe 649	-6.313
Euxanthone	Ser 876	Glu 802, Phe 1013, Phe 649	-6.275
Eugenol	Thr 1010	Phe 1009, Phe 914	-6.079
Naringenin	Lys 771, Glu 802	Glu 802, Phe 649	-6.047
Allopurinol	Glu 802, Thr 1010,Arg 888	Arg 880, Phe 914, Phe 1009	-6.021

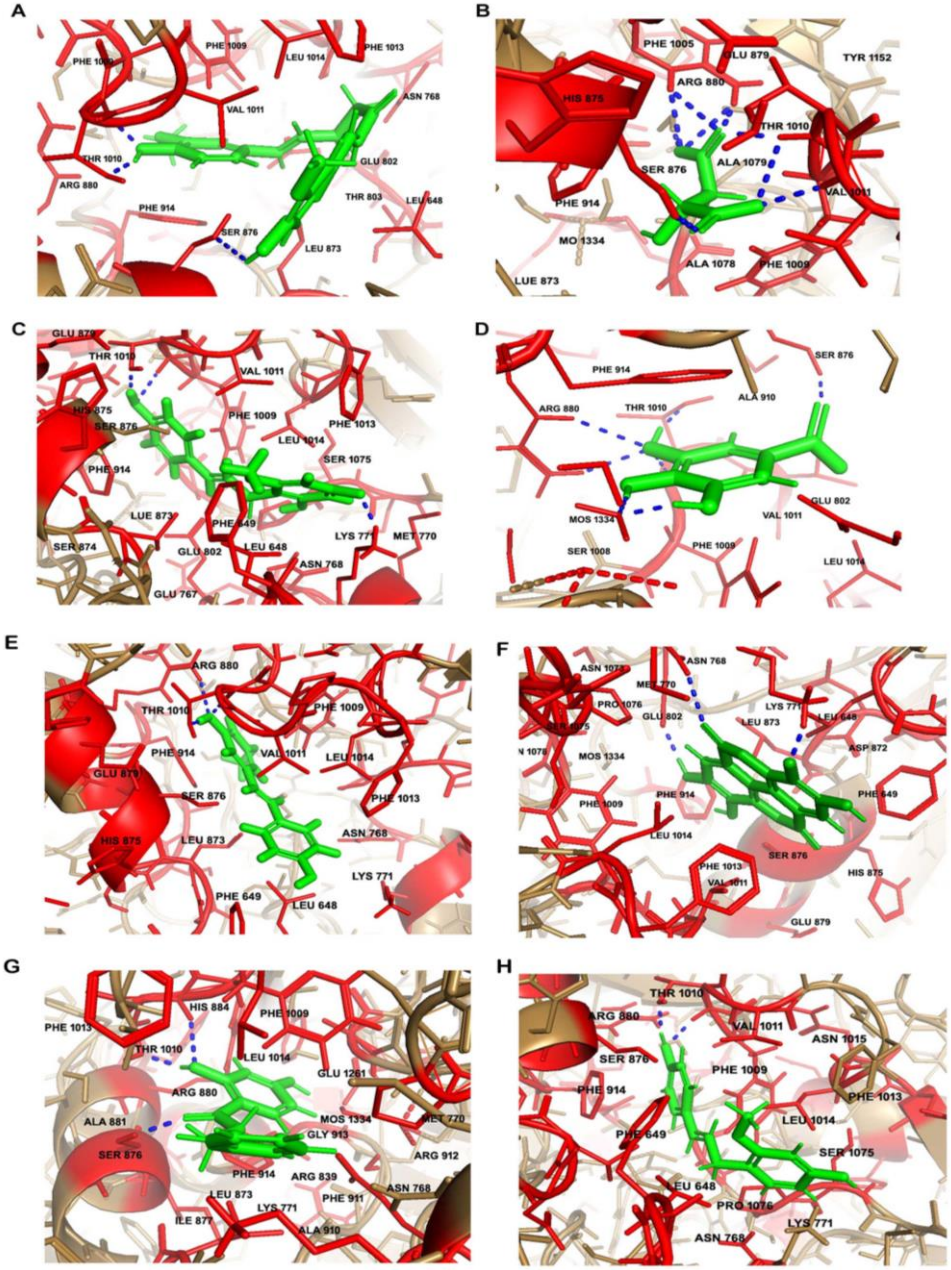

Figure 1. Binding pose view of different compounds with the active site of XO. (**A**) Curcumin; (**B**) D-malic acid; (**C**) Helichrysetin; (**D**) Gallic acid; (**E**) Pinostilbene; (**F**) Ellagic acid; (**G**) 2',4'-Dihydroxy dihydrochalcone; (**H**) Xenognosin. The green color denoted the ligand molecule, and the blue-colored dashes denoted the hydrogen bond interactions.

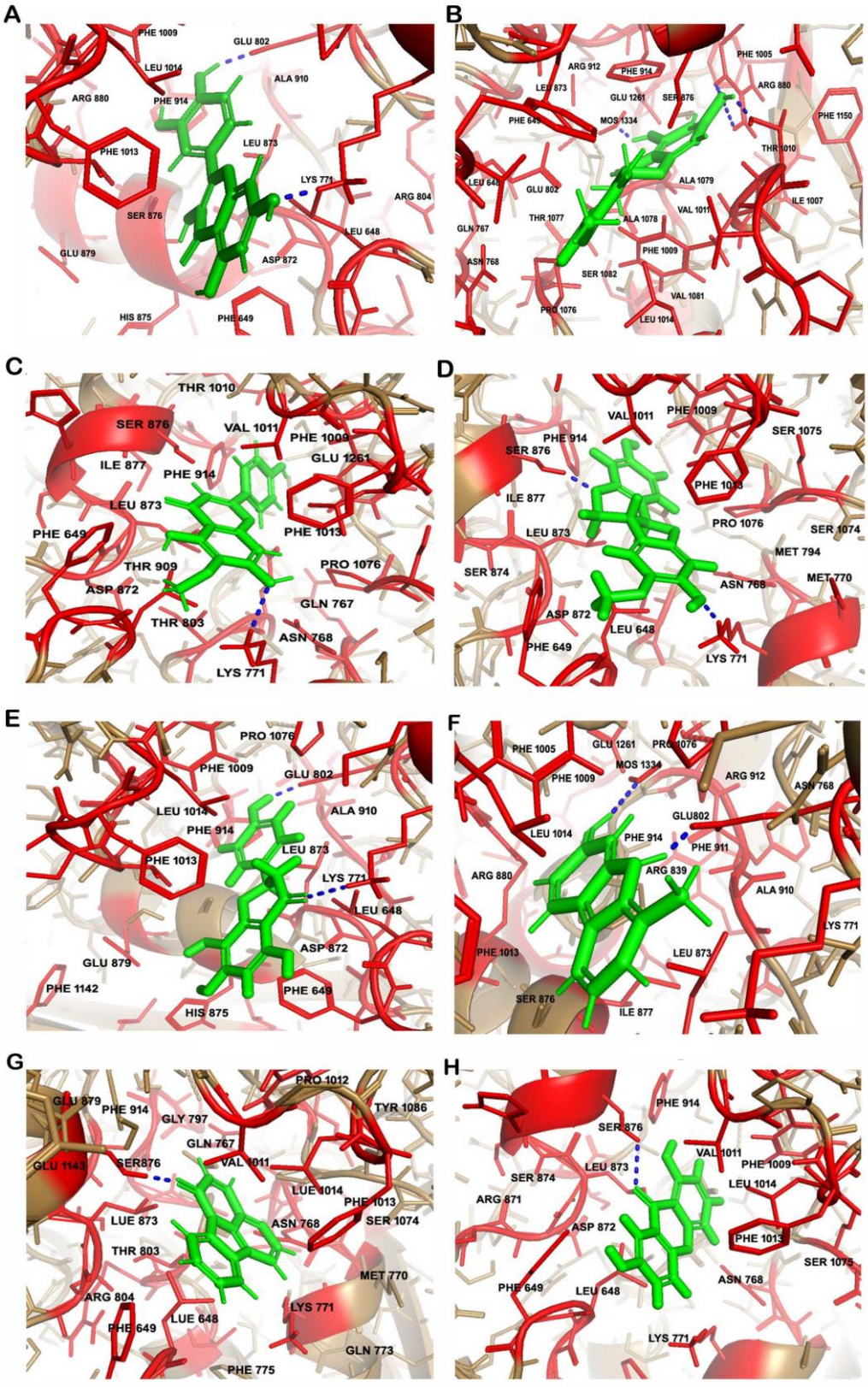

Figure 2. Binding pose view of different compounds with the active site of XO. (**A**) Quercetin; (**B**) Hesperetin; (**C**) Hispidulin; (**D**) Kushenin; (**E**) 8-hydroxynaringenin; (**F**) Harmol; (**G**) Canthin-6-one; (**H**) Euxanthone. The green color denoted the ligand molecule, and the blue-colored dashes denoted the hydrogen bond interactions.

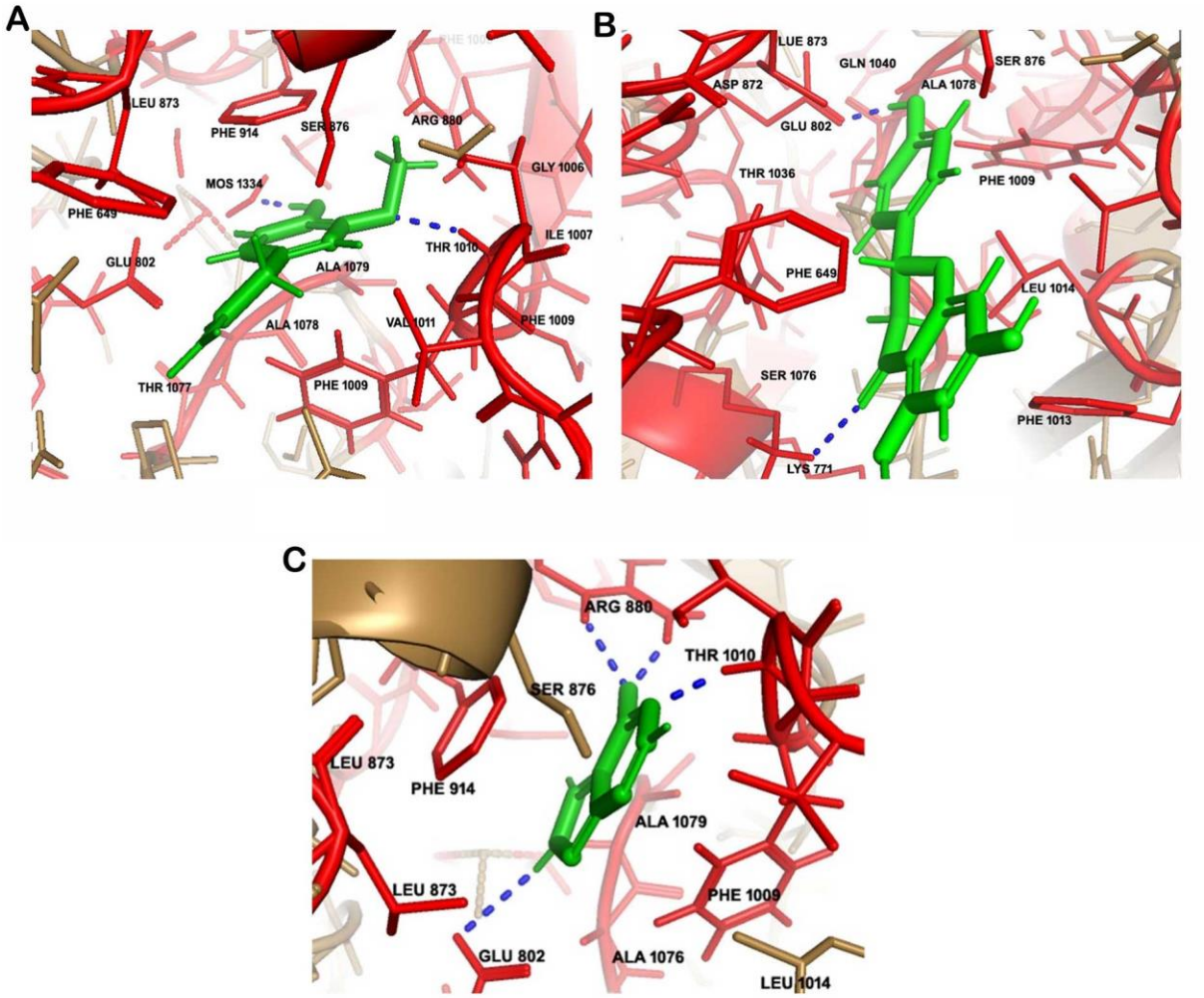

Figure 3. Binding pose view of different compounds with the active site of XO. (**A**) Eugenol; (**B**) Naringenin; (**C**) Allopurinol. The green color denoted the ligand molecule, and the blue-colored dashes denoted the hydrogen bond interactions.

4. Conclusions

Natural molecules can play a major role in drug development. Numerous studies have shown that such molecules have many advantages and few side effects. The current research was conducted by xanthine oxidase binding studies of natural molecules with anticancer activity. Eighteen natural molecules were screened out based on docking studies and ADME analysis. It can be predicted that these natural molecules with anticancer activity can act as xanthine oxidase inhibitors very effectively. These may be successfully developed into new drugs in the future based on further studies.

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Conflicts of Interest

The authors declare no conflict of interest.

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