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Structure-Based Virtual Screening and Molecular Dynamics of Quercetin and Its Natural Derivatives as Potent Oxidative Stress Modulators in ROS-induced Cancer

Abd. Kakhar Umar[1,*], James H. Zothantluanga[2]

[1]Department of Pharmacy, Faculty of Math and Natural Sciences, Universitas Tadulako, Palu 94148, Indonesia

[2]Department of Pharmaceutical Sciences, Faculty of Science and Engineering, Dibrugarh University, Dibrugarh 786004, Assam, India

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Abstract

Quercetin derivatives are known to have significant anticancer activity. The activity is strongly influenced by the type and position of the substituent group. By studying the structural pattern of quercetin and its impact on their binding affinity, the development of quercetin-based drugs can be optimized. The study aimed to determine the impact of 3D structure, type, and position of quercetin moiety on its activity against ROS-modulating enzymes that play a role in the induction and growth of ROS-induced cancer. The 23 natural quercetin derivatives were docked to 7 ROS-modulating enzymes using Autodock Vina to determine their binding affinity and interaction. The interaction stability was further studied through molecular dynamics simulation using the CABS Flex 2.0 server. Determination of crucial amino acid targets of the quercetin group was determined using DockFlin. Finally, the toxicity of each test ligand was determined using the pkCSM server. The highest binding affinity for SOD and NOX was produced by quercetin 3'-glucoside with the binding energy of -10.2 and -12.8 kcal/mol. Quercetin 3,4'-diglucoside had the highest binding affinity for CAT and GR at -11.5 and -10.5 kcal/mol, respectively. Routine produced the highest binding affinity at LOX (-10.9). Quercetin 3-O-xyloside and quercetin 3-O-rhamnoside-7-O-glucoside had the highest binding affinity in XO with a value of -10.4 kcal/mol. The glucose and prenyl groups are beneficial for quercetin in interacting with all ROS-modulating enzymes except XO. In contrast, the methoxy group negatively affects all interactions of quercetin with receptors. The perfect fit between the binding pocket and the 3D structure of the ligand greatly benefits the ligand in accessing more amino acids in the binding pocket. Their interaction stability and toxicity show that quercetin 3'-glucoside, quercetin 3,4'-diglucoside, and rutin are potent oxidative stress modulators in treating ROS-induced cancer.

Keywords: ROS-induced cancer, Quercetin derivates, Oxidative stress modulator, ROS-modulating enzymes

*Corresponding author,
e-mail : abdulkaharumar@gmail.com (A. K. Umar)

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1. Introduction

The imbalance between the number of free radicals (ROS) and reactive metabolites (antioxidants) in the body causes oxidative stress. High amounts of ROS can accelerate the oxidation process in normal cells, leading to cell damage. Premature aging and the onset of chronic diseases such as cancer are the most prominent outcomes. It is widely acknowledged that oxidative stress promotes tumor genesis and growth by causing genetic instability [36]. As a result, focusing on redox-sensitive pathways and transcription factors has significant potential for cancer prevention and treatment [10, 27]. Lipoxygenase (LOX), NADPH oxidase (NOX), and xanthine oxidase (XO) are enzymes regulating ROS generation. The inhibition of these three enzymes significantly impacts the suppression of ROS generation and cancer progression [5, 10, 16, 22, 25, 31]. Likewise, induction of the catalase (CAT), glutathione reductase (GR), glutathione peroxidase (GPx), and superoxide dismutase (SOD) can reduce ROS levels and prevent cell damage [3, 7, 11, 12, 14, 34].

One of the powerful natural antioxidants is quercetin. Quercetin has been shown to effectively prevent and inhibit cancer growth via the regulation of ROS [33, 35, 37]. Quercetin was reported to increase catalase activity up to 28.6% in 3-NP treated animals [29]. In addition, quercetin also inhibits the activity of several pro-oxidant enzymes such as LOX, NOX, and XO [4, 9, 17, 18, 28, 30, 37]. The activity is strongly influenced by the structure and position of functional group on quercetin (see Figure 1). The substitution of functional groups of quercetin impacts its biochemical and pharmacological properties [20, 26, 33]. Therefore, this research was conducted to study the impact of 3D structure, type, and position of quercetin moiety on its activity against ROS-modulating enzymes to develop more optimal quercetin-based drugs in treating ROS-induced cancer. The 23 natural quercetin derivates are present in fruit, seeds, tubers, and honey [21]. In this study, 23 natural quercetin derivatives were docked on 7 ROS-modulating enzymes, and then a molecular dynamics study was conducted to determine the stability of the ligand-protein interactions. Toxicity studies were also carried out to assess the safety of the test ligands.

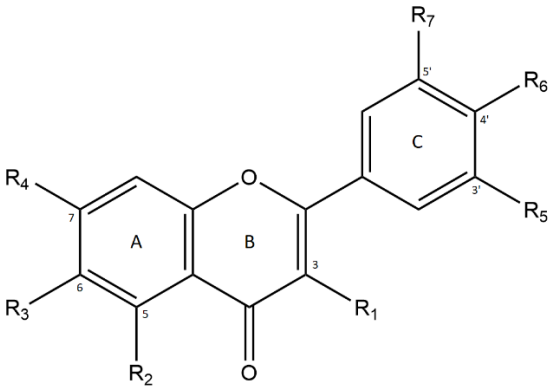

Figure 1. Quercetin basic structure.

2. Methods

2.1 Ligand Preparation

The test ligands were quercetin and its derivates found in plants [21]. Some ligand structures were downloaded from PubChem, and the rest were drawn using ChemDraw Pro 12.0 (PerkinElmer Informatics, PerkinElmer Inc, USA). Structure errors were checked using the "Check Structure" feature, and then the structures were cleaned using the "Clean Up Structure" feature in ChemDraw. The default geometry of each ligand was removed using the "Clean Geometry" feature in Discovery Studio 2021 Client (DS) (BIOVIA, San Diego, CA, USA).

Energy minimization (MM2) was performed using Chem3D. Each ligand was then optimized using AutoDockTools 1.5.6 (ADT) (TheScripps Research Institute, USA) to add Gasteiger charges, set rotatable bonds, and TORSDOF. Furthermore, the ligands are stored in PDBQT format.

2.2 Protein Preparation

The structure of the proteins was obtained from the Protein Data Bank (PDB) website. The code for each protein used is lipoxygenase (3O8Y), NADPH oxidase (5O0X), xanthine oxidase (3BDJ), catalase (1DGF), glutathione reductase (1XAN), glutathione peroxidase (6ELW), and superoxide dismutase (2C9V). Native ligand and protein were separated using the DS. The protein was optimized using ADT to remove water, regulate the charges (Kollman charges), and add polar hydrogen. The protein was then stored in PDBQT format. The grid position was arranged based on the active site attached by the native ligand. The grid dimension was set to 40 x 40 x 40 magnification with a spacing of 0.375Å. Gridbox parameters can be seen in Table 1.

Table 1. The ROS enzymes' gridbox parameters.

Enzymes	**Active sites Coordinate (**Å)
Lipoxygenase	4,976 x 21,401 x 0,286
NADPH Oxidase	65,738 x 0,875 x 62,590
Xanthin Oxidase	17,175 x -17,782 x 16,527
Catalase	14,877 x 15,793 x 78,537
Glutathione Reductase	82,170 x -5,827 x 36,154
Glutathione Peroxidase	42,474 x 14,099 x -18,061
Superoxide Dismutase	95,611 x 47,146 x 112,872

2.3 Molecular Docking

Molecular docking was performed using DockFlin software (ETFLIN, Indonesia). This software is a tool for systematically scheduling multi-ligand and multi-protein docking processes by the Autodock Vina. Ligands and proteins were added to the respective list panel, then the docking parameters per protein were loaded in the order in the grid list panel. The docking parameters used were energy range of 4 and exhaustiveness of 8. The operating system used was Windows 10 Home Single Language 64 bit with AMD Ryzen 5 3500U, Radeon Vega Mobile Gfx 2.10 GHz, and RAM of 8 GB.

2.4 Molecular Dynamics Study

The protein's stable structure was studied using the CABS Flex 2.0 server, based on coarse-grained simulations of protein motion [15]. Distance restraints generator mode was SS2 with minimal restraint length of 3.8 Å and maximal restraint length of 8.0 Å. The number of cycles and trajectory frames was set to 50, with a global weight of 1.0 and a temperature of 1.4. The distance restraints generator was set to default values. The output of this step is ten structural models for each enzyme based on their flexibility. Each model of each enzyme was then re-docked with potent ligands obtained from the molecular docking results. The fluctuation of the binding energy of each ligand-protein interaction is presented in

the form of a line graph. This test aimed to see whether the ligand-protein interaction remains stable during attachment without losing binding energy on all models [2].

2.5 Determination of Crucial Amino Acid

This test was carried out to determine the most active amino acid residues at the binding site of the proteins based on the habit of a group of compounds interacting with the protein. The output file of the docking process (Autodock Vina) produced nine ligand-protein interaction models for each ligand. The active amino acid of each protein receptor was determined based on its occurrence (binding ligands via hydrogen bonds) in each model and all ligand-protein complexes. The number of occurrences has been scored using DockFlin. If an amino acid has a score of more than 9, it is easily accessible and preferred as a target for binding [32].

2.6 Toxicity prediction of quercetin derivates

Prediction of acute oral toxicity (LD50) was carried out using pkCSM ADMET to determine the safety of quercetin and its derivates. The SMILES string for each ligand was obtained from a PDB file converted to SMI format using DS.

3. Results

3.1 Molecular Docking

Based on the molecular docking results, it was found that quercetin has strong interactions with antioxidant and pro-oxidant enzymes, especially with CAT, LOX, and NOX. Quercetin and its derivatives produced binding energy in CAT of -8.9 to -11.5 kcal/mol, GR of -7.6 to -10.5 kcal/mol, GPx of -6.9 to -8.8 kcal/mol, SOD of -7.5 to -10.2 kcal/mol, LOX of -7.6 to -10.9 kcal/mol, NOX of -9.7 to -12.8 kcal/mol, and XO of -7.4 to -10.4 kcal/mol. Each binding energy of the test ligands can be seen in Table 2.

Table 2. Binding energy of test ligand on each ROS-modulating enzymes.

Compound Name	Ligand Code	CAT	GR	GPx	SOD	LOX	NOX	XO
Quercetin	Ligand 0	-9.5	-8.8	-7.5	-9	-9.1	-10.8	-9.6
Quercetin 3-O-galactoside	Ligand 1	-9.7	-8.7	-7.6	-8.3	-10.2	-10.4	-8
Quercetin 3-O-glucoside	Ligand 2	-9.5	-8.6	-7.5	-8.2	-10.2	-10.3	-9.3
Quercetin 3-O-rhamnoside	Ligand 3	-9.6	-8.7	-7.8	-8.9	-9.8	-10.8	-7.9
Quercetin 3-O-rhamnozyl-(1→6)-glucoside	Ligand 4	-10.4	-8.2	-8.8	-8.1	-10.9	-10.9	-9.2
Quercetin 7-*O*- glucoside	Ligand 5	-10.9	-9.3	-8.6	-8.9	-10.4	-11.5	-8.3
Quercetin 3-O-rhamnoside-7-O-glucoside	Ligand 6	-9.5	-8	-7.3	-7.7	-8.7	-9.9	-10.4
Quercetin 6-C- glucoside	Ligand 7	-10.5	-9.9	-8.1	-9	-8.9	-11.1	-8.1
Quercetin 3-(2’’-acetylgalactoside)	Ligand 8	-9	-7.6	-7.5	-7.5	-7.6	-11	-7.4
Quercetin 3-sulfate-7-O-arabinoside	Ligand 9	-10.3	-9.2	-8.7	-9.1	-9.9	-10.1	-8.1
Quercetin 3-O-glucoside-3’-sulfate	Ligand 10	-9.5	-9.2	-7.9	-8.3	-9.8	-10.3	-8
Quercetin 5-methyl ether	Ligand 11	-9.4	-8.5	-7.3	-7.8	-8.9	-9.7	-8.9
Quercetin 7- methyl ether	Ligand 12	-9.4	-7.8	-7.4	-8.8	-8.9	-10.6	-7.7
Quercetin 3’- methyl ether	Ligand 13	-9.4	-8.5	-7.3	-8.8	-8.3	-10.7	-8.7
Quercetin 4’- methyl ether	Ligand 14	-9.4	-8.5	-7.5	-8.5	-8.7	-10.6	-9.5
Quercetin 7-methoxy-3-O-glucoside	Ligand 15	-10	-8.2	-7.6	-8.2	-9.7	-10.7	-8

Quercetin 3'- methoxy -3-O-galactoside	Ligand 16	-9.9	-8	-7.5	-8.3	-9	-10.3	-7.9
6,5'-Di-C-prenylquercetin	Ligand 17	-10.3	-9.1	-8.4	-9.4	-10.6	-11.7	-9.1
Quercetin 3-O-xyloside	Ligand 18	-9.8	-8.2	-7.5	-8.3	-9.5	-9.9	-10.4
Quercetin 3-O-glucuronide	Ligand 19	-9.9	-8.3	-8.4	-8.4	-10.1	-10.9	-9.9
Quercetin 3,4'-diglucoside	Ligand 20	-11.5	-10.5	-8.7	-9.3	-10.6	-10	-8.3
Quercetin 3-O-6''-acetylglucoside	Ligand 21	-11	-8.4	-7.8	-7.9	-10.3	-11.2	-7.8
Quercetin 3,3'-dimethyl ether	Ligand 22	-8.9	-8	-6.9	-8.6	-8.1	-10.3	-8.8
Quercetin 3'-glucoside	Ligand 23	-11.2	-9.8	-8.8	-10.2	-10.7	-12.8	-8.8

In the XO enzyme, structural modification of quercetin will generally decrease its binding energy except for the substitution of xylose and glucuronate at the C-3 atom. In other enzymes, the substitution of glucose, prenyl, arabinose, and glucuronate groups generally increases the binding energy of quercetin. However, the binding energy of quercetin can be decreased if there is a methoxy group as an alkyl group. The increasing or decreasing percentage in the binding energy of quercetin based on its functional group can be seen in Table 3.

Table 3. Changes in binding energy due to the influence of substituents

Ligand Code	Moiety position							Differences in binding energy (%)*							
	R1	R2	R3	R4	R5	R6	R7	CAT	GR	GPx	SOD	LOX	NOX	XO	Average
Ligand 23	OH	OH	H	OH	Glu	OH	H	17.9	11.4	17.3	13.3	17.6	18.5	-8.3	12.5
Ligand 20	Glu	OH	H	OH	OH	Glu	H	21.1	19.3	16.0	3.3	16.5	-7.4	-13.5	7.9
Ligand 17	OH	OH	Pre	OH	OH	OH	Pre	8.4	3.4	12.0	4.4	16.5	8.3	-5.2	6.8
Ligand 5	OH	OH	H	Glu	OH	OH	H	14.7	5.7	14.7	-1.1	14.3	6.5	-13.5	5.9
Ligand 4	Glu & Rha	OH	H	OH	OH	OH	H	9.5	-6.8	17.3	-10.0	19.8	0.9	-4.2	3.8
Ligand 19	Gcr	OH	H	OH	OH	OH	H	4.2	-5.7	12.0	-6.7	11.0	0.9	3.1	2.7
Ligand 9	Sul	OH	H	Ara	OH	OH	H	8.4	4.5	16.0	1.1	8.8	-6.5	-15.6	2.4
Ligand 7	OH	OH	Glu	OH	OH	OH	H	10.5	12.5	8.0	0.0	-2.2	2.8	-15.6	2.3
Ligand 21	6-A	OH	H	OH	OH	OH	H	15.8	-4.5	4.0	-12.2	13.2	3.7	-18.8	0.2
Ligand 0	OH	OH	H	OH	OH	OH	H	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ligand 2	Glu	OH	H	OH	OH	OH	H	0.0	-2.3	0.0	-8.9	12.1	-4.6	-3.1	-1.0
Ligand 18	Xyl	OH	H	OH	OH	OH	H	3.2	-6.8	0.0	-7.8	4.4	-8.3	8.3	-1.0
Ligand 3	Rha	OH	H	OH	OH	OH	H	1.1	-1.1	4.0	-1.1	7.7	0.0	-17.7	-1.0
Ligand 10	Glu	OH	H	OH	Sul	OH	H	0.0	4.5	5.3	-7.8	7.7	-4.6	-16.7	-1.6
Ligand 1	Gal	OH	H	OH	OH	OH	H	2.1	-1.1	1.3	-7.8	12.1	-3.7	-16.7	-2.0
Ligand 14	OH	OH	H	OH	OH	Met	H	-1.1	-3.4	0.0	-5.6	-4.4	-1.9	-1.0	-2.5
Ligand 15	Glu	OH	H	Met	OH	OH	H	5.3	-6.8	1.3	-8.9	6.6	-0.9	-16.7	-2.9
Ligand 13	OH	OH	H	OH	Met	OH	H	-1.1	-3.4	-2.7	-2.2	-8.8	-0.9	-9.4	-4.1
Ligand 6	Rha	OH	H	Glu	OH	OH	H	0.0	-9.1	-2.7	-14.4	-4.4	-8.3	8.3	-4.4
Ligand 16	Gal	OH	H	OH	Met	OH	H	4.2	-9.1	0.0	-7.8	-1.1	-4.6	-17.7	-5.2
Ligand 12	OH	OH	H	Met	OH	OH	H	-1.1	-11.4	-1.3	-2.2	-2.2	-1.9	-19.8	-5.7
Ligand 11	OH	Met	H	OH	OH	OH	H	-1.1	-3.4	-2.7	-13.3	-2.2	-10.2	-7.3	-5.7
Ligand 22	Met	OH	H	OH	Met	OH	H	-6.3	-9.1	-8.0	-4.4	-11.0	-4.6	-8.3	-7.4

Ligand 8	2-A	OH	H	OH	OH	OH	H	-5.3	-13.6	0.0	-16.7	-16.5	1.9	-22.9	-10.4

Note: Glu = glucose, Met = methoxy, Rha = rhamnose, Sul = sulfate, Gal = galactose, Pre = prenyl, Gcr = glucuronate, Ara = arabinose, 6-A = 6-acetylglucose, 2-A = 2-acetylgalactose, and Xyl = xylose. *A negative percentage indicates a reduction in binding affinity and a positive percentage indicates an increase in binding affinity (compared to basic quercetin).

3.2 Molecular Dynamics Study

Molecular dynamics simulations were only carried out on enzymes that have strong potential to become targets of quercetin derivatives, namely CAT, LOX, and NOX. Based on the molecular dynamics simulation, it can be seen that the flexibility of the protein structure does not change the 3D pattern of the enzyme significantly (see Figure 2). Each enzyme's binding site retains a similar shape and coordinates to not interfere with the ligand binding.

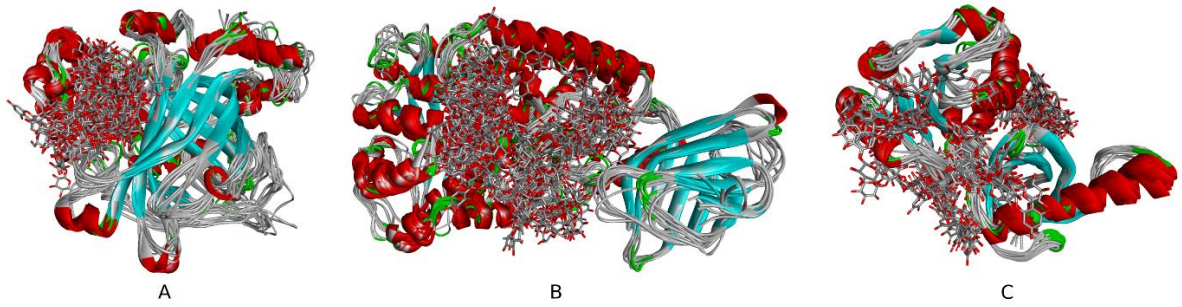

Figure 2. Simulation of protein flexibility and ligand-protein dynamics interaction of (A) quercetin 3,4'-diglucoside and CAT, (B) quercetin 3-O-rhamnozyl-(1→6)-glucoside and LOX, and (C) quercetin 3'-glucoside and NOX.

After re-docking the potent ligands on each model of the three target enzymes, it was found that the interactions of CAT-Ligand 20, LOX-Ligand 4, and NOX-Ligand 23 remained stable without losing binding energy in any of the models. The average binding energies of CAT-Ligand 20, LOX-Ligand 4, and NOX-Ligand 23 were -9.06 ± 0.55, -9.02 ± 0.97, and 8.77 ± 0.48 kcal/mol. The fluctuations of the three interactions can be seen in Figure 2.

3.3 Determination of Crucial Amino Acid

Based on the scoring results, it is known that the CAT enzyme has ten crucial amino acids that were active in forming hydrogen bonds with compounds from the quercetin group. In LOX, there are four crucial amino acids, while in NOX, there are nine crucial amino acids. The average bond length, hydrogen bond types, and scores of each amino acid in each enzyme can be seen in Table 4.

3.4 Toxicity prediction of quercetin derivates

Toxicity data of each test ligand can be seen in Table 5. Ligand 22 has the highest dose tolerance, while ligand 4 has the lowest dose tolerance. The predicted dose is recommended for use in phase I clinical trials. All tested ligands are non-toxic to the liver and do not induce skin sensitization.

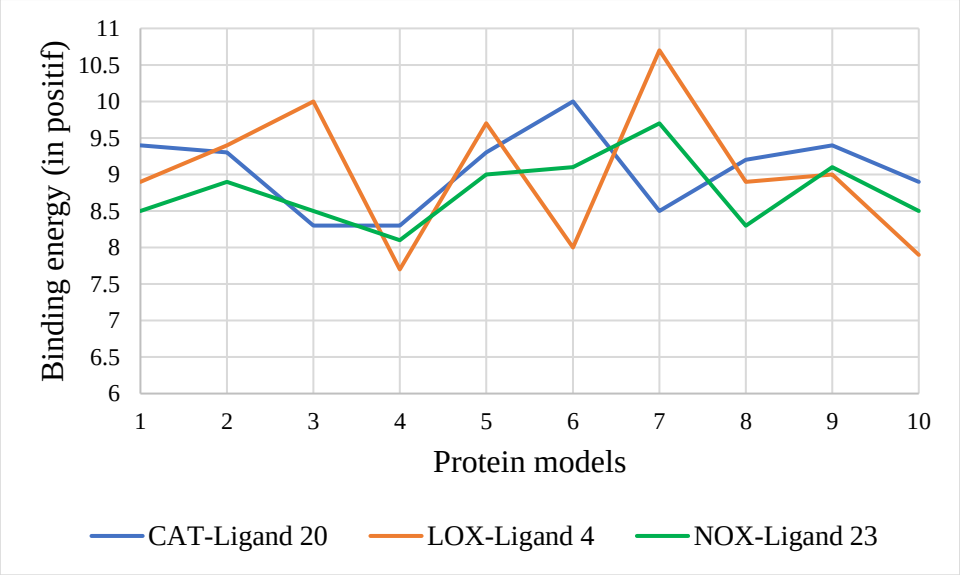

Figure 3. Fluctuations in the binding energy of CAT-Ligand 20, LOX-Ligand 4, and NOX-Ligand 23 in each model.

Table 4. The crucial amino acid at the binding sites of CAT, LOX, and NOX.

Enzyme	Crucial Amino Acids	Average Bond Length (Å)	Average Bond Type	Score
CAT	His305	4.638	Conventional Hydrogen	36.91
	Arg203	4.475	Conventional Hydrogen	22.64
	His305	4.587	Carbon Donor Hydrogen	19.73
	Phe446	4.695	Carbon Donor Hydrogen	19.55
	Phe198	4.52	Carbon Donor Hydrogen	19.09
	Ser201	3.971	Conventional Hydrogen	14.36
	Asp202	4.715	Conventional Hydrogen	11.64
	His194	4.487	Conventional Hydrogen	11.09
	Gln442	4.38	Conventional Hydrogen	9.41
	Ala445	5.076	Carbon Donor Hydrogen	9.14
LOX	Asp170	4.672	Conventional Hydrogen	16.50
	Val243	4.598	Conventional Hydrogen	16.27
	Asp442	4.728	Conventional Hydrogen	9.41
	Ser447	4.544	Conventional Hydrogen	9.00
NOX	Thr462	3.74	Conventional Hydrogen	29.05
	Arg478	4.09	Conventional Hydrogen	23.14
	Pro460	4.41	Conventional Hydrogen	23.14
	Phe461	4.44	Carbon Donor Hydrogen	18.68
	Thr462	4.66	Carbon Donor Hydrogen	12.18
	His459	4.81	Carbon Donor Hydrogen	10.82
	Pro460	5.17	Carbon Donor Hydrogen	10.32
	Thr484	4.24	Conventional Hydrogen	9.77
	Trp695	4.43	Conventional Hydrogen	9.41

Table 5. Toxicity prediction of quercetin derivates.

Ligand	Max. tolerated human dose (mg/KgBW/day)	Hepatotoxicity	Skin Sensitisation
Ligand 0	5.13	No	No
Ligand 1	15.10	No	No
Ligand 2	12.85	No	No
Ligand 3	8.93	No	No
Ligand 4	2.62	No	No
Ligand 5	8.38	No	No
Ligand 6	10.02	No	No
Ligand 7	3.05	No	No
Ligand 8	5.77	No	No
Ligand 9	3.23	No	No
Ligand 10	8.93	No	No
Ligand 11	5.62	No	No
Ligand 12	4.15	No	No
Ligand 13	3.14	No	No
Ligand 14	5.19	No	No
Ligand 15	4.36	No	No
Ligand 16	6.59	No	No
Ligand 17	6.59	No	No
Ligand 18	6.92	No	No
Ligand 19	6.55	No	No
Ligand 20	7.67	No	No
Ligand 21	8.83	No	No
Ligand 22	7.48	No	No
Ligand 23	12.42	No	No

4. Discussion

Quercetin is a powerful natural antioxidant. Changes in functional groups in the basic structure of quercetin will have a significant effect on its pharmacological activity [20, 26, 33]. This study found that the dimensions, position, and type of substituent functional groups of quercetin significantly affect their interactions with ROS-modulating enzymes. The most abundant substituent groups in quercetin derivatives were glucose (9 compounds), followed by methoxy (7 compounds), rhamnose (3 compounds), sulfate (2 compounds), galactose (2 compounds), prenyl, glucuronate, arabinose, 6-acetylglucose, 2-acetylgalactose, and xylose. The basic structure of quercetin can be seen in Figure 1.

Based on the molecular docking results, it was found that, on average, quercetin and its derivatives only bind strongly to CAT, LOX, and NOX enzymes. The mean binding energies for GR, GPx, SOD, and XO were -8.67 ± 0.708, -7.85 ± 0.566, -8.56 ± 0.61, and -8.67 ± 0.854 kcal/mol, respectively. Meanwhile, the average

binding energy of CAT, LOX, and NOX were -9.938 ± 0.69, -9,538 ± 0.9, and -10,688 ± 0.676 kcal/mol, respectively. Quercetin was reported to have no significant effect on glutathione reductase and glutathione peroxidase [8]. SOD activity is also said to not increase significantly after being given quercetin [19]. However, some quercetin derivatives still provide high binding affinity for all enzymes, except GPx.

Quercetin 3,4'-diglucoside produced the highest binding energies for CAT and GR at -11.5 and -10.5 kcal/mol, respectively. The highest binding affinity for SOD and NOX was produced by quercetin 3'-glucoside with a binding energy of -10.2 and -12.8 kcal/mol. Rutin (ligand 4) had the highest binding affinity at LOX (-10.9). Quercetin 3-O-xyloside and quercetin 3-O-rhamnoside-7-O-glucoside produced the highest binding affinity in XO with a value of -10.4 kcal/mol. Based on these values, it can be seen that all quercetin derivatives that have a glucose group as a substituent are potent ligands for all enzymes. The effect depends strongly on the position of glucose [38, 39]. Glucose substituents in rings A and C are the main contributors to quercetin activity. In Table 3, it can be seen that the substitution of glucose on the 3-O atom did not significantly increase the binding affinity of all enzymes, except LOX. The glucose group on the C ring of quercetin (see Figure 1) increases binding affinity significantly in all enzymes except XO. Still, it decreases considerably in NOX if there is another group on the 3-O atom. It is because other groups on the 3-O atom affect the position of the ligand entry into the NOX binding pocket (see Figure 4). The 3-O atom's glucose group appears to bind the residue outside the binding pocket, causing the ligand to become stranded outside. It caused quercetin 3,4'-diglucoside (see Figure 4B) to lose the four hydrogen bonds it would have formed if it had managed to fit snugly into the binding pocket like quercetin 3'-glucoside (see Figure 4A).

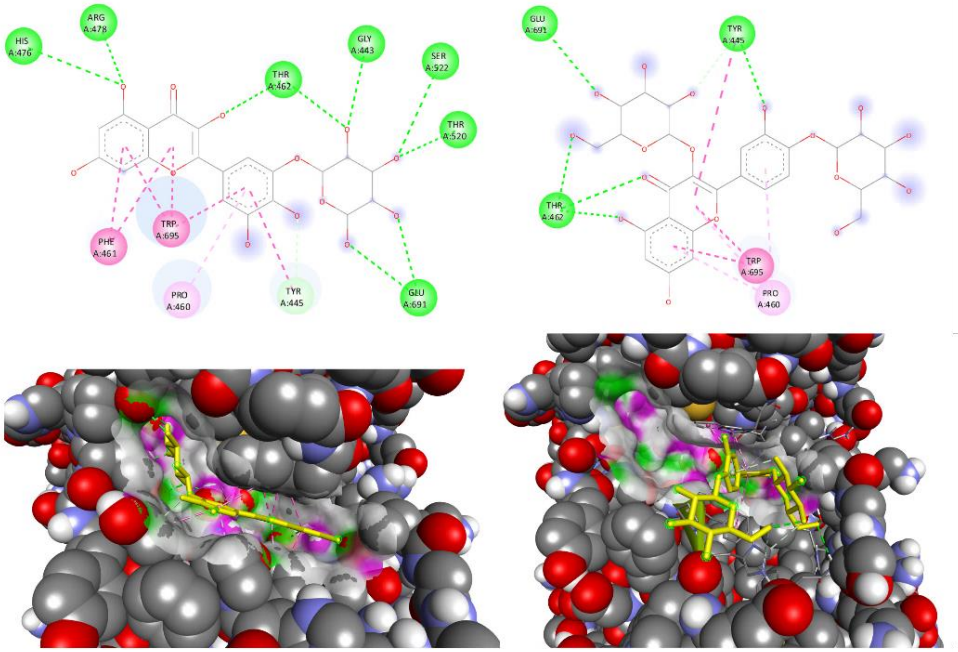

Figure 4. Quercetin 3'-glucoside (A) and quercetin 3,4'-diglucoside (B) on NOX binding pocket.

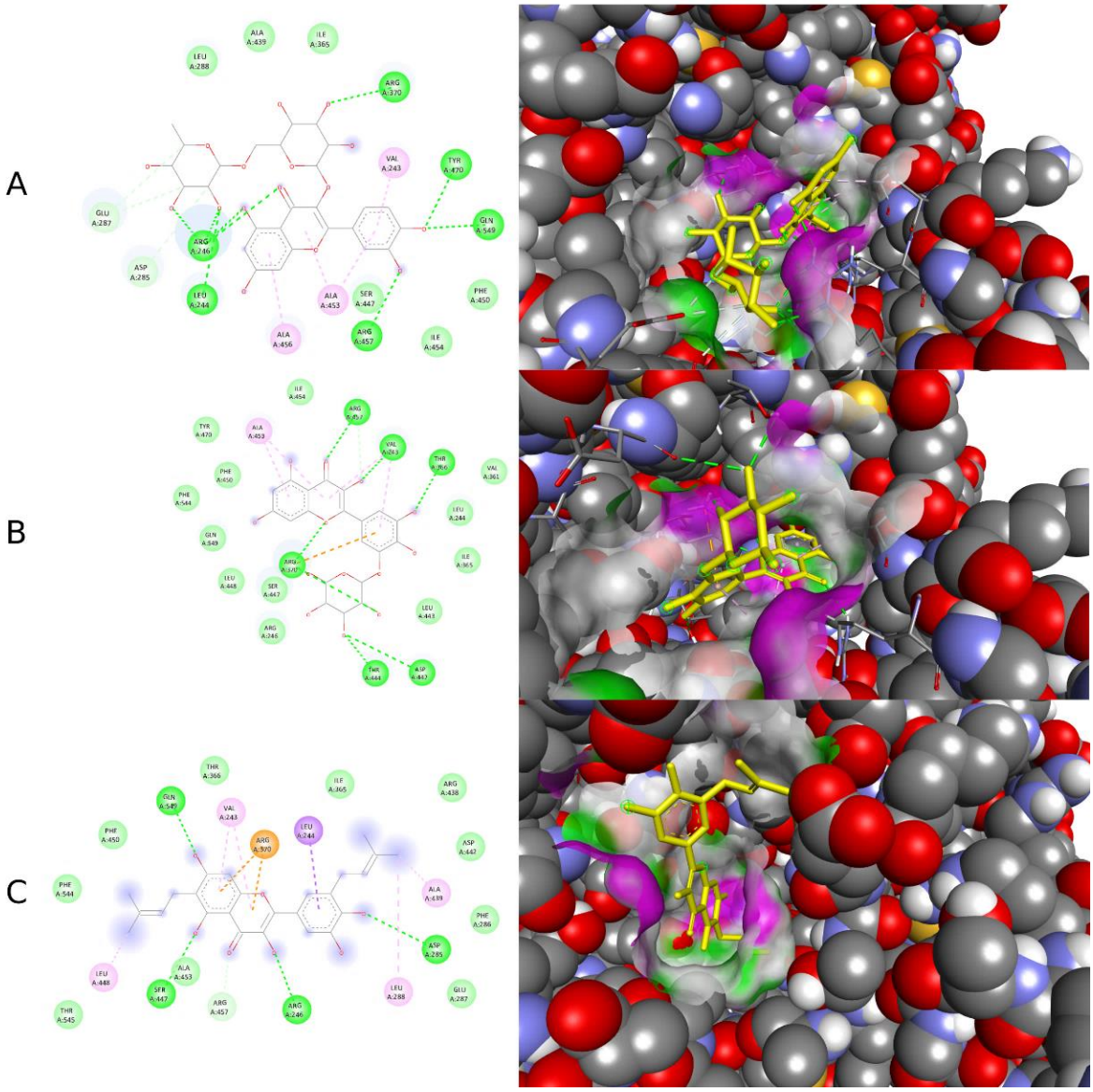

Figure 4. Interaction of rutin (A), quercetin 3'-glucoside (B), and 6,5'-Di-C-prenylquercetin (C) on LOX binding pocket in 2D and 3D.

In the LOX enzyme, glucose and rhamnose complex (rutin) produced the highest binding affinity, followed by quercetin 3'-glucoside and 6,5'-Di-C-prenylquercetin. This is due to the broader dimensions of the binding pocket so that ligand with large 3D volumes can reach and interact more with amino acid residues. In Figure 5, it can be seen that rutin forms nine conventional hydrogen bonds and two hydrogen carbons, quercetin 3'-glucoside forms seven conventional hydrogen bonds and one hydrogen carbon. In comparison, Di-C-prenylquercetin only includes four conventional hydrogen bonds and one hydrogen carbon. In contrast to rutin, quercetin 3'-glucoside and 6,5'-Di-C-prenylquercetin have a slender and elongated structure so that they bind amino acids that are only in the elongation pathway. For that, it is more suited to the extended binding pocket like the NOX.

In addition to the glucose group, the prenyl group also significantly increased the binding affinity of quercetin in all enzymes except XO. The compound 6,5'-Di-C-prenylquercetin binds strongly to the LOX.

The two prenyl groups at the ends of the quercetin structure act as anchors and anchors to the LOX binding pocket and form pi-alkyl bonds with the amino acids Ala439 and Leu448 (see Figure 5C). Adding a prenyl group often increases the pharmacological activity of aromatic compounds such as quercetin [1, 6, 13]. Prenylation of the flavonoid structure can also enhance the bioavailability, allowing the effect to last longer [24].

The 2-acetylgalactose, methoxy, xylose, and sulphate groups have a terrible effect on the activity of quercetin. The acetyl group at the C-2 position prevents the formation of hydrogen bonds from the galactose hydroxy group. All ligands with a methoxy group had their hydrogen bonds with all enzymes weaken. Research conducted by Z. Sroka et al. (2017) also showed decreased activity due to a methoxy group in ring B of quercetin [30]. The methoxy group can block the formation of hydrogen bonds or weaken them [23]. In XO, all substitutions on atoms other than C-3 (R3) will decrease the binding affinity of quercetin. Xylose, rhamnose, and glucuronate are favorable C-3 substituents for quercetin and XO interactions.

Crucial amino acids were obtained by scoring their presence in binding to ligands in each Autodock Vina docking output model. Amino acids that get a score of more than nine can be said to be easily accesible and form hydrogen bonds in every interaction model. The more derivatives used, the more accurate the results for that group of compounds. Molecular docking results showed that potent ligands such as quercetin 3'-glucoside, quercetin 3,4'-diglucoside, and rutin formed hydrogen bonds with crucial amino acid residues to produce stable bonds in each flexible model of the enzyme. Based on toxicity studies, quercetin 3'-glucoside and quercetin 3,4'-diglucoside have a higher dose tolerance than quercetin, are non-hepatotoxic, and do not induce skin sensitization.

5. Conclusion

Some quercetin derivatives produce greater binding affinity than basic quercetin. The 3D volume of the structure, type, and position of the substituent groups plays a significant role in determining the interaction of quercetin and ROS-modulating enzymes. The glucose and prenyl groups are beneficial for quercetin in interacting with all ROS-modulating enzymes except XO. In contrast, the methoxy group negatively affects all interactions of quercetin with receptors. Based on molecular docking studies, interaction stability, and toxicity, we conclude that quercetin 3'-glucoside , quercetin 3,4'-diglucoside, and rutin are potent oxidative stress modulators in treating ROS-induced cancer with the binding energy of -12.8 kcal/mol, -11.5 and -10.5 kcal/mol, respectively.

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