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Molecular Docking Studies Flavonoid (Quercetin, Isoquercetin, and Kaempferol) of Single Bulb Garlic (*Allium sativum*) to Inhibit Lanosterol Synthase as Anti-hypercholesterol Therapeutic Strategies

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Abstract. Hypercholesterolemia is the highest risk of CVD which is the biggest disease leading death. One of the Indonesian medicinal plants is single bulb garlic with high flavonoids concentration. Lanosterol synthase, an enzyme on the final stage of cholesterol synthesis are the appropriate inhibition stage for drug. This study aimed to analyze the potential of single bulb garlic flavonoids (quercetin, isoquercetin, and kaempferol) in inhibiting lanosterol synthase. Computational docking analysis was performed using Pyrx, Pymol, Discovery studio, also webserver to predict ADMET and biological activity. Lanosterol synthase was obtained from PDB (PDB ID: 1W6J) with RO 48-8071 as native ligand used for control. The results showed binding affinity RO 48-8071 (-10.3 kcal/mol), quercetin (-9.8 kcal/mol), isoquercetin (-6.8 kcal/mol), and kaempferol (-9.9 kcal/mol). Based on interaction and bonding distance, flavonoids have more stable than control. Flavonoids also have potential as APOA1, HMOX1 enhancers, lipid peroxidase inhibitors, cardioprotectant and hepatoprotectant, high distribution volumes, low toxicity, and clearance. This result indicated that quercetin, isoquercetin, and kaempferol from single bulb garlic could be potential ligand to treat hypercholesterolemia, and could proceed to in vitro and in vivo study by improving the absorption.

INTRODUCTION

Hypercholesterolemia is one of risk factors of cardiovascular disease occurrence [1]. Mostly used chemical drugs to treat hypercholesterolemia are from statin group [2,3]. The use of statin in a long term could trigger a decrease in memory function, cause muscle weakness, and reduce Q10 coenzyme that played role in electron transfer in mitochondria; thus it brings impact on oxidative stress increase [4,5]. A candidate of non-statin drug that becomes a Lanosterol synthase enzyme inhibitor and is currently developed from native ligand is RO 48-8071 [6]. The drug candidate has not clinically been tested as well as its pharmacokinetic; thus, its toxicity and safety level for human consumption are still unknown. Indonesia has many medicinal plant potentials. One of them with potential of reducing blood cholesterol level is single bulb garlic (*Allium sativum*).

Single bulb garlic contains high organosulfur compounds and flavonoids. A research result indicated that single bulb garlic had higher antioxidant activity than local garlic from Ciwedey variety and imported garlic [7]. Flavonoids content in the garlic likely can support organosulfur compound work since drug composed from various compounds (multiple drug) could reduce toxic effect, increase compound bioavailability or synergistic effect in the body [8,9]. Of all flavonoid components, compounds having potential for cardiovascular disease are kaempferol, quercetin, and isoquercetin. Lanosterol synthase enzyme is an appropriate cholesterol synthesis inhibition stage since it does not inhibit important isoprenoid synthesis, such as Farnesylpyrophosphate (FPP) and Geranylgeranylpyrophosphate (GPP) that will become Dolichol, Ubiquinon, Heme-a, Guanosine triphosphate (GTP), RhoA, Rac1, CDC42 protein that function in cell formation, motility, secretion, proliferation, and signaling [10].

Bioinformatics plays essential role in drug development. One of techniques in bioinformatics is molecular docking [11]. Molecular Docking is a technique to design drug using computer to predict ligand binding position that corresponds to protein [12]. The discovery of herbal drug candidate requires strong assessment on pharmacological quality, including absorption, distribution, metabolism, excretion, and toxicity [13–15]. This study aimed to analyze the potential of single bulb garlic flavonoids (quercetin, isoquercetin, and kaempferol) in inhibiting lanosterol synthase about the conformation, binding affinity, and also pharmacological data.

MATERIALS AND METHODS

Lanosterol Synthase was downloaded on PDB (ID : IW6J). Ligand compounds, such us kaempferol (CID 5280863), quercetin (CID 5280343), isoquercetin (CID 5280804) were downloaded from Pubchem with sdf format. The next step was target protein sterilization, water removal, and ligand native separation using pymol software. Further, compound prediction was performed using PASS online web server with canonical smiles to find out the Probability Activity (Pa) value, a value that indicates a compound's biological activity. Pa value used was $Pa > 0.7$ since it is a compound activity that has potential for wet lab experiment. Biological activity chosen was the one that supported hypercholesterolemia therapy. Pharmacokinetic property prediction was carried out using pkCSM web server, whereas compound toxicity used protox-II.

Software used in docking process was Pyrex 8.0. It produced binding affinity (ΔG) in kcal/mol unit and fixation result saved in .pdb format for visualization. Binding site confirmation used pymol software. Protein was sterilized in the preparation stage and the result of ligand– protein fixation in docking stage was opened simultaneously to observe whether or not the compounds' binding position and control used were in one fixation location. The last step was result visualization using Pymol software to find out the conformation of compound bound to enzyme and Discovery Studio software to see the established bind, amino acid residue, and bond distance. Data collected were compared to control.

RESULTS

Lanosterol Synthase in complex with Ro 48-8071 (Fig. 1), *Homo sapiens* organism. The enzyme was downloaded in the form of binding to native ligands and water solvent.

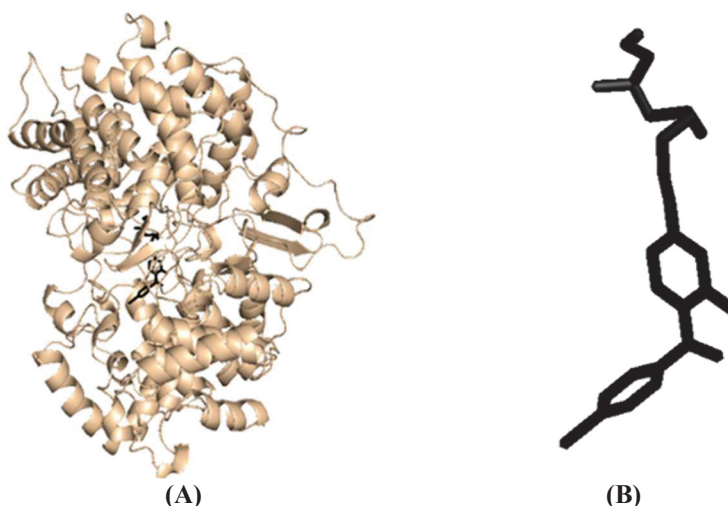


FIGURE 1. (A) Structure of *Lanosterol Synthase* Target Enzyme with RO 48-8071 and (B) RO 48-8071 Structure (control).

Molecular properties of ligand compound were an important Lipinski parameter as a comparison in designing drug candidate to fulfill oral bioavailability property (Table 1).

TABLE 1. Molecular Properties (Drug-likeness) of Ligand Compounds

Ligand	Molecular Weight (g/mol)	Acceptor of H-bond	Donor of H bond	LogP	Number of rotatable bond	Topological Polar Surface Area TPSA (Å ²)
<i>References</i>	≤500	≤10	≤ 5	<5	<10	< 140
Quercetin	302.238	7	5	1.98	1	127
Isoquercetin	464.379	12	8	-0.54	4	207
Kaempferol	286.239	6	4	2.28	1	107
RO 48-8071	448.376	4	0	5.87	12	29.5

According to Table 1, quercetin and kaempferol met all the parameters. It indicated that the quercetin and kaempferol had good absorbability and bioavailability as an oral drug. Isoquercetin does not have good results in Lipinski rule, but still counting to analyze other parameters and potency. The biological activity potential of single bulb garlic flavonoid compound is presented in Table 2.

TABLE 2. Prediction of Ligand Compound Potential

No	Biological Activity Potential	PASS (Pa) Value Prediction			
		Quercetin	Isoquercetin	Kaempferol	RO 48-8071
1.	<i>Antihypercholesterolemia</i>		0.871		0.182
2.	<i>APOA1 expression enhancer</i>	0.776		0.777	0.311
3.	<i>Vasoprotector</i>	0.824	0.947	0.807	
4.	<i>Cardioprotectant</i>	0.851	0.986	0.813	
5.	<i>HMOX1 expression enhancer</i>	0.963	0.774	0.945	
6.	<i>Lipid peroxidase inhibitor</i>	0.813	0.976	0.783	0.198
7.	<i>Free radical Scavenger</i>	0.816	0.978	0.774	0.143
8.	<i>TP53 expression enhancer</i>	0.939	0.959	0.931	

Probability Activity value (Pa) > 0.7 indicated high likelihood compound to be tested experimentally. Based on Table 2, all single bulb garlic flavonoid compounds had better potential than control, and isoquercetin had better potential than other compounds. Pharmacokinetic Properties of Ligand Compound are shown in Table 3. Pharmacokinetic properties are important for the consideration of drug safety and effectiveness consisted of distribution, metabolism, expression, and toxicity (ADMET). It used pkCSM web server [16] (Table 3), and LD50 used ProTox-II* webserver with reference of Globally Harmonized System [17].

TABLE 3. Pharmacokinetic Properties of Ligand Compounds

	Parameter	Reference	Quercetin	Isoquercetin	Kaempferol	RO 48-8071
Absorption	Absorption of human digestion (%)	> 30%: perfectly absorbed	77.207	47.99	74.29	92.32
	Permeability Caco-2 cell (Log Papp in 10-6cm/s)	Value > 0.90: well absorbed	-0.229	0.242	0.032	1.063
Distribution	Distribution Volume in human (VDSS) (logL/Kg)	Value > 0.45: high distribution	1.559	1.846	1.274	1.331
	Blood Brain Barrier Permeability (BBB) (LogBB)	Value < -1: few distribution	-1.098	-1.688	-0.939	0.776
Metabolism	Substrate & inhibitor of CYP3A4	-	No	No	No	Yes
Excretion	ROCT2 Substrate	-	No	No	No	No
	Clearance (log ml/min/kg)	-	0.407	0.394	0.477	1.026
Toxicity	Hepatotoxicity	No	No	No	No	Yes
	AMES	No	No	No	No	No
	LD50(mg/kg)*	-	159	5000	3919	1300

Molecular docking was performed using Pyrx 8.0 software with blind docking of native ligand to identify the active side of lanosterol synthase enzyme. Next, matching approach was conducted in accordance with the blind docking result coordinates, which were at X: 16.4741 Å, Y: 14.4133 Å, and Z of 16.7797 Å (Fig. 2). Molecular docking results were in form of binding affinity values (Table 5).

TABLE 4. Ligand Binding Affinity Values

<i>Ligand</i>	Binding Affinity Values (kcal/mol)
Quercetin	-9.8
Isoquercetin	-6.8
Kaempferol	-9.9
RO 48-8071	-10.3

The control compound had the lowest binding affinity value of -10.3 kcal/mol, whereas kaempferol had the lowest value of the three flavonoid compounds of single bulb garlic, which was -9.9 kcal/mol. Drug with better therapeutic potential had the lowest binding affinity. The lower the binding affinity value, the lower energy required to bind to targets.

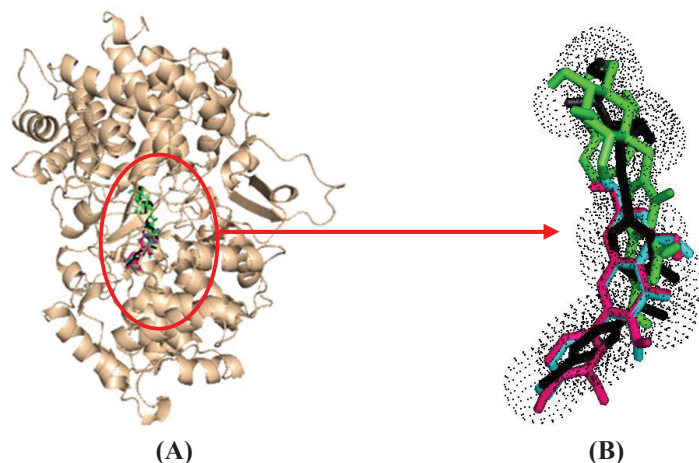


FIGURE 2. (A) Binding position of ligand compounds and control to the active side of lanosterol synthase enzyme (B) RO 48-8071 (black), quercetin (pink), isoquercetin (green), and kaempferol (cyan).

TABLE 5. Bond Type and Bond Distance between Ligands and Lanosterol Synthase Enzyme

Ligand	Amino acid residue		
	Hydrogen	Hydrophobic	Alkyl
RO- 48-8071		Phe : 521, Phe : 696, Trp : 192, His : 232	Trp : 192, Phe : 521, Phe : 444, Trp : 387, Ile : 524
Isoquercein	Tyr : 98, Asp : 455, Tyr : 704	Phe : 696, His 232	Val :453
Quercetin		Phe : 696, His : 232, Trp : 230, Trp : 192	Ile: 524
Kaempferol	Gly :380	Phe: 696, His : 232, Trp : 192, Trp : 230	Ile : 524

DISCUSSION

Water molecule needed to be cleaned and ligand must be separated before docking (Fig. 1) because the water molecule could disturb fixation process also had interaction with residue amino acid [18,19]. Based drug-likeness properties (Table 1), quercetin and kaempferol had good absorbability and bioavailability as an oral drug. RO 48-8071 had low bioavailability since it had excess log P and rotation bond values that indicated too lipophilic. Biological activity of Single bulb garlic flavonoid had better than control (Table 2). The activity namely, APOA1 enhancer

potential was found in quercetin, kaempferol, and RO 48-8071 that functioned to increase synthesis of Apolipoprotein-A playing role in reverse cholesterol transport (RCT) process [20]. The potential of Apolipoprotein-A is to prevent cardiovascular diseases with cardioprotectant, Vasoprotector and HMOX1 enhancer activities. Flavonoid compounds and RO-48-8071 had potential of atherosclerosis prevention, namely lipid peroxidase inhibitor and free radical scavenger.

The pharmacokinetics properties and single bulb garlic flavonoid compounds were perfectly absorbed however, they were low in the permeability in Caco-2 cells. Low BBB indicated flavonoid compounds means did not targeting CNS. Distribution volume of the flavonoid was high, so that it influenced half-life of a drug [21]. Flavonoid compounds were Renal OAT substrates. According to drug-to-drug interaction, flavonoid compounds could not be consumed together with drugs that are Renal OAT inhibitor for maximal effect. The clearance of single bulb garlic had lower than control. Low clearance would increase the half-life of a drug and indicate high oral bioavailability due to optimal metabolism [22]. RO 48-8071 had hepatotoxicity potential. It was related to RO 48-8071 that is the substrate as well as inhibitor of CYP3A4 enzyme (Table 3). Thus, its concentration in plasma was high that increased the possibility of damaging the liver. LD50 acute toxic parameter. Flavonoid compounds and the drug candidate did not have mutagenic and carcinogenic potential (Table 2).

Molecular docking result, amino acid residue compounds, and control had similarity to the active side of lanosterol synthase enzyme according to COFACTOR web server [23], namely Tyr98, Trp192, Trp230, His232, Cys 233, Val236, Ile338, Gly380, Thr381, Trp387, Phe444, Asp455, Thr502, Ile524, Cys533, Trp581, Phe696, Asn697, Tyr704 except Phe521 in RO 48-8071 and Tyr 503, Val453 in isoquercetin. Control had the lowest binding affinity whereas kaempferol had the lowest binding affinity than the other flavonoids. Low binding affinity has a good efficacy for new drug discovery and is more effective for face complex disease, such as hypercholesterolemia, because the drug can work for several targets at the same time [24]. Based on Table 6, hydrogen bond and hydrophobic interaction were found in single bulb garlic flavonoid compounds, the both interactions act to optimize binding affinity since it plays role in macromolecular recognition, folding, and stability of complex bond between drug and target [25,26]. This research still analyzes predicting level; development can be done through in vitro and in vivo testing according to the potentials of single bulb garlic flavonoids (isoquercetin, quercetin, and kaempferol).

SUMMARY

Single bulb garlic flavonoid had a potential as anti-hypercholesterolemia according to the biological activity potentials, druglikeness criteria fulfilment, and ligand bonding. According to molecular docking, kaempferol had the lowest binding affinity value compared to quercetin and isoquercetin and the most stable bond compared to quercetin, isoquercetin, and control. Kaempferol and isoquercetin had low toxicity and did not cause hepatotoxicity; however flavonoid compounds had lower absorption than control.

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