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Potential of Flavonoid Compounds from *Rhodomyrtus tomentosa* as Anticholesterol: An In Silico Study

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Abstract

Indonesia's biodiversity ranks second largest in the world. This vast potential, if managed well, can be utilized as raw materials for medicines. Currently, 96% of drug raw materials in Indonesia are still imported, making medicines expensive. Therefore, efforts are needed to achieve self-sufficiency in drug raw materials, including researching Indonesian plants with potential as hypercholesterolemia drugs to be used as native raw materials. This study aims to determine the potential of flavonoid compounds in *Rhodomyrtus tomentosa* fruit as an alternative hypercholesterolemia drug. Six flavonoids were identified: myricetin, quercetin, quercetin 7,4'-diglucoside, dihydromyricetin, kaempferol, and vitexin in *R. tomentosa* (Karamunting) fruit. The method used was an in silico study. In silico studies are used to screen compounds based on their mechanism of action against target proteins. In this study, myricetin, quercetin, dihydromyricetin, kaempferol, quercetin 7,4'-diglucoside, and vitexin were subjected to molecular docking using Autodock Vina software to determine the affinity and interaction of these six compounds with the HMG-CoA reductase enzyme, which acts as an intermediary in the mevalonate pathway for cholesterol metabolism. Simvastatin, a drug used for hypercholesterolemia treatment, was used as a comparison. The molecular docking results showed that the binding energy values for myricetin, quercetin, dihydromyricetin, kaempferol, quercetin 7,4'-diglucoside, and vitexin were -10.0, -9.4, -9.6, -9.2, -11.4, and -9.9 kcal/mol, respectively. These six flavonoid derivatives from Karamunting fruit have better inhibition scores compared to simvastatin, indicating that these flavonoid derivatives can inhibit cholesterol biosynthesis better than simvastatin and have potential as anticholesterol drugs.

Keyword: Flavonoid compounds, *Rhodomyrtus tomentosa*, anticholesterol agents, silico analysis

INTRODUCTION

Heart attacks, caused by the sudden blockage of coronary blood vessels by blood clots, are a major health concern worldwide. Typically, patients receive blood thinners to prevent further blockages and are advised to rest in the hospital for two weeks. Despite this treatment, 20% of patients do not survive the hospital stay. However, a study on angioplasty, a procedure to quickly restore blood flow through arteries after an acute myocardial infarction linked high patency rates to increased survival rates for heart attack patients (Smilowitz & Feit, 2016). One significant cause of blood vessel blockage is hypercholesterolemia, which is characterized by elevated blood lipid levels due to excessive fat consumption, leading to an imbalance between fat intake and breakdown (Poredos et al., 2007).

Hypercholesterolemia is a major risk factor for cardiovascular diseases, responsible for 18% of cerebrovascular diseases and approximately 56% of ischemic heart diseases globally. Cardiovascular diseases are the leading cause of death worldwide. According to the previous study, The number of deaths due to cardiovascular diseases (CVDs) rose from 12.1 million in 1990 to 18.6 million in 2019, marking a 53.81% increase. Additionally, the prevalence of CVDs nearly doubled, with cases increasing from 271 million to 523 million during the same period (Roth et al., 2020). Hypercholesterolemia leads to complications such as arteriosclerosis, hypertension, increased stroke risk, and fatty liver (Baharvand et al., 2016).

Elevated blood cholesterol, known as hypercholesterolemia, occurs when cholesterol levels surpass normal thresholds. Excessive total cholesterol in the bloodstream can lead to arterial plaque buildup, potentially compromising cardiovascular health and function. Dyslipidemia, a disorder in lipoprotein metabolism, can result in increased levels of total cholesterol, LDL, and triglycerides, and decreased levels of HDL in the blood (Devi & Jyothi, 2017) which is subsequently converted into cholesterol, thereby lowering total cholesterol levels in the blood (Bulbul et al., 2017).

Previous study showed that the antioxidant activity of *Rhodomyrtus tomentosa* fruit extract significantly improves lipid profiles by reducing triglycerides, total cholesterol, and low-density lipoprotein (LDL) while increasing high-density lipoprotein (HDL) in hypercholesterolemic rabbits (Maskam et al., 2014). The extract also helps reduce lipid peroxidation by decreasing the formation of MDA complexes, indicating strong antioxidant activity and the potential to prevent atherosclerosis. Previous studies have suggested that a high-cholesterol diet can induce high serum cholesterol levels in rabbits, and *R. tomentosa* extract effectively lowers total cholesterol levels, making it a potential candidate for preventing cardiovascular diseases.

METHOD

1. Materials

   Ligands: The three-dimensional structures of the ligands used in this research were flavonoid compounds and simvastatin as a positive control. These structures were obtained from PubChem in .sdf format. Receptor: The three-dimensional structure of the HMG-CoA reductase receptor (PDB ID: 1DQ8) was downloaded from the Protein Data Bank (PDB) in .pdb format.

2. Protein Structure Preparation

   The 3D structure of HMG-CoA reductase (PDB ID: 1DQ8) was downloaded from the Protein Data Bank. The structure was optimized by removing water molecules and setting the active site using Discovery Studio. The optimized structure was saved in .pdb format.

3. Preparation of Ligand and Protein

   The ligand structures were downloaded from PubChem in .sdf format. These files were opened and converted to .pdb format using Discovery Studio. The ligand files in .pdb format were opened in Autodock Tools. Hydrogen atoms were added, torsion angles were set, and the optimized ligands were saved in .pdbqt format. The receptor enzyme HMG-CoA reductase, separated from docking inhibitors and saved in .pdb format, was opened in Autodock Tools. Hydrogen atoms were added, and grid box parameters were set. The optimized file was saved in .pdbqt format.

4. Grid Box Preparation for Molecular Docking

   The receptor HMG-CoA reductase and the original ligand in .pdbqt format were opened in Autodock Tools 1.5.6. The size and position of the 3D grid map were set, representing the

receptor area where the ligand can dock. The 3D grid map values were recorded and saved in a notepad.

5. Molecular Docking

Docking was performed using Autodock Vina. Since Autodock Vina does not support a graphical interface, the docking process was executed using the command prompt (cmd) with specific commands for Autodock Vina. The docking results, including affinity energy values, were automatically saved in a log.txt file. Molecular docking analysis was conducted using Autodock Vina. Autodock Tools were used to prepare the protein by setting electron charges and arranging polar hydrogen atoms. The ligands were prepared to have flexible angles, and both protein and ligand were saved in .pdbqt format. Specific molecular docking between HMG-CoA reductase and the flavonoid compounds was performed, resulting in binding energy values involving total intermolecular energy, including hydrogen bonding, Van der Waals energy, desolvation energy, and electrostatic energy. The docking results were compared based on binding energy, Root Mean Square Deviation (RMSD), and interactions between ligands and proteins. The compound with the lowest binding energy was considered the most effective inhibitor of HMG-CoA reductase, indicating its potential as an anticholesterol drug candidate (Fatmawaty et al., 2015).

6. Analysis of Flavonoid Compounds Based on Quantitative Structure–Property Relationship (QSPR)

The flavonoid compounds and their six derivatives (myricetin, quercetin, dihydromyricetin, kaempferol, quercetin 7,4'-diglucoside, and vitexin) were analyzed for their physicochemical properties using Molsoft Drug-Likeness software. These properties are essential in drug development, from design studies to preclinical tests. The absorption, distribution, metabolism, and excretion (ADME) characteristics were predicted using ACD/I-lab and FAF-Drugs3 programs.

RESULT

Analysis of Flavonoid Compounds Based on Quantitative Structure–Activity Relationship (QSPR)

Based on the physicochemical analysis of flavonoids, intrinsic properties of the six compounds were identified. The proton donors in these structures ranged from 4 to 11, and the proton acceptors ranged from 6 to 17. The molecular weights varied between 286.05 and 432.11, with the number of rotatable bonds ranging from 1 to 7. The refractive index values were between 1.530 and 1.864. The density of the six compounds ranged from 1.51 to 1.9 g/cm^3, and the surface tension ranged from 98.9 to 133.0 dyne/cm (Table 1).

Table 1. Physicochemical properties of flavonoid derivatives and commercial cholesterol drugs

No	Molecule Name	H-bond Donor	H-bond Acceptor	Molecular Weight	Rotatable Bonds	Refractive Index	Density (g/cm^3)	Surface Tension (dyne/cm)
1	Myricetin	6	8	318.237	1	1.864	1.9 ± 0.1	133.0 ± 3.0
2	Quercetin	5	7	302.23	1	1.752	1.799 ± 0.1	107.0 ± 3.0

No	Molecule Name	H-bond Donor	H-bond Acceptor	Molecular Weight	Rotatable Bonds	Refractive Index	Density (g/cm^3)	Surface Tension (dyne/cm)
3	Quercetin 7,4'-diglucoside	11	17	626.5	7	1.752	1.8 ± 0.1	116.7 ± 3.0
4	Kaempferol	4	6	286.05	1	1.785	1.7 ± 0.1	98.9 ± 3.0
5	Dihydromyricetin	6	8	320.05	1	1.798	1.8 ± 0.1	115.7 ± 3.0
6	Vitexin	7	10	432.11	3	1.743	1.7 ± 0.1	99.1 ± 3.0
7	Simvastatin	1	5	418.566	7	1.530	1.1 ± 0.1	43.1 ± 5.0

Molecular Docking

The molecular docking study aimed to evaluate the binding affinity and interaction of six flavonoid compounds from Rhodomyrtus tomentosa (myricetin, quercetin, dihydromyricetin, kaempferol, quercetin 7,4'-diglucoside, and vitexin) with the HMG-CoA reductase enzyme. Simvastatin, a known hypercholesterolemia drug, was used as a reference compound. The docking results are summarized in Table 2.

Table 2. Molecular docking results of flavonoid compounds with HMG-CoA reductase

No.	Compound	Binding Energy (kcal/mol)	Amino Acid Residues Involved in Interaction	Number of Hydrogen Bonds
1	Myricetin	-10.0	GLY806(C), GLY808(C), GLY765(C), GLY807(C), GLY808(C), GLU557(D), MET657(C)	3
2	Quercetin	-9.4	GLU700(D), LYS606(A), ALA585(D), LYS633(D), SER705(D), HOH2267(A)	4
3	Quercetin 7,4'-diglucoside	-11.4	SER493(B), ARG496(B), TYR533(B), ASP516(A), ARG515(A)	4
4	Kaempferol	-9.2	SER705(C), LYS606(B), HIS635(B), GLU610(B), ALA585(C), SER635(B), PRO798(C)	1
5	Dihydromyricetin	-9.6	GLY785(D), GLY806(D), GLY807(D), VAL805(D), GLU559(C), ALA625(D), MET665(D)	4
6	Vitexin	-9.9	GLU700(C), ALA585(C), ASP586(C), LEU584(C), GLU610(B)	3
7	Simvastatin	-8.8	LEU634(B), LYS633(C), ALA585(C), HIS635(B)	1

The best binding free energy was shown by the compound quercetin 7,4'-diglucoside, with a ΔG of -11.4 kcal/mol, featuring a hydroxyl group substitution at atom B number 515.

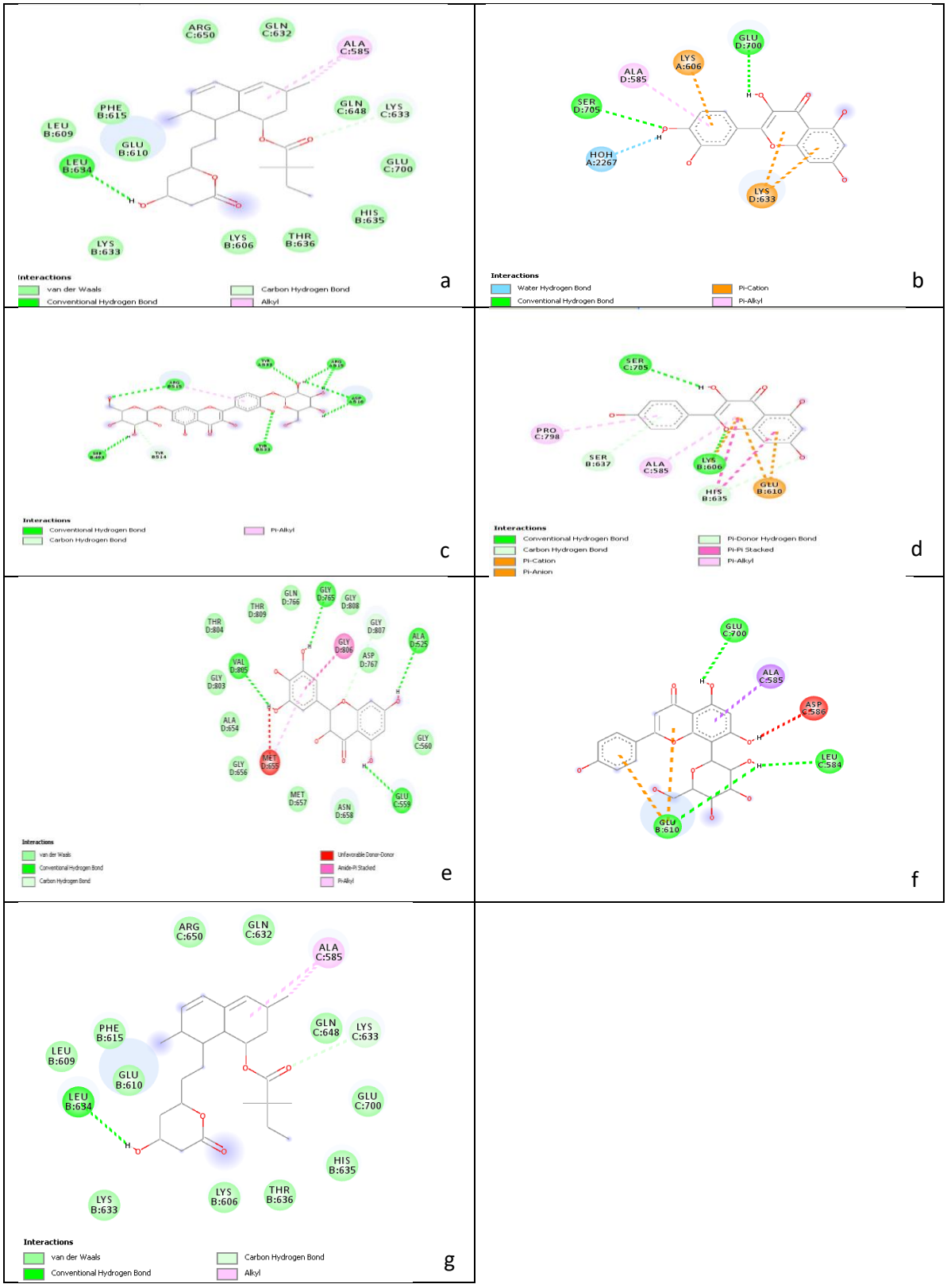

Figure 2. Visualization of Ligand and HMG-CoA Enzyme Interactions. a. Myricetin, b. Quercetin, c. Quercetin 7,4'-diglucoside, d. Kaempferol, e. Dihydromyricetin, f. Vitexin, g. Simvastatin.

From this study, it showed that the interaction model of flavonoid derivative compounds on the inhibition activity of HMG-CoA shows the formation of hydrogen bonds between the ligand and the amino acids of HMG-CoA at the binding site. In comparison, the interaction bonds occurring with simvastatin show that simvastatin only has one hydrogen bond at molecule B position 634.

DISCUSSION

The study's findings highlight the significant potential of flavonoid derivatives as inhibitors of HMG-CoA reductase, a key enzyme in cholesterol biosynthesis. The molecular docking results demonstrated that these compounds, including myricetin, quercetin, dihydromyricetin, kaempferol, quercetin 7,4′-diglucoside, and vitexin, form multiple hydrogen bonds with the enzyme's active site. This interaction is crucial for their inhibitory activity. In contrast, simvastatin, a well-known cholesterol-lowering drug, forms only a single hydrogen bond at molecule B position 634, indicating a comparatively weaker interaction.

The Gibbs free energy values obtained from the docking studies further underscore the superior binding affinity of these flavonoid derivatives. With values ranging from -9.2 to -11.4 kcal/mol, these compounds exhibit stronger binding and inhibitory potential compared to simvastatin, which has a Gibbs energy of -8.8 kcal/mol. Notably, quercetin 7,4′-diglucoside displayed the lowest Gibbs energy, suggesting it as the most potent inhibitor among the tested flavonoids.

The interaction analysis revealed that while most flavonoid derivatives engaged in hydrogen or ionic bonds, kaempferol uniquely forms hydrophobic interactions with the amino acid alanine (AlaA478). This distinct interaction contributes to its stronger binding affinity compared to simvastatin, which also interacts with alanine but lacks the additional serine bond present in kaempferol. Serine plays a vital role in enzyme catalysis, enhancing the binding strength of kaempferol to HMG-CoA reductase (Ramesh et al., 2021).

Quercetin 7,4’-diglucoside's interaction with HMG-CoA reductase involves different amino acids compared to simvastatin, yet it still achieves superior binding strength. This compound formed four hydrogen bonds and an additional serine bond at atom B position 493, further supporting its potential as a potent cholesterol synthesis inhibitor.

These findings align with previous studies, such as those by Istvan and Deisenhofer (2001), which highlighted the importance of amino acids like serine, lysine, and glutamic acid in ionic bonding with statins. Alanine's role in hydrophobic interactions also contributes to the inhibitory action on HMG-CoA reductase, preventing cholesterol biosynthesis.

Simvastatin, a competitive inhibitor of HMG-CoA reductase, functions by slowing cholesterol production and enhancing the liver's ability to remove cholesterol from the blood (Jiang et al., 2018). It achieves this by forming an open-ring mevalonate acid, inhibiting cholesterol synthesis, increasing LDL receptor expression, and reducing LDL receptor degradation (Giacomini et al., 2021). However, the study's results indicate that the flavonoid derivatives from *Rhodomyrtus tomentosa* exhibit stronger binding and inhibitory potential, suggesting their promise as alternative cholesterol-lowering agents.

Rhodomyrtus tomentosa, commonly known as "karamunting," is a plant native to Southeast Asia, recognized for its rich content of bioactive compounds with potential health benefits (Kamarudin et al., 2021). Recent studies have focused on the anticholesterol properties of the flavonoid compounds found in this plant (Hz et al., 2022; Rini & Aurora, 2021). Flavonoids such as myricetin, quercetin, quercetin 7,4'-diglucoside, dihydromyricetin, kaempferol, and vitexin have been identified in *R. tomentosa* and are known for their antioxidant activities (Vo & Ngo, 2019).

These flavonoids have been shown to significantly reduce triglycerides, total cholesterol, and low-density lipoprotein (LDL) levels while increasing high-density lipoprotein (HDL) levels in

hypercholesterolemic models (Maheswari, 2020). This suggests that *R. tomentosa* extracts can improve lipid profiles and potentially prevent cardiovascular diseases. The antioxidant properties of these compounds help reduce lipid peroxidation, which is crucial in preventing atherosclerosis (Sinaga et al., 2021).

In silico studies using molecular docking have demonstrated that these flavonoids exhibit strong binding affinities to the HMG-CoA reductase enzyme, a key player in cholesterol biosynthesis (Islam et al, 2015; Ressaissi et al, 2017). The binding energy values of these flavonoids indicate a higher inhibitory potential compared to simvastatin, a commonly used cholesterol-lowering drug. For instance, quercetin showed the lowest Gibbs energy value, suggesting it as a potent inhibitor of cholesterol biosynthesis (Riyad et al., 2023).

The presence of hydrogen bonds and ionic interactions between the flavonoids and HMG-CoA reductase enhances their inhibitory effects (Charan et al., 2022; Ozalp et al., 2020). Notably, kaempferol forms hydrophobic interactions, contributing to its strong binding affinity. These interactions suggest that flavonoids from *R. tomentosa* could serve as effective anticholesterol agents, warranting further in vivo and in vitro studies to confirm their therapeutic potential.

CONCLUSION

Based on the molecular docking study, myricetin, quercetin, quercetin 7,4'-diglucoside, kaempferol, dihydromyricetin, and vitexin show potential as anti-cholesterol drug candidates. The research findings indicate that flavonoids have better affinity than simvastatin, especially in the case of quercetin 7,4'-diglucoside. Therefore, further in vivo and in vitro studies are recommended.

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