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ANALYSIS OF THE POTENTIAL OF NATURAL STEROL COMPOUNDS FROM TIN (FICUS CARICA) LEAVES AS ANTI-HYPERCHOLESTEROLEMIA WITH IN SILICO TESTS

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ABSTRACT

This study aims to determine the potential of natural sterol compounds from Tin (*Ficus carica*) leaves as anticholesterolemic using in silico test. This study employed an in silico approach using molecular docking methods to evaluate the potential of sterol compounds as anti-hypercholesterolemic agents, in comparison to other references compounds. Data processing and interpretation were conducted using molecular databases for both ligands and target proteins. The analysis utilized several computational tools, including PyRx 0.8, PyMOL, LigPlus, and Discovery Studio 2016 Client. The compounds used in this research were sterol, fluvastatin and simvastatin (as control), with HMG CoA reductase as the target protein. The results showed that the highest binding affinity value was fluvastatin which is -8.3 kcal/mol. Sterol compounds are compounds with lower binding affinity which is -7.7 kcal/mol. From the visualization results, it is known that the binding distance between sterol compounds with target proteins (HMG CoA reductase) is between 2.69 to 5.49 Å, and the binding distance between simvastatin compounds with target proteins (HMG CoA reductase) is between 2.76 to 5.36 Å, and the binding distance between fluvastatin compounds with target proteins (HMG CoA reductase) is between 1.95 to 5.26 Armstrong. While based on the comparison of the binding side of strerol, simvastatin and fluvastatin, it is known that the three compounds have the same site because they bind to the same amino acid residues, namely ARG (B: 515), TYR (A: 533), TYR (B: 533), TYR (B: 517), PRO (A: 511). The results of this research indicate that the natural sterol compounds found in Tin (*Ficus carica*) leaves have potential as anti-hypercholesterolemic agents, based on reverse docking analysis. These sterol compounds bind to the same active site as the control drugs, simvastatin and fluvastatin, and exhibit comparable binding affinity values

Keywords: Anti-hypercholesterolemia, Cholesterol , *Ficus carica,* In silico test, Tin, Sterol compound.

INTRODUCTION

Regular consumption of foods high in cholesterol can lead to elevated blood cholesterol levels. A significant increase in total cholesterol contributes to the development of atherosclerotic plaques in the arterial walls. This atherosclerosis plays a key role in the pathogenesis of hypertension and vascular obstruction in vital organs such as the brain, heart, and extremities. Obstruction in the cerebral blood vessels may result in cerebrovascular diseases such as stroke, while blockage in the coronary arteries can lead to cardiovascular conditions such as coronary heart disease. Meanwhile, obstruction in the peripheral arteries, particularly in the lower limbs, often causes peripheral vascular disease, which is

indonesian.biodivers.j.
Vol. 6, No. 1, April 2025
ISSN : 2722-2659

commonly characterized by pain and cramping in the legs (Garnadi, 2012).

The treatment commonly used to lower cholesterol levels involves synthetic drugs. These synthetic drugs are still widely used today in the management of hypercholesterolemia due to their proven effectiveness through various phased clinical trials and large-scale scientific studies. Commonly used synthetic drugs include fibric acid derivatives (e.g., gemfibrozil), bile acid sequestrants (e.g., cholestyramine and colestipol), HMG-CoA (3-hydroxy-3-methylglutary-CoA) reductase inhibitors (statin), and nicotinic acid (Tjay, 2007). Additional advantages of synthetic drugs include accurate dosage standardization, consistent product quality, and availability in various pharmaceutical forms that facilitate patient use. However, these synthetic drugs also have several drawbacks, such as high cost, adverse side effects—such as muscle and liver disorders—and dependence on long-term use. These limitations have encouraged efforts to explore alternative treatments using traditional medicines derived from medicinal plants that contain natural active compounds.

Hypercholesterolemia is a metabolic disorder characterized by high levels of cholesterol in the blood, especially low-density lipoprotein (LDL) cholesterol, which can cause various cardiovascular complications such as atherosclerosis, heart attack, and stroke. Based on a recent report from the World Health Organization (2023), cardiovascular disease remains the number one cause of death in the world, with hypercholesterolemia as one of the main risk factors. The commonly used first-line therapy today is statins, but their use is often associated with side effects such as muscle pain, liver disorders, and long-term resistance (Thompson et al., 2022)

Therefore, alternative approaches based on natural substances are increasingly gaining attention in the development of safer antihypercholesterolemic agents. One group of compounds that shows promising potential is phytosterols—plant-derived sterols such as β-sitosterol, stigmasterol, and campesterol—which have the ability to lower serum cholesterol levels by inhibiting cholesterol absorption in the intestine (Joerin et al., 2014). Research on the sterol content of tin leaves (Ficus carica) continues to expand due to their potential as natural therapeutic agents, particularly for lowering cholesterol levels, as well as for their anti-inflammatory and antioxidant properties.

One of the medicinal plants that is thought to have properties as anticholesterolemia is the Tin plant (*Ficus carica*), Tin plant is one of the plants that has been widely cultivated because it is believed to treat many diseases. With the development of science, there are many studies on the content and benefits of Tin trees both leaves, fruits and roots. *Ficus carica*, or the tin plant, is known to contain various bioactive compounds, including phytosterols. Recent pharmacological studies have shown that tin leaf extract has the potential to lower total and LDL cholesterol, and increase high-density lipoprotein (HDL) cholesterol levels, both in animal models and limited clinical trials (Boukhalfa, 2018). In addition, the flavonoids and phenolic contents in these leaves also support antioxidant and anti-inflammatory activities that contribute to vascular protection.

To strengthen the understanding of the molecular mechanisms of active compounds found in tin leaves, *in silico* approaches have become an essential tool in the modern era of drug discovery. This

indonesian.biodivers.j.
Vol. 6, No. 1, April 2025
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method enables the modeling and prediction of interactions between phytosterol compounds and key protein targets involved in cholesterol regulation, such as HMG-CoA reductase (a key enzyme in cholesterol biosynthesis), NPC1L1 (an intestinal cholesterol transporter), and the LDL receptor. Through molecular docking techniques, the binding affinities of compounds such as stigmasterol and lupeol to these targets can be efficiently analyzed, providing preliminary data for the further development of potential therapeutic agents (PubChem, 2023).

This study aims to investigate the sterol content of *Ficus carica* leaves and evaluate their potential as antihypercholesterolemic agents using an *in silico* approach. The findings are expected to provide a scientific basis for the development of more effective and safer phytotherapeutic strategies in the management of hypercholesterolemia.

RESEARCH METHOD

The research method used in this study is descriptive qualitative, to process and interpret the data obtained from the database and software used, as follows:

Ligand Preparation

The 3D chemical structures and SMILES of sterol ligands were taken from the compound database on the server (https://pubchem.ncbi.nlm.nih.gov/) with ID number: 1107

Target Selection

The selection of target proteins was carried out with the assistance of several web-based tools to ensure the accuracy of the chosen protein targets. First, the SMILES notation of the sterol ligands was input into **PharmMapper** (http://59.78.96.61/pharmmapper/) to identify potential protein targets. Second, the identified targets were cross-checked using **SwissTargetPrediction** (http://www.swisstargetprediction.ch/) to confirm consistency with the results obtained from the first server. Third, a further validation was conducted using **SuperPred** (http://prediction.charite.de/) to determine whether the same target proteins appeared as in the previous two platforms. This multi-step verification process was repeated to ensure the accuracy of the selected protein targets and to minimize the risk of failure in the subsequent molecular docking analysis.

Molecular Docking

Then docking of sterol molecules, target proteins, and control compounds which are chemical drugs in the treatment of hypercholesterolemia using PyRx 0.8 software.

Visualization and Molecular Interaction

The interactions between sterols, target proteins, and control compounds (simvastatin and fluvastatin), as identified through protein visualization, were subsequently analyzed using PyMOL, LigPlus, and Discovery Studio 2016 Client software

RESULTS AND DISCUSSION

The results of target selection using PharmMapper, SwissTargetPrediction, and SuperPred indicated that sterols interact with the target protein HMG-CoA reductase enzyme. Sterols, also known as steroid alcohols, are a subgroup of steroids and represent an important class of organic molecules. They

indonesian.biodivers.j.
Vol. 6, No. 1, April 2025
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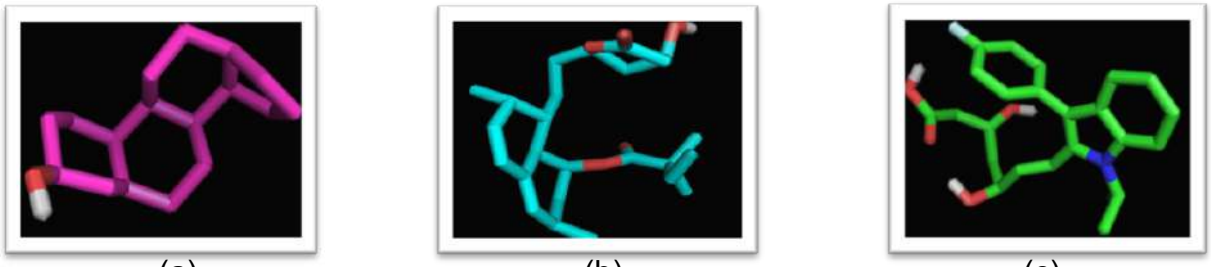
naturally occur in plants, animals, and fungi, with cholesterol being the most well-known form. The three-dimensional structures of the natural compounds were obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov) in Sybyl Data File (*.sdf) format.



(a) (b) (c)

Figure. 1 (a) 3D structure of Sterol natural compound. (b) 3D structure of simvastatin control compound (c) 3D structure of fluvastatin control compound modeled with PyMol software.

Target protein prediction results were obtained through several web-based servers, including the PharmMapper web server. PharmMapper predicts potential target proteins based on pharmacophore-based similarity between the compound of interest (query structure) and known drug compounds, including FDA-approved drugs and non-drug compounds that have been analyzed in vitro and in vivo. Additional servers used in this study include **SuperPred** (http://prediction.charite.de/) and **SwissTargetPrediction** (http://www.swisstargetprediction.ch/), both of which predict target proteins based on structure-based similarity between the query compound and compounds with known biological activities (Gfeller et al., 2014). Furthermore, the predicted target proteins obtained from PharmMapper, SuperPred, and SwissTargetPrediction were supplemented with additional information such as **Gene ID**, **molecular function**, and the **involvement of each protein in biological processes** in the human body, using data provided by the **UniProt database** (http://www.uniprot.org)

Affinity Comparison

Based on the results of target selection using Pharmmapper, SwissTargetPrediction and SuperPret, it is known that sterol compounds are able to bind to the HMG CoA reductase enzyme by forming a bond at one end of its side with the control compounds simvastatin and fluvastatin. Furthermore, PyMoland LigPlus software is used to visualize the 3D structure of a molecule, combining the docking result compound with the target protein and the following image is produced

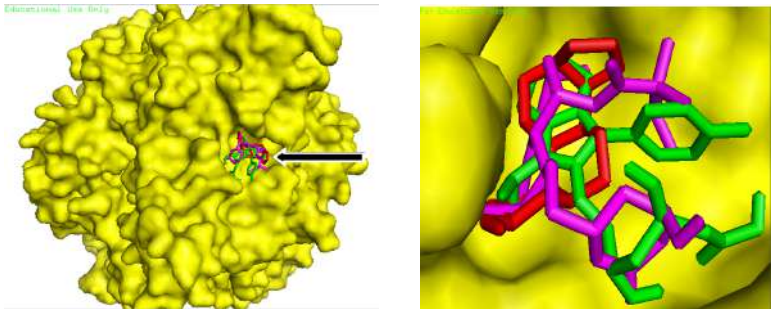


Figure 2. Binding of target protein, sterol natural compound (red) and control compounds simvastatin (magenta) and fluvastatin (green)

indonesian.biodivers.j.
Vol. 6, No. 1, April 2025
ISSN : 2722-2659

Based on the analysis using PyMOL and LigPlus software, it was found that the natural sterol compound binds to the same active site as the control compounds commonly used as anti-hypercholesterolemic drugs, namely simvastatin and fluvastatin. These drugs exert their therapeutic effect by inhibiting cholesterol biosynthesis through the inhibition of the enzyme HMG-CoA reductase. However, long-term use of these synthetic drugs may lead to several adverse effects, including hepatotoxicity, malaise, rhabdomyolysis, myopathy, and others. Due to the potential for multiple side effects associated with prolonged use of chemical drugs, there is a growing shift toward the use of herbal medicines as alternative therapies (Hidayati, et al., 2020).

The test results indicate that natural sterol compounds may function similarly to synthetic antihypercholesterolemic drugs such as simvastatin and fluvastatin. The binding affinity values between natural sterol compounds and the HMG-CoA reductase target protein, when compared to those of simvastatin and fluvastatin, show only minor differences. This suggests that natural sterols have the potential to be developed as alternative therapeutic agents for hypercholesterolemia. The binding affinity values, representing the strength of interaction between the ligands and the target protein, were calculated using PyRx 0.8 software presented in Table 1.

Table.1 Interaction strength of sterol compounds against HMG CoA reductase target protein

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
Sterol-*HMG CoA reduktase*	-7.7	0.0	0.0
Sterol-*HMG CoA reduktase*	-6.7	15.038	11.656
Sterol-*HMG CoA reduktase*	-6.7	13.18	11.076
Sterol-*HMG CoA reduktase*	-6.6	56.012	53.4
Sterol-*HMG CoA reduktase*	-6.6	4.356	2.566
Sterol-*HMG CoA reduktase*	-6.5	90.985	89.656
Sterol-*HMG CoA reduktase*	-6.4	96.358	94.575
Sterol-*HMG CoA reduktase*	-6,4	89.682	87.751
Sterol-*HMG CoA reduktase*	-6,4	72.981,00	70.335

Table. 2 Interaction strength of simvastatin compound against HMG CoA reductase target protein

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
Simvastatin-HMG CoA reduktase	-8.0	0.0	0.0
Simvastatin-HMG CoA reduktase	-7.9	90.245	87.438
Simvastatin-HMG CoA reduktase	-7.8	4.743	1.864
Simvastatin-HMG CoA reduktase	-7.6	4.975	2.098
Simvastatin-HMG CoA reduktase	-7.5	3.671	2.343
Simvastatin-HMG CoA reduktase	-7.4	3.018	1.637
Simvastatin-HMG CoA reduktase	7.4	14.466	10.619
Simvastatin-HMG CoA reduktase	-7.2	83.708	80.626
Simvastatin-HMG CoA reduktase	-7.1	90.617	88.304

indonesian.biodivers.j.
Vol. 6, No. 1, April 2025
ISSN : 2722-2659

Table. 3 Interaction strength of fluvastatin compound against HMG CoA reductase target protein

Ligand	Binding Affinity	rmsd/ub	rmsd/lb
Fluvastatin-HMG CoA reduktase	-8.3	0.0	0.0
Fluvastatin-HMG CoA reduktase	-8.0	90.183	87.508
Fluvastatin-HMG CoA reduktase	-7.9	54.899	51.644
Fluvastatin-HMG CoA reduktase	-7.7	27.809	24.78
Fluvastatin-HMG CoA reduktase	-7.6	53.586	50.705
Fluvastatin-HMG CoA reduktase	-7.6	3.977	2.564
Fluvastatin-HMG CoA reduktase	-7.6	2.167	1.551
Fluvastatin-HMG CoA reduktase	-7.4	90.294	88.041
Fluvastatin-HMG CoA reduktase	-7.4	90.258	87.659

Based on the data table above, the binding affinity values of both natural and control compounds range from the lowest to the highest, where the binding affinity indicates the strength of the interaction between the ligand and the target protein. A more negative binding affinity value suggests that the ligand binds more easily to the target protein, as it requires less energy to form the interaction. According to the binding strength results obtained using PyRx 0.8 software, the compound with the highest binding affinity is fluvastatin, which binds to the target protein with the lowest energy requirement of -8.3 kcal/mol. In comparison, sterol compounds show a lower binding affinity, requiring a higher binding energy of -7.7 kcal/mol. While sterols are slightly less potent than the control compound in terms of binding affinity, their potential as safer, side-effect-free phytochemicals should be taken into consideration in the development of alternative antihypercholesterolemic agents.

1. Bond distances and amino acid residues in ligand and target protein interactions.

Visualization of intermolecular bonds using Discovery Studio 2016 Client software shows the bond distance and amino acid residues at the interaction of ligand and target protein. The results of 2D visualization with Discovery Studio 2016 Client application are as follows:

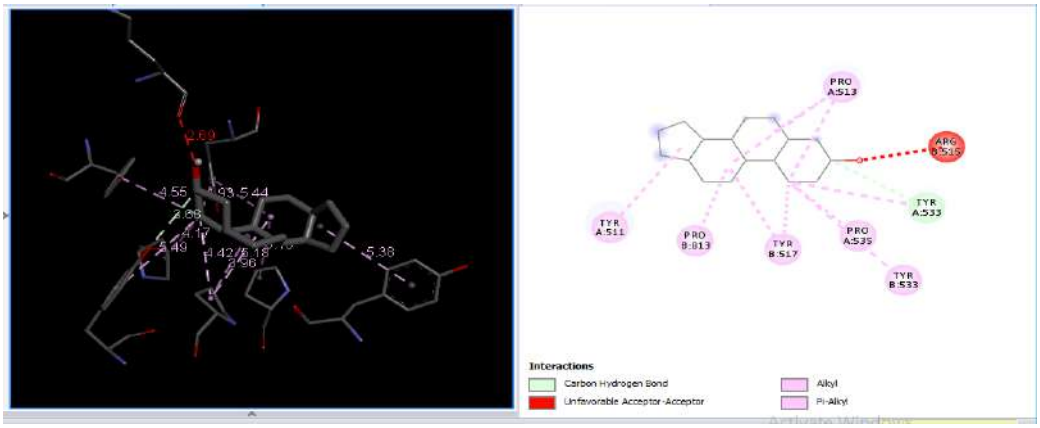


Figure. 3 2D visualization of bond distances and amino acid residues at the interaction of sterol and target protein (HMG CoA reductase)

indonesian.biodivers.j.
Vol. 6, No. 1, April 2025
ISSN : 2722-2659

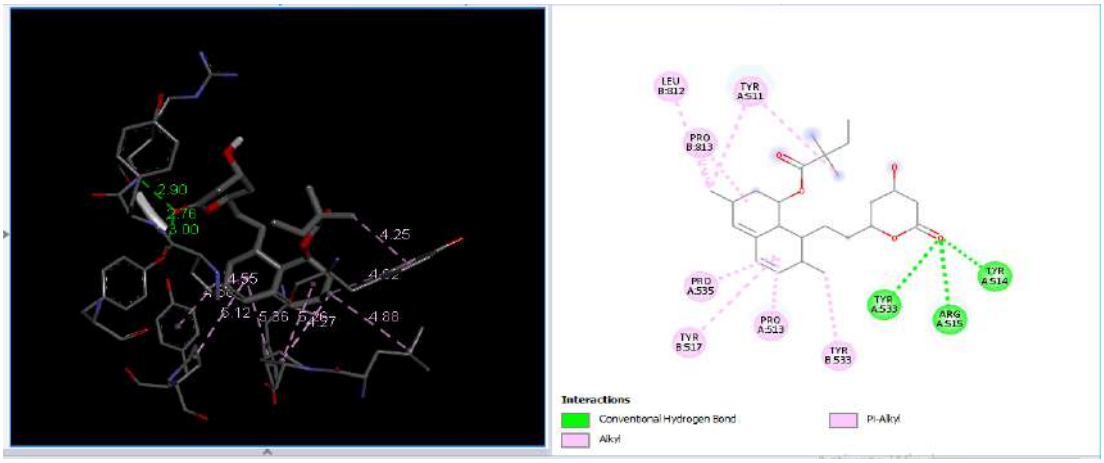


Figure. 4 2D visualization of bond distance and amino acid residues at the interaction of simvastatin and target protein (HMG CoA reductase)

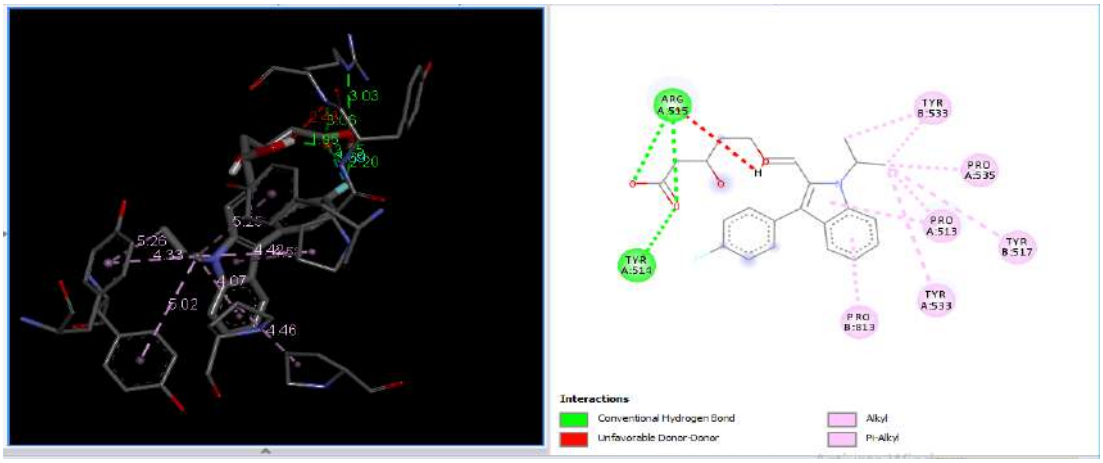


Figure. 5 2D visualization of bond distance and amino acid residues at the interaction of fluvastatin and target protein (HMG CoA reductase)

From the visualization results above, it is known that the bond distance between sterol compounds and target proteins (HMG CoA reductase) varies between 2.69 to 5.49 Armstrong with the chemical interaction types of hydrogen carbon, unfavorable acceptor, alkyl and pi-alkyl and it is known that the bond distance between simvastatin compounds and target proteins (HMG CoA reductase) varies between 2, 76 to 5.36 Armstrong with conventional hydrogen, alkyl and pi-alkyl chemical interactions while the bond distance between fluvastatin compounds with target proteins (HMG CoA reductase) also varies between 1.95 to 5.26 Armstrong with conventional hydrogen, unfavorable donor, alkyl and pi-alkyl chemical interactions. Meanwhile, based on the comparison of the binding side of strerol, simvastatin and fluvastatin, it is known that the three compounds have the same site because they bind to the same amino acid residues namely ARG (B: 515), TYR (A: 533), TYR (B: 533), TYR (B: 517), PRO (A: 511). This indicates that, sterols have the same potential as simvastatin and fluvastatin as anti-cholesterolemia drugs.

Although natural sterol compounds demonstrate similar potential to that of the control compounds,

indonesian.biodivers.j.
Vol. 6, No. 1, April 2025
ISSN : 2722-2659

it is also essential to determine their safety for human use. Therefore, toxicity testing was conducted using Toxtree v2.6.13 software, and the following results were obtained.

Table. 4 Predicted toxicological hazard (when administered orally) of molecular structure based on cramer's rule.

Class	Class Prediction of toxicological hazards
Low (Class I)	Substances with simple chemical structures and in which efficient modes of metabolism exist, exhibiting a low order of oral toxicity
Intermediate (Class II)	Substances that have a less hazardous structure than class I substances, but do not contain structural features indicative of toxicity like substances in class III
High (Class III)	Substances with chemical structures that allow no strong initial assumption of safety or may even exhibit significant toxicity or have reactive functional groups.

The results of *in silico* analysis using several web servers, databases, and software indicate that sterols, simvastatin, and fluvastatin are capable of binding to HMG-CoA reductase at the same binding site and through the same types of interactions, namely hydrogen bonds, alkyl, and π-alkyl bonds. This may explain the similarity in their binding energy values. Although the potential of sterol compounds is slightly lower than that of the control compounds, it is important to consider the numerous side effects associated with long-term use of synthetic drugs. Therefore, the use of natural remedies such as sterol-based phytochemicals is increasingly being promoted as a safer alternative in the management of hypercholesterolemia.

CONCLUSION

Based on the results of the *in silico* analysis, it can be concluded that the natural sterol compounds found in Tin (*Ficus carica*) leaves have potential as antihypercholesterolemic agents. This conclusion is supported by reverse docking results, which show that sterol compounds bind to the same active site as the control compounds, simvastatin and fluvastatin, with comparable binding affinities. The sterols exhibited binding affinities approaching –8.0 kcal/mol to the target protein HMG-CoA reductase, indicating relevant bioactive potential.

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indonesian.biodivers.j.
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