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Anti-atherosclerosis potential activity from Alphitobius diaperinus: a molecular docking study on NLRP3 receptor

Potensi aktivitas anti-aterosklerosis dari ulat kandang Alphitobius diaperinus: studi molecular docking pada reseptor NLRP3

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ABSTRACT

This study aimed to analyze the potential activity anti-atherosclerosis of Alphitobius diaperinus larvae using molecular docking. The target receptor used was NLRP3, with β-sitosterol and campesterol as the test ligands, and simvastatin as the control ligand. The results showed that the binding affinity energies of β-sitosterol and campesterol were more negative than simvastatin, with values of -9.3, -9.3, and -8.6 kcal/mol, respectively. The inhibition constants of β-sitosterol and campesterol were lower than that of simvastatin, with values of 0.213, 0.213, and 0.494 μM, respectively. The percentage of binding site similarity with the native ligand for β-sitosterol and campesterol was 60%, while simvastatin showed 0%. Molecular probability analysis using Lipinski's rules revealed that simvastatin met all five criteria, whereas β-sitosterol and campesterol met four criteria. The LD50 analysis indicated that simvastatin fell under category III, while β-sitosterol and campesterol were in category I. All three molecules β-sitosterol, campesterol, and simvastatin were found to be non-carcinogenic. The conclusion of this study was β-sitosterol and campesterol from A. diaperinus larvae have potential as anti-atherosclerosis candidates.

Kata kunci:
Larva Alphitobius diaperinus
Aterosklerosis
Molekular docking

ABSTRAK

Penelitian ini bertujuan untuk menganalisis potensi aktivitas anti-aterosklerosis dari ulat kandang Alphitobius diaperinus melalui pemodelan molecular docking. Target reseptor yang digunakan adalah NLRP3. Ligan uji yang digunakan adalah β-sitosterol dan campesterol dengan simvastatin sebagai ligan pembanding. Hasil penelitian menunjukkan bahwa energi afinitas β-sitosterol dan campesterol lebih negatif dibandingkan simvastatin, dengan nilai masing-masing -9,3; -9,3; dan -8,6 kcal/mol. Konstanta inhibisi β-sitosterol dan campesterol lebih rendah dibandingkan simvastatin, yaitu 0,213; 0,213; dan 0,494 μM. Persentase binding site similiarity dengan ligan alami, β-sitosterol dan campesterol sebesar 60%, sedangkan simvastatin 0%. Analisis probabiltas molekul dengan aturan Lipinski, simvastatin memenuhi lima kriteria, sementara β-sitosterol dan campesterol memenuhi empat kriteria. Analisis LD50 menunjukkan simvastatin termasuk dalam kategori III, sedangkan β-sitosterol dan campesterol termasuk dalam kategori I. Molekul β-sitosterol, campesterol, dan simvastatin bersifat non-karsinogenik. Kesimpulan dari penelitian ini adalah β-sitosterol dan campesterol dari ulat kandang A. diaperinus berpotensi sebagai kandidat anti-aterosklerosis.

INTRODUCTION

Atherosclerosis is a progressive inflammatory condition characterized by the accumulation of lipids on the arterial walls (atherosclerotic plaque). The buildup of atherosclerotic plaque leads to the narrowing of the arterial lumen, increasing the risk of blood vessel rupture and thrombosis. In addition, atherosclerotic plaques are associated with cardiovascular disease, myocardial infarction, and stroke, which are the leading causes of death worldwide. In Indonesia, the mortality rate from cardiovascular disease, stroke, and atherosclerotic diseases is notably high, with an estimated 470,000 deaths annually. Atherosclerotic cases have also been observed in animals such as rats, rabbits, pigs, and primates (Hussain et al. 2016).

The development of atherosclerosis was influenced by several factors, including genetics, lifestyle, and inflammatory processes within the body (Gusev and Sarapultsev 2023). Inflammation plays a key role in the pathogenesis of atherosclerosis, with the nucleotide-binding oligomerization domain-like receptor family pyrin domain containing 3 (NLRP3) inflammasome acting as a key receptor responsible for this inflammation. The increasing prevalence of atherosclerosis, along with a more detailed understanding of its pathogenesis, has attracted researchers to explore prevention and treatment solutions for the disease. In particular, the exploration of bioactive compounds from natural resources has gained significant attention, especially those that modulate NLRP3 activity, as they are believed to hold great potential as anti-atherosclerosis drug candidates.

Insects are one such natural resource, known for their diverse bioactive compounds, including amino acids, fatty acids, vitamins, minerals, and antioxidants that exhibit anti-inflammatory, anti-diabetic, antihypertensive, and antilipidemic properties. Alphitobius diaperinus (A. diaperinus), commonly known as the lesser mealworm, is an insect considered parasitic and a potential threat to livestock and agriculture. However, recent research has shown increased interest in utilizing A. diaperinus as an innovative protein source in animal feed. The high antioxidant content in A. diaperinus suggests its potential as an anti-atherosclerosis drug candidate. Research on the anti-atherosclerosis activity of the lesser mealworm is still limited, particularly in the context of NLRP3 inhibition through molecular docking studies, which have yet to be conducted. Thus far, the exploration of natural resources for anti-atherosclerosis drug candidates have largely focused on synthetic compounds or plant extracts. Therefore, further exploration of the potential anti-atherosclerosis activity of A. diaperinus using a computational molecular docking approach on the NLRP3 receptor is necessary.

Molecular docking was an insilico research method used to predict the interaction between small molecules and target proteins (Aritonang 2024). Through this approach, bioactive compounds from A. diaperinus that effectively inhibit NLRP3-induced inflammation may be identified. The results of this study could serve as a reference for further in vitro and in vivo studies using animal models.

MATERIALS AND METHODS

Molecular Docking Computation

This study was an experimental research using computational molecular docking modelling. The molecular docking simulations were conducted using Discovery Studio (v17.2.0.16349), AutoDockTools (v.1.5.6), AutodockVina (v.1.5.6), and command prompt applications. Three-dimensional visualizations were performed using PyMOL software (v.2.5.4). Ligand probability analysis was conducted via the Lipinski rules website (SCFBio) (http://www.scfbio-iitd.res.in/software/drugdesign/lipinski.jsp) (Jayaram et al. 2012; Lipinski 2004). Information on the biological content of A. diaperinus and the atherosclerosis target receptor was obtained from literature reviews of various scientific articles. The receptor used in this study was the nucleotide-binding oligomerization domain-like receptor family pyrin domain containing 3 (NLRP3) inflammasome with PDB ID 8ETR. The native ligand bound to the receptor is 6,7-dihydro-5H-pyrazolo[5,1-b][1,3]oxazinesulfonylureas. The receptor protein structure was downloaded from the RCSB PDB website (https://www.rcsb.org/). The test ligands used are β-sitosterol and campesterol, which are compounds found in A. diaperinus (Siddiqui et al. 2024). The control ligand used

simvastatin. The chemical structures of the test and control ligands were downloaded from the PubChem website (https://pubchem.ncbi.nlm.nih.gov/).

The computational molecular docking method in this study was divided into five stages. The first stage was the selection of the target receptor, control ligand, and test ligands. The second stage involved docking preparation, where water molecules were removed, and hydrogen atoms were added. The grid box dimensions used for docking were set at 30 with a spacing of 1 angstrom. The third stage was validation through the calculation of binding affinity energy (ΔG) and root mean square deviation (RMSD) values. The fourth stage involved docking the test and control ligands, followed by analyzing the ΔG values of each ligand. The ΔG value for the native ligand was analyzed based on the first (smallest) conformation, while the ΔG values for the control and test ligands were based on the second conformation (Trott and Olson 2010). The fifth stage was model validation and the visualization of two- and three-dimensional structures.

Molecular Probability and Safety Testing

The molecular probability test was conducted using the five Lipinski's rules (Ro5) on the native, test, and control ligands. The Ro5 criteria consist of five requirements: a molecular weight of less than 500 Daltons, lipophilicity < 5, hydrogen bond donors < 5, hydrogen bond acceptors < 10, and molar refractivity between 40 and 300. The safety analysis of the molecules was conducted through acute oral toxicity LD50 testing and carcinogenicity testing. The stability of the molecules was analyzed by uploading the ligand file in PDB format through the SCFBio website(-Jayaram et al. 2012; Lipinski 2004).

The safety assessment was performed by classifying molecules based on their acute oral toxicity lethal dose 50 (LD50) and carcinogenicity. The acute oral toxicity LD50 test was divided into four categories: category 1 (LD50 occurs with compound doses ≤ 50 mg/kg), category 2 (LD50 occurs with doses > 50 mg/kg, but < 500 mg/kg), category 3 (LD50 occurs with doses > 500 mg/kg, but < 5000 mg/kg), and category 4 (LD50 occurs with doses > 5000 mg/kg). The toxicity test was categorized into carcinogenic and non-carcinogenic. The molecular safety analysis was conducted by uploading the ligand file in SMILES format through the admetSAR website (http://lmmd.ecust.edu.cn/admetsar1/predict/) (Cheng et al. 2012).

Model Quality Analysis

Model validation was carried out by comparing the values of ΔG, Ki, bonding categories (hydrogen and hydrophobic bonds), and %BSS (binding site similarity). The Ki value was calculated using the equation ΔG = RTlnKi (R = 1.986 cal/mol·K, T = 298.15 K) (Kalontong, Safithri, and Tarman 2022). The %BSS was calculated by dividing the number of amino acid bonds of the test ligand that matched the native ligand by the total number of amino acid bonds of the native ligand, then multiplying by 100%. Molecular probability analysis states that a molecule was considered drug-like and safe if it meets at least 3 out of the 5 Ro5 criteria. The safety analysis of the molecule was conducted by comparing the LD50 category and carcinogenicity of the native, test, and control ligands. The results for the model validation variables in molecular docking were analyzed descriptively.

RESULTS AND DISCUSSION

The ΔG values for β-sitosterol and campesterol showed more negative values compared to simvastatin, the control ligand. The ΔG values for β-sitosterol and campesterol exhibited only a small difference from the native ligand (Table 1). ΔG serves as a predictor of a ligand's binding affinity to a receptor. More negative ΔG values indicated stronger binding capabilities with the receptor (Aritonang et al. 2024). Based on the docking ΔG values, β-sitosterol and campesterol demonstrated better binding abilities to the NLRP3 receptor compared to simvastatin. The strong binding potential between β-sitosterol and campesterol to the NLRP3 receptor suggests their ability to reduce the expression and migration of the receptor, potentially lowering atherosclerosis effects in patients.

The Ki values for β-sitosterol and campesterol were smaller than simvastatin's (Table 1). Ki was a predictor of a compound's ability to inhibit a receptor, with smaller Ki values indicated a stronger inhibitory capacity (Umamaheswari, Madeswaran, and Asokkumar 2013). Based on

the docking Ki values, β-sitosterol and campesterol exhibited better inhibition of the NLRP3 receptor than simvastatin. This higher inhibition potential of the NLRP3 receptor may contribute to reduce the occurrence of atherosclerosis in patients.

The amino acid bonds between the test ligands and the NLRP3 receptor were categorized into two types: hydrogen bonds and hydrophobic interactions. Hydrogen bonding of the native ligand at the receptor's active site occurred at ARG167 and WTN703. β-Sitosterol formed one hydrogen bond, whereas campesterol did not form any. The hydrogen bond of β-sitosterol did not correspond to the native ligand's hydrogen bond at the receptor's active site. Simvastatin formed three hydrogen bonds, but none matched the native ligand's bonds (Table 1). Hydrogen bonds were relatively strong form of interaction between a receptor and a ligand, mediated by hydrogen ions (Aritonang 2024).

The native ligand's hydrophobic interactions at the active site occurred at ILE234, PRO412, and WTN703. β-Sitosterol formed nine hydrophobic interactions, with three matching those of the native ligand at the receptor's active site (ILE234, ARG167, and PRO412). Campesterol formed eight hydrophobic interactions, also matching the native ligand at ILE234, ARG167, and PRO412. Simvastatin formed 14 hydrophobic interactions, but none matched the natural ligand at the active site (Table 1). Hydrophobic interactions reflected the stability of the bond between a ligand and a receptor (Aritonang 2024). Based on the hydrophobic bond analysis, β-sitosterol and campesterol demonstrated better bonding stability with the NLRP3 receptor than simvastatin.

The %BSS values for β-sitosterol and campesterol were 60%, which was mean that 60% of the amino acid bonds formed by these ligands were similar to those of the native ligand. Simvastatin had a %BSS value of 0%, indicating no similarity with the native ligand at the receptor's active site (Table 1). The relatively high %BSS values for β-sitosterol and campesterol compared to simvastatin suggest their better binding potential. These higher %BSS values compared to the commercial drug simvastatin indicated the potential of β-sitosterol and campesterol from A. diaperinus as a candidate for anti-atherosclerosis drugs. The %BSS served as an indicator of amino acid similarity between the natural ligand and the test ligand at the receptor's active site (Klara, Purwono, and Achmadi 2023). The %BSS closer to 100% indicated a higher similarity to the native ligand at the receptor's active site.

Table 1. Molecular docking modeling variables for the native, test, and control ligand with the NLRP3 receptor.

Value of model quality variables	Native ligand (control)	Test ligand		Simvastatin (control)
		β-sitosterol	Campasterol	
ΔG (kcal/mol)	-9,4	-9,3	-9,3	-8,6
Ki (μM)	0,128	0,213	0,213	0,494
Hydrogen bonds	ARG167, WTN703	GLU511	-	ARG578, GLN624, GLU629
Hydrophobic bonds	ILE234, PRO412, WTN703	ILE234*, PRO412, LEU413, ILE151, ARG167*, PRO412*, PHE373, TYR381, TRP416	ILE234*, PRO412*, LEU413, ILE151, ARG167*, PHE373, TYR381, TRP416,	ALA227, ALA228, MET408, ILE411, MET661, ILE411, MET408, MET661, LEU 403, VAL414, PHE410, PHE575, PHE579, TYR632
%BSS	100	60	60	0

Note: *indicates the similarity of amino acids that bind at the active site of the ligand with the receptor.

The variable of molecules with a mass > 500 Dalton would have difficulty penetrating cell membranes. The research results indicated that all test molecules meet the Ro5 criteria. The lipophilicity variable of a molecule was an indicator of its ability to dissolve in fats or water. Higher lipophilicity mean it was more difficult to dissolve in fats or water and may potentially cause toxic

effects due to the molecule being trapped in the lipid bilayer. The results showed that only simvastatin meets the lipophilicity criterion of < 5. The donor and acceptor hydrogen bond variables were indicators of the energy requirement in the absorption process. The greater the hydrogen bond capacity of a molecule, the more energy was needed during absorption. The molar refractivity of a molecule was an indicator of its ability in the absorption mechanism. Higher refractivity means the molecule will be more difficult to absorb in the body. According to the research, all test ligands met the Ro5 criteria for hydrogen bonding and molar refractivity (Klara et al. 2023; Muhammad, Rahmayanti, and Isfanda 2021).

The molecular probability test results indicated similarity in meeting the Ro5 criteria between the test ligands and the native ligand, with four criteria fulfilled. Simvastatin, as a control ligand, met all five Ro5 criteria (Table 2). A molecule was considered to have good probability if it met ≥ 3 out of 5 criteria. Ro5 was a molecular analysis method to predict a molecule's probability as a drug candidate, its solubility, and oral permeability which were related to its similarity to a drug molecule. Drug molecules that meet the Ro5 criteria are predicted to easily penetrate digestive membranes, cell membranes, and reach target receptors. Based on the molecular probability test results, it was known that β-sitosterol and campesterol have a good probability for development as safe drug candidates with effective potential for oral administration to patients. Simvastatin has a good probability value as it meets all Ro5 requirements so that it was a drug widely used as an anti-atherosclerosis therapy.

The acute oral toxicity variable LD50 indicated that the native ligand and simvastatin fall into category III, meaning that LD50 can occur at doses > 500 mg/kg but < 5000 mg/kg. Meanwhile, β-sitosterol and campesterol fall into category I, indicating that LD50 can occur at doses ≤ 50 mg/kg (Table 3). These results suggest that β-sitosterol and campesterol should be further studied in research involving animal trials at low doses, not exceeding 50 mg/kg. LD50 was an acute toxicity test that caused death in 50% of test animals (Sulastra, Khaerati, and Ihwan 2020). The carcinogenicity variable showed that all tested ligands have non-carcinogenic activity (Table 3). Carcinogenic substances could trigger the onset of cancer when consumed (Irmawan et al. 2023). The safety testing of β-sitosterol and campesterol indicated their potential for development but still requires further research to determine their activity in vitro or in vivo.

Table 2. Molecular probability testing with Lipinski's five rules (Ro5)

Variabel Ro5	Native ligand	Test ligand		Simvastatin (control)
		β-sitosterol	Campasterol	
Molecular weight (Dalton)	406	414	400	418
Lipofilitas (LogP)	25,836	8,024	7,634	4,582
Hydrogen donor bonds	0	1	1	1
Hydrogen acceptor bonds	9	1	1	5
Molar refractivy	207,599	128,216	123,599	115,454
Fulfillment of the Ro5	4	4	4	5

Tabel 3. Molecular safety testing on native, test, and control ligands

Safety variabel	Native ligand	Test ligand		Simvastatin (control)
		β-sitosterol	Campasterol	
Acute oral toxicity categories (LD50)	III	I	I	III
Carcinogenic	Non carcinogenic	Non carcinogenic	Non carcinogenic	Non carcinogenic

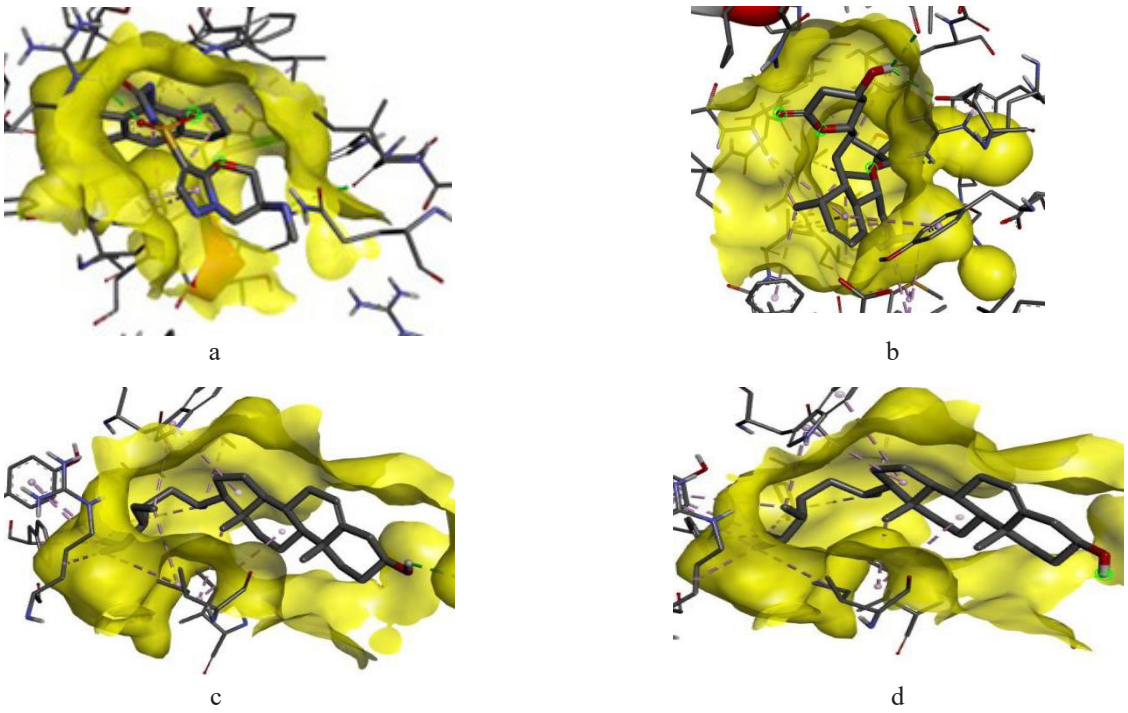

Figure 1. Two-dimensional interactions of ligands with the receptor: naturanative ligand (a), simvastatin (b), β-sitosterol (c), and campasterol (d).

Three-dimensional visualization showed that all tested ligands (native, β-sitosterol, campasterol, and simvastatin) have interactions or bonds with the NLRP3 receptor. The area was colored yellow represented the ligands. The area outside the yellow region was the receptor, while the yellow area indicated the active site where the interaction between the ligands and the receptor occured.

CONCLUSIONS

β-sitosterol and campasterol from the A. diaperinus larvae showed potential as anti-atherosclerosis agents based on computational molecular docking modeling, evaluated through the variables of ΔG values, Ki, binding categories, molecular probability testing using the Ro5 method, LD50 category, and carcinogenicity. Further research in vitro and in vivo on animal models is needed for a comprehensive evaluation.

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