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Original Research Article

Molecular docking of sterol derivatives in *Tagetes erecta* Linn. as an antiatherosclerotic agents through activation of PPARγ and LXRα receptors

I Wayan Surya Rahadi - *https://orcid.org/0009-0006-7858-4749*
Department of Chemistry, Faculty of Pharmacy, Universitas Mahasaraswati Denpasar, Denpasar 80233, Indonesia

Ni Kadek Ayu Mas Ratnadewi
Department of Phamacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Mahasaraswati Denpasar, Denpasar 80233, Indonesia

Ketut Agus Adrianta - *https://orcid.org/0000-0003-1837-3705*
Department of Phamacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Mahasaraswati Denpasar, Denpasar 80233, Indonesia

Corresponding authors: I Wayan Surya Rahadi: suryarahadi@unmas.ac.id)

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ABSTRACT

Atherosclerosis is a condition characterized by inflammation in the arteries, which is linked with the accumulation of lipids and alterations in metabolism. Considering that atherosclerosis is the main factor causing death in the world, it is necessary to carry out immediate prevention and treatment to reduce the risk of developing clinical severity. The creation of foam cells, which originate from macrophages, is considered a key element in cardiovascular ailments, particularly in the advancement of atherosclerosis. Two types of the nuclear receptors known as Peroxisome proliferator-activated receptor γ (PPARγ) and liver X receptor α (LXRα), which serve as a primary regulator of cholesterol, intracellular lipid homeostasis and they are instrumental in the process of reverse cholesterol transport (RCT). Activation of these receptors could potentially decrease foam cell formation, consequently lowering the risk of atherogenesis and reducing cardiovascular disease risk. This research aims to determine sterol derivative compounds in Tagetes erecta Linn. which have the best interaction and potential as anti-atherosclerosis through peroxisome proliferator-activated receptor γ (PPARγ) and liver X receptor α (LXRα) activation. The analysis of this study is using docking molecular analysis. The parameters observed in this study were docking score, visualization results, absorption, distribution, metabolism, excretion profile, and toxicity value. The molecular docking outcomes indicate that β-sitosterol and 7β-hydroxysitosterol possess the most favorable binding energy values. They exhibit a positive pharmacokinetic profile, with the exception of gastrointestinal absorption and respiratory toxicity.

Introduction

Atherosclerotic disease occurs due to the accumulation of plaque in the walls of arteries. Atherosclerotic plaque formation consists of four stages, starting with endothelial damage, migration of Low Density Lipoprotein (LDL) into the intima, inflammatory response, and fibrous cap formation (Pramatama et al., 2014). The initial phase of atherosclerosis is marked by the accumulation and modification of LDL, which is oxidized to form oxLDL, triggering endothelial inflammation. This is followed by the migration of monocytes into the tunica intima, where they transform into macrophages and phagocytize oxidized LDL (oxLDL) via scavenger receptors. This procedure leads to the creation of foam cells, which over time transform into fatty streaks. The activation of this system leads to the production of cytokines and growth factors, which in turn stimulate the growth and movement of smooth muscle cells from the tunica media to the tunica intima. This can lead to plaque enlargement, fibrous cap formation, and ultimately, atherosclerosis (Ramadhian and Rahmatia, 2017).

LXR is a transcription factor that is part of the extensive family of Nuclear Receptors (NRs), which serves as a primary regulator of cholesterol and intracellular lipid homeostasis. LXR serves as the primary cholesterol sensor, addressing excess sterols by enhancing the regulation of ATP Binding Cassette (ABC) transporters, including ATP Binding Cassette A1 (ABCA1) and ATP Binding Cassette G1 (ABCG1, which subsequently leads to the formation of High Density Lipoprotein (HDL) from macrophages (Franceschelli et al., 2023). The Peroxisome Proliferator Activated Receptor Gamma (PPARγ) has the ability to boost cholesterol efflux by triggering the transcription of the LXRα gene. Activated PPARγ leads the expression of ABCA1, ABCG1, and SR-B1, which are associated with increased cholesterol efflux from macrophages through a transcriptional cascade mediated by LXRα (Fruchart et al., 2019). The role of ABCA1 and ABCG1 in maintaining cholesterol homeostasis is widely acknowledged, the expression of ABCA1 and ABCG1 can speed up the efflux of cholesterol (Lin et al., 2018).

Tagetes erecta Linn. also known as the Gemitir flower or Marigold flower, is a plant that has anti-atherosclerosis potential. Gemitir flowers are widely cultivated as traditional medicine, ornamental plants, and natural dyes. Gemitir flowers

in Bali are used as a traditional ceremonial offering, namely canang. There are 22 phytochemical components of the ethanol extract of Gemitir flower compounds which were tested using silica gel column chromatography, including daucosterol, erythrodiol-3palmitate, β-sitosterol, ethylene glycollinoleate, 7β-hydroxysitosterol, erythrodiol, 1-[5-(1-propyn-1-yl)[2, 2-bithiophen]-5-yl]-ethanone, α-terthienyl, lupeol, quercetagetin, quercetagetin-7-O-glucoside, quercetagetin-7-methylether, syringic acid, gallic acid, 3-α-galactosyl disyringic acid, 3-βgalactosyl disyringic acid, 6-ethoxy-2, 4-dimethylquinoline, oplodiol, (3S, 6R, 7E)-hydroxy4,7-megastigmadien-9-one, kaempferol, n-hexadecane, and palmitin (Singh et al., 2020).

Based on research conducted by Kresnapati et al. (2021), Gemitir flower ethanol extract at a dose of 200mg/kg and 400mg/kg induced in Rattus norvegicus can reduce the value of total cholesterol, Low Density Lipoprotein (LDL), malondialdehyde (MDA), and Apolipoprotein B (Apo B) significantly (p<0, 05). Previous research may support the potential of sterol derivatives in Tagetes erecta L. to be developed as anti-atherosclerosis.

The initial stage in the discovery of new drugs which is currently widely studied is in silico interaction studies which begin with molecular docking (Damayanti et al., 2021). Molecular docking studies are one of the methods that can be used to discover new drugs. Molecular docking is a computational process of searching for ligands that match protein binding sites. This method is used to imitate the interaction of a ligand molecule with the target protein from in vitro test through a computer simulation model. Research using sterol derivatives in gemitir flower (*Tagetes erecta* Linn) compounds for anti-atherosclerosis has never been carried out before, so this research can be used as a starting point to find out more about the sterol derivatives in *Tagetes erecta* Linn which have the potential to activate LXRα and PPARγ receptors.

Methods

Ligand and Protein Preparation

The four sterol derivative compounds isolated from *Tagetes erecta* Linn. were chosen as tested ligands in the study. The 3D structures of each ligand were downloaded on the PubChem website. Ligand preparation started with hydrogen added and Gasteiger charges. The protein structure used in this research was PPARγ (PDB ID: 8HUP) and LXRα (PDB ID: 3IPQ) downloaded from Protein Data Bank (RSCB PDB) (https://www.rcsb.org). Each protein substrate was prepared to dock using AutoDockTools 1.5.7. by removing unnecessary amino acid chains, removing water molecules, hydrogen added, and addition of Kollman charges. The grid box coordinate used is 43.079 X 16.295 X -6.463, the grid center coordinate are 46, 22, 24 (xyz coordinate) for PDB ID: 3IPQ and 4.661 X 2.342 X -11.773, the grid center coordinate are 40,26,18 (xyz coordinate) for PDB ID: 8HUP.

Molecular Docking

Data obtained from the results of this study stated the bond energy strength, the number of hydrogens, and the number of hydrophobic interactions. The docking procedure was configured to yield the top 100 conformations utilizing the Lamarckian Genetic Algorithm incorporated in the AutoDock suite. The optimal conformation was selected according to the minimum binding energy value, and the 2D interaction representation was created using the Discovery Studio Visualizer software.

Validation Method

The validation of molecular docking in this research utilizes the overlay method with the Pymol application. The overlay method involves ensuring that the conformation of the original ligand perfectly aligns with the crystallographic ligand (Shah et al., 2019). This validation aims to assess the performance of the docking program to avoid deviations or errors. The Root Mean Square Deviation (RMSD) value is a common way to measure the average distance between the ligands from redocking results and the crystallographic ligands. The commonly accepted maximum limit for RMSD to evaluate the docking program's capability is 2Å (Rimac et al., 2021). A smaller RMSD value (approaching zero), indicates a higher similarity to the original ligand.

ADME and Toxicity Properties

Each of the ligands was transformed into SMILES format utilizing the Open Babel software, and subsequently, they were individually submitted to the ADMETLab and SwissADME web servers. The software supplied the data, which included the outcome of the Lipinski rule of five computations, as well as absorption, distribution, metabolism, excretion, and toxicity data for every ligand.

Results and Discussion

Molecular Docking Analysis

Sterols are type of steroid compound characterized by a hydroxyl group at the C-3 atom of the A ring. Four sterol derivatives have been identified in *Tagetes erecta* Linn., namely β-sitosterol, daucosterol, erythrodiol, and 7β-hydroxysitosterol. The structures of the macromolecules were obtained from the Protein Data Bank (PDB), with specific macromolecules used for the LXRα receptor (PDB ID: 3IPQ) and the PPARγ receptor (PDB ID: 8HUP). The resolution value for 8HUP is 2.36Å, while for 3IPQ, is 2.00Å. Both of these resolution values are in the category of good resolution. Generally, lower resolution values indicate better defined structures (Fatimah et al., 2020).

The preparation of macromolecules begins with the removal of unnecessary chains, elimination of water molecules, the addition of hydrogen, and the incorporation of Kollman charges. Water molecules have the potential to interfere with the binding process between the ligand and the receptor by forming hydrogen bonds with the receptor. Therefore, macromolecular preparation always starts with the removal of water molecules (Suwandi et al., 2023). Hydrogen atoms are

added to enhance the interactions between the ligand and the protein. Furthermore, the addition of hydrogen can ensure that the bonds formed during molecular docking have the same conditions with the body's pH (pH 7) (Suwandi et al., 2023). The addition of Kollman charges aims to assign values to each amino acid, then the macromolecule can adapt to the computational environment. Additionally, the incorporation of Gasteiger charges during ligand preparation serves to optimize the interactions between the ligand and the receptor (Prasetiawati et al., 2023).

Based on the validation method, LXRα protein has an RMSD value of 1.769Å, while the PPARγ protein has an RMSD value of 0.000. This indicates that both specific proteins have crystallographic ligands closely similar to the original ligands and have been validated. The grid box settings serve to prevent changes in the binding site (Ravi and Krishnan, 2016). The stability parameter under observation is the Gibbs free binding energy (ΔGbinding), which signifies the free energy involved in the interaction between the ligand and a particular protein. Table 1 displays the binding energy measurement results for both the test ligand and the original ligand against the LXRα and PPARγ receptors. A smaller or negative value of ΔGbinding implies a more stable interaction between the ligand and the protein, indicating the formation of a stronger bond (Weni et al., 2020). The ΔGbinding parameter is considered good if it has a value ≤ -4.0 kcal/mol.

Table 1. Molecular docking results of ligands through LXRα and PPARγ receptor

Macromolecul	Tested Ligand	$\Delta G_{binding}$ (kkal/mol)
LXRα	965*	-14.93
	T0901317**	-8.14
	β-sitosterol***	-13.37
	Daucosterol***	-6.77
	Erythrodiol***	-8.20
	7β-hydroxysitosterol***	-13.46
PPARγ	KKB*	-7.90
	Pioglitazone**	-8.34
	β-sitosterol***	-9.69
	Daucosterol***	-5.71
	Erythrodiol***	+3.79
	7β-hydroxysitosterol***	-9.32

* = Original Ligand; ** = Positif Control; *** = Compound

LXRα agonists can be used as a therapy for atherosclerosis by reducing foam cells within macrophages. An imbalance or dysregulation of LXRα within the body can potentially lead to the onset of metabolic disorders. This includes conditions such as hyperlipidemia and atherosclerosis. It is important to maintain the regulation of LXRα to prevent these health complications (Arifuzzaman et al., 2023). According to Tsou et al. (2014), 3-(5′-hydroxymethyl-2′-furyl)-1-benzyl indazole (YC-1), a synthetic activator of soluble guanylyl cyclase (sGC), led to an increase in ABCA1 expression induced by YC-1. This coincided with elevated levels of LXRα, resulting in the inhibition of foam cell formation. The findings of the current study suggest that andrographolide might be a potential contender for preventing atherosclerosis. This is achieved by increasing the mRNA and protein expression of ABCA1 and BCG1, which in turn leads to the prevention of macrophage foam cell formation induced by oxLDL (Lin et al., 2018).

The molecular docking results for the positive control T0901317, which is a chemical agonist of LXRα, show a ΔGbinding value of -8.14 kcal/mol. In comparison, the sterol derivatives from gemitir flowers against the specific protein 3IPQ indicate that 7β-hydroxysitosterol and β-sitosterol have the lowest ΔGbinding values, -13.46 and -13.37 kcal/mol, respectively. When compared to the original ligand, these values are slightly higher. The original ligand 3IPQ has a ΔGbinding of -14.94, indicating that the compounds 7β-hydroxysitosterol and β-sitosterol can bind to the 3IPQ protein, but the formed bonds are not more stable than the original ligand. The research conducted by Susanti (2019) by testing compounds Hibiscetin 3-glucoside, Gossypitrin, Quercetin, and Luteolin found in Hibiscus sabdariffa as LXRα agonists, it is revealed that Gossypitrin has the lowest ΔGbinding value, which is -9.1 kcal/mol. This binding energy value is still higher compared to the compounds 7β-hydroxysitosterol and β-sitosterol.

Increased ligands with agonistic activity towards the PPARγ receptor stimulate the formation of dimeric structures with the Retinoid X Receptor (RXR), followed by conformational changes in the receptor that form a transcription complex to activate target genes (Khotimah et al., 2020). Activation of PPARγ can inhibit the development of atherosclerosis through various mechanisms, which is by enhancing reverse cholesterol transport. The molecular docking outcomes of the test compounds against the specific receptor 8HUP indicate that both 7β-hydroxysitosterol and β-sitosterol have lower ΔGbinding values compared to the original ligand and pioglitazone as a positive control. This suggests that these two compounds can bind to the 8HUP protein and form more stable bonds than the original ligand.

Visualization Result

The identification of ligand interactions in the active pocket is crucial. This aims to illustrate the types of bonds formed between the ligand and the target protein. Some interactions interactions encompass hydrogen bonds, hydrophobic associations, and electrostatic connections, these are key in understanding the relationship between the ligand and its target protein. Comparing hydrogen bonding patterns between the test ligand and the reference ligand reveals similarities in the types of interactions, which is related to the similarity in activity between the original ligand and the test ligand. Fig-1 and Fig-2 show that a bond forms between the ligand and various amino acid residues on the LXRα and PPARγ receptors. Compounds 7β-hydroxysitosterol and β-sitosterol create hydrogen bonds with the amino acid residue GLU267 in the protein 3IPQ. However, β-sitosterol does not form a hydrogen bond with any amino acid residues in the protein 8HUP. The hydrogen bond formed by the compound 7β-hydroxysitosterol with the specific protein 8HUP involves CYS285, and this amino acid

residue is also present in the original ligand and the positive control (pioglitazone). According to Gholam (2022), hydrogen bonds formed with amino acid residues can maintain the stability of molecular complexes, thereby stimulating the biological response to the target protein.

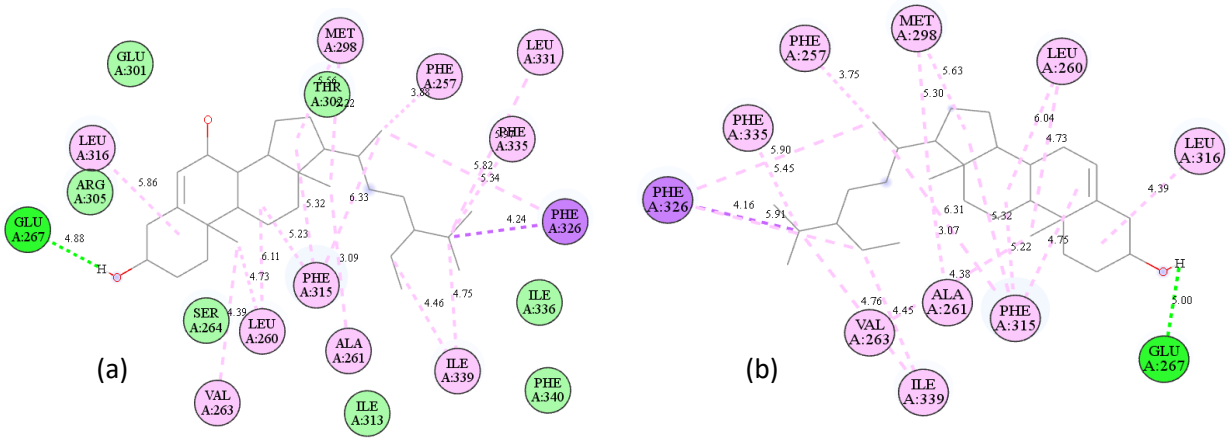

Fig-1. 2D interaction of ligands (a) β-sitosterol and (b) 7β-hydroxysitosterol against LXRα receptor

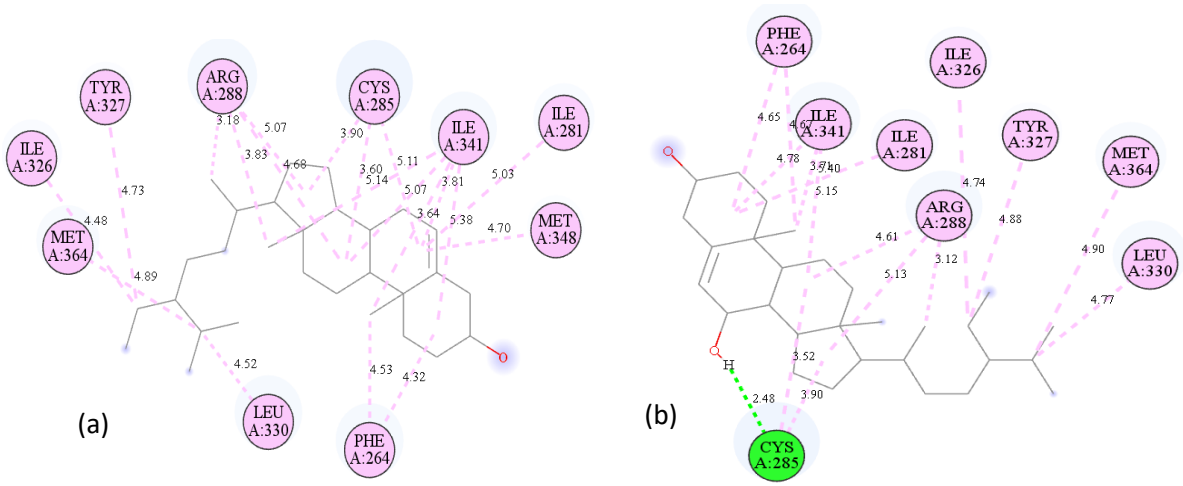

Fig-2. 2D interaction of ligands a) β-sitosterol and b) 7β-hydroxysitosterol against PPARγ receptor

The presence of hydrophobic interactions and electrostatic interactions can enhance the stability of conformation (Naufa et al., 2022). Hydrophobic interactions are a type of interaction that tends to avoid aqueous environments and prefers to gather within the globular protein structure. The formation of hydrophobic bonds aims to reduce interactions between non-polar residues and water. Types of hydrophobic interactions include pi-alkyl, alkyl, pi-pi stacked, pi-pi T-shaped, and pi-sigma (Arifin and Febriansah, 2022). The hydrophobic interactions of β-sitosterol and 7β-hydroxysitosterol compounds with the same protein 3IPQ complex as the original ligand complex and positive control is ILE339. There are three residues which results from the interaction from both compounds with 8HUP protein, these include ILE281, ARG288, and ILE341. Additionally, the pi-sigma interaction occurring between the compounds 7β-hydroxysitosterol and β-sitosterol with the amino acid residue PHE326 against protein 3IPQ is also considered a hydrophobic interaction.

ADME and Toxicity Properties

In recent drug development and discovery, the evaluation of drug-like properties in early stages of the research process has been conducted. This evaluation involves various approaches, such as Lipinski's Rules of Five. Lipinski's Rules of Five is a simple set of guidelines aimed at assessing the similarity of a chemical compound with specific pharmacological or biological activities that make it active through oral administration in humans. The rules state that a compound with drug-like activity should have a molecular weight (MW) <500g/mol, a log P value <5 to represent its hydrophobicity, should have a maximum of 5 Hydrogen Bond Donors (HBDs), a maximum of 10 Hydrogen Bond Acceptors (HBAs), and its Molar Refractivity value should fall within the 40-130 cm^3/mol range. Compounds meeting these five principles have high pharmacokinetic and bioavailability levels in the metabolic process. A compound is considered not to comply with Lipinski's Rule if it fails to meet the requirements of two out of the five criteria (Ivanović et al., 2020). The results of the drug-likeness evaluation using Lipinski's Rule of Five indicate that three compounds, β-sitosterol, erythrodiol, and 7β-hydroxysitosterol, meet the criteria presented in Table 2.

Monitoring the pharmacokinetic properties of test compounds in the early stages of drug discovery can reduce failures related to pharmacokinetics in later phases. The pharmacokinetic profile of compounds is predicted using the ADMETlab and SwissADME web servers, considering absorption, distribution, metabolism, and excretion profiles, as shown in Table 3. The

observed absorption profiles include GI Absorption, P-glycoprotein inhibitor, and P-glycoprotein substrate. All four compounds have low GI absorption values. This may result in poor absorption by the intestines and inadequate distribution throughout the body. Further research to enhance GI absorption involves structural modification by adding a base to ensure that the compound's structure have same conditions with intestinal. Factors influencing GI absorption and bioavailability are not limited to the structure of the active compound alone, several external factors can also affect GI absorption.

Table 2. Drug likeness lipinski's rule of five

Compounds	Molecular Weight (MW)	H-Bond Acceptors	H-Bond Donors	logP	Molar Refractivity	Acceptability
	< 500 g/mol	< 10	< 5	< 5	40-130 cm^3/mol	
β-sitosterol	414.71	1	1	7.663	133.23	*Accepted*
Daucosterol	576.44	6	4	5.970	165.61	*Rejected*
Erythrodiol	442.38	2	2	6.755	136.04	*Accepted*
7β-hydroxy sitosterol	430.38	2	2	6.225	134.39	*Accepted*

Table 3. Pharmacokinetic Profile of Test Compounds with ADMETLab and SwissADME

Parameter	Compounds			
	β-sitosterol	Daucosterol	Erythrodiol	7β-hydroxy sitosterol
Absorbtion				
GI absorption	Low	Low	Low	Low
P-glycoprotein inhibitor	-	--	---	-
P-glycoprotein substrate	---	---	---	---
Distribution				
Plasma Protein Binding (PBB) (%)	98.314	97.236	98.952	98.616
Blood brain barrier penetration	++	---	++	++
Volume Distribution (VD) (L/Kg)	1.963	1.440	1.175	1.287
Fraction unbound in plasma (Fu) (%)	1.485	1.807	2.427	1.881
Metabolism				
CYP3A4 substrate	++	-	-	++
CYP3A4 inhibitor	--	---	-	--
CYP2D6 substrate	-	-	+	+
CYP2D6 inhibitor	---	---	---	---
CYP2C9 substrate	-	--	--	+
CYP2C9 inhibitor	---	---	--	--
CYP2C19 substrate	+++	+++	+++	+++
CYP2C19 inhibitor	---	---	---	---
CYP1A2 substrate	-	-	--	+
CYP1A2 inhibitor	---	---	---	---
Ekskretion				
Clearance (Cl) (mL/min/kg)	16.686	5.939	13.615	13.518
Half Life (T1/2)	0.013	0.016	0.014	0.018

Description: Probability value prediction: --- : 0 – 0.1; -- : 0.1 – 0.3; - : 0.3 – 0.5; + : 0.5 – 0.7; ++ : 0.7 – 0.9; +++ : 0.9 – 1.0

The distribution profile observed in this study includes %Plasma Protein Binding (PPB), Blood-Brain Barrier penetration, Volume of distribution, and Fraction unbound in plasma. Erythrodiol has a strong binding with plasma receptors with a %PBB value of 98.952%, followed by 7β-hydroxysitosterol with a value of 98.616%, and β-sitosterol with a value of 98.314%. The %PPB value indicates the portion of the drug bound to plasma receptors that cannot diffuse across cell membranes, thereby being unable to interact with target sites (Ruswanto et al., 2020). The Blood-Brain Barrier represents the ability of a compound to be absorbed into the Central Nervous System (CNS). Compounds β-sitosterol, Erythrodiol, and 7β-hydroxysitosterol show BBB values of 0.7-0.9, indicating that these three compounds are considered easy to pass through the blood-brain barrier (Winardi et al., 2023).

The volume of distribution (Vd) of a drug is highly dependent on the characteristics of the drug. Lipophilic compounds have a larger Vd as they can distribute into adipose tissues and intracellular compartments, while hydrophilic drugs have a smaller Vd as they can only spread in the interstitial area, i.e., extracellular fluid (Setiawan et al., 2019). The volume of distribution for the three compounds indicating that the volume of distribution for these compounds categories into the high category.

The prediction of P-glycoprotein (P-gp) substrates indicates that all four test compounds are not classified as substrates for P-gp. The objective of determining if a compound is a substrate or not for P-glycoprotein (P-gp) is to predict the active expulsion across biological barriers, for instance, the transition from the gastrointestinal wall to the lumen, this is a vital

factor in understanding how a drug might be absorbed and distributed within the body. P-glycoprotein can limit drug uptake by cells, potentially leading to therapy failure due to lower-than-expected drug concentrations (Agustin and Wahjudi, 2023).

The metabolic profile is marked by the presence or absence of inhibition of cytochrome P450 enzymes, especially isoforms CYP2D6 and CYP3A4. Cytochrome P450 enzymes are crucial in the metabolism of drugs within the human body. Compounds acting as substrates for CYP450 enzymes will undergo metabolism by these enzymes, while compounds acting as inhibitors may weaken the metabolism activity and increase the toxicity of the compound. Additionally, when the activity of these Cytochrome P450 isoenzymes is inhibited, it can lead to an accumulation of the drug or its metabolites in the body due to reduced metabolism, this can result in increased drug potency, prolonged drug effect, and potential toxicity (Agustin and Wahjudi, 2023). β-sitosterol, Erythrodiol, and 7β-hydroxysitosterol do not act as inhibitors for CYP2D6, CYP3A4, CYP1A2, CYP2C19, and CYP2C9, indicating that these three compounds are more easily excreted and are non-toxic.

The compound's excretion profile can be observed through parameters such as Clearance and Half-Life (T1/2). Total Clearance is a combination of hepatic clearance, biliary clearance, and renal clearance (Agustin and Wahjudi, 2023). Clearance values, based on the ADMETLab parameters, are divided into three categories: high clearance with values >15 mL/min/kg, moderate with values between 5-15 mL/min/kg, and low with values <5 mL/min/kg. Table 4 shows that β-sitosterol has a high clearance value of 16.686 ml/min/kg, indicating a lower risk of toxicity.

Table 4. Compound's toxicity

Parameter	Compounds			
	β-sitosterol	Daucosterol	Erythrodiol	7β-hydroxy sitosterol
Respiratory toxicity	+	++	+++	++
Non-genotoxic rule	No Alert	No Alert	No Alert	No Alert
Human hepatotoxicity	--	--	--	--
Genotoxic rule	No Alert	No Alert	No alert	No Alert
Drug Induced Liver Injury (DILI)	--	---	---	---
Carcinogenicity	---	---	---	---
AMES toxicity	---	---	--	---
Acute toxicity rule	No Alert	No Alert	No Alert	No Alert
LC_{50}FM	5.365	5.307	6.414	5.893

Description: Probability value prediction: --- : 0 – 0.1; -- : 0.1 – 0.3; - : 0.3 – 0.5; + : 0.5 – 0.7; ++ : 0.7 – 0.9; +++ : 0.9 – 1.0

The toxicity analysis of the test compounds is assessed based on parameters such as AMES toxicity, carcinogenicity, genotoxic rule, non-genotoxic rule, acute toxicity rule, Drug-Induced Liver Injury (DILI), LC_{50}FM, respiratory toxicity, and Human Hepatotoxicity (H-HT). All compounds have a very low probability of causing mutagenicity, do not cause carcinogenicity, and have no warnings for the genotoxic rule, non-genotoxic rule, and acute toxicity. All compounds indicate safety regarding human hepatotoxicity and Drug Induced Liver Injury (DILI). In contrast to the previous analysis, all three test compounds, β-sitosterol, Erythrodiol, and 7β-hydroxysitosterol indicate that they can cause respiratory toxicity.

Conclusion

In this study, the focus of investigation is the potential antiatherosclerotic properties of natural compounds derived from *Tagetes erecta* Linn. Our findings revealed that the compounds β-sitosterol and 7β-hydroxysitosterol, isolated from *Tagetes erecta* Linn., exhibited remarkable inhibitory activity against atherosclerosis using in silico methods. Furthermore, in silico molecular docking simulations indicated strong binding affinity between β-sitosterol and 7β-hydroxysitosterol and specific molecular targets: 8HUP from Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) and 3IPQ from Liver X Receptor Alpha (LXRα). These receptors serve as key regulators in antiatherosclerotic processes, including cholesterol homeostasis and reverse cholesterol transport (RCT). Based on the results of ΔGbinding, pharmacokinetic profiles, and toxicity, these findings suggest that β-sitosterol and 7β-hydroxysitosterol merit further exploration as promising candidates for drug development in antiatherosclerotic therapy. Future studies should focus on in vivo validation and elucidating the underlying mechanisms of their action.

Conflict of Interests

The author(s) declares that there is no conflict of interest in this research and manuscript.

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