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Integrating Toxicity Prediction, Protein-Protein Interaction Analysis, and Molecular Docking to Explore the Potential Anti-Atherosclerotic Properties of Crude Palm Oil Minor Constituents

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Abstract

Atherosclerosis is a complex cardiovascular disease characterized by lipid accumulation, chronic inflammation, oxidative stress, and endothelial dysfunction. Crude palm oil (CPO) or oil of *Elaeis guineensis* contains diverse bioactive compounds that may exert protective effects against atherosclerosis. This study aimed to evaluate the anti-atherosclerotic potential of CPO constituents using toxicity prediction, protein-protein interaction (PPI) analysis, and molecular docking. Nineteen compounds identified in CPO were screened for toxicity using ProTox 3.0. Atherosclerosis-related target proteins were identified through literature review and analyzed using the STRING database. Molecular docking was subsequently performed to predict the binding affinity of CPO compounds toward these targets. Toxicity prediction showed that most compounds exhibited low to moderate toxicity, whereas oleic acid was excluded from further analysis due to its predicted high toxicity (LD_{50} = 48 mg/kg). The PPI network revealed 21 highly interconnected proteins involved in lipid metabolism, inflammation, and vascular homeostasis. Among the evaluated compounds, α-carotene and β-carotene demonstrated the strongest binding affinities toward SCARB1, RSAD2, and CETP. α-Carotene exhibited binding affinities of −16.34, −13.10, and −12.91 kcal/mol, while β-carotene showed affinities of −16.11, −13.14, and −12.42 kcal/mol against SCARB1, RSAD2, and CETP, respectively. Interaction analyses revealed extensive hydrophobic contacts with amino acid residues critical for cholesterol transport, inflammatory signaling, and lipid metabolism. These findings suggest that α-carotene and β-carotene are promising anti-atherosclerotic constituents of CPO and may contribute to cardiovascular protection through modulation of multiple molecular targets involved in atherosclerosis.

Keywords: α-carotene, atherosclerosis, β-carotene, crude palm oil, molecular docking.

INTRODUCTION

Atherosclerosis is a chronic inflammatory disease of the arterial wall characterized by lipid accumulation, endothelial dysfunction, oxidative stress, and persistent inflammation that ultimately leads to plaque formation and vascular obstruction [1,2]. This disease is the principal underlying cause of cardiovascular disorders, including myocardial infarction and ischemic stroke, which remain among the leading causes of death worldwide [3,4]. The pathogenesis of atherosclerosis is complex and involves multiple molecular pathways associated with cholesterol transport, lipoprotein metabolism, inflammatory responses, and vascular homeostasis [5,6]. Proteins such as scavenger receptors, lipid transfer proteins, cytokines, and adhesion molecules have been identified as important regulators of atherogenesis and are considered attractive therapeutic targets [7].

Current pharmacological interventions, including statins, antiplatelet agents, and anti-inflammatory therapies, have significantly improved cardiovascular outcomes [8,9,10]. However, residual cardiovascular risk remains substantial, even among patients receiving optimal treatment [11]. Furthermore, long-term drug administration may be associated with adverse effects and variable therapeutic responses. Consequently, the

search for novel bioactive compounds capable of modulating multiple molecular targets involved in atherosclerosis has become an important area of research [12]. Natural products have gained considerable attention because they contain structurally diverse compounds that often exhibit antioxidant, anti-inflammatory, and cardioprotective activities [13,14].

Crude palm oil (CPO) (Figure 1), obtained from the mesocarp of *Elaeis guineensis*, is a rich source of biologically active compounds. Minor compounds of CPO have been identified, including tocopherols, tocotrienols, carotenoids, squalene, phenolic compounds, phytosterols, and phytostanols [15]. Among these constituents, α-carotene and β-carotene are particularly abundant and have been reported to possess potent antioxidant activity through scavenging reactive oxygen species and reducing oxidative stress [16]. Tocopherols and tocotrienols contribute to antioxidant and anti-inflammatory effects, whereas phytosterols have been associated with cholesterol-lowering properties [17,18]. In addition, phenolic compounds such as gallic acid, ferulic acid, vanillic acid, and protocatechuic acid have demonstrated antioxidant and cardioprotective activities [19]. Since oxidative stress and inflammation are closely involved in the initiation and progression of atherosclerosis, the bioactive constituents present in CPO may represent promising candidates for anti-atherosclerotic drug discovery.

Recent advances in computational biology have accelerated the identification of potential therapeutic compounds through *in silico* approaches. Molecular docking is a widely used computational method that predicts the binding orientation and binding affinity between a ligand and a target protein, providing insights into potential molecular mechanisms and biological activities. The approach enables rapid screening of multiple compounds against multiple targets while reducing the time and cost associated with experimental drug discovery [20]. In addition, protein-protein interaction (PPI) analysis can be used to identify key proteins involved in disease pathways and prioritize targets for molecular docking studies [21]. Hence, integrating PPI analysis with molecular docking represents a valuable strategy for discovering novel compounds that may regulate complex diseases such as atherosclerosis.

In the present study, nineteen compounds previously reported in CPO were subjected to toxicity prediction analysis to identify compounds with favorable safety profiles. Subsequently, atherosclerosis-associated proteins were identified through PPI network analysis using the STRING database, resulting in the selection of twenty-one candidate proteins involved in lipid metabolism, inflammation, and vascular regulation. Molecular docking analysis was then performed to evaluate the binding affinities of CPO compounds toward these targets. The results identified α-carotene and β-carotene as the most promising compounds, exhibiting strong binding affinities toward SCARB1 (Scavenger Receptor Class B Member 1), RSAD2 (Radical S-Adenosyl Methionine Domain Containing 2), and CETP (Cholesteryl Ester Transfer Protein). These proteins are known to participate in cholesterol transport, inflammatory signaling, and lipoprotein metabolism, suggesting that carotenoids from CPO may contribute to anti-atherosclerotic activity through multiple molecular mechanisms. Therefore, this study aimed to evaluate the anti-atherosclerotic potential of CPO constituents and to elucidate their interactions with key proteins involved in atherosclerosis using an integrated *in silico* approach.

EXPERIMENTAL SECTION

Methods

Protein-Protein Interaction Analysis

Proteins associated with atherosclerosis were identified through literature mining and subsequently analyzed using the STRING database version 12.0 (https://string-db.org/) [22]. The selected proteins were uploaded to STRING using *Homo sapiens* as the target organism. Protein interaction networks were generated to identify functional associations among proteins involved in lipid metabolism, cholesterol transport, endothelial dysfunction, inflammation, and vascular homeostasis. The interaction network was evaluated based on node connectivity and interaction confidence scores. Proteins within the resulting network were selected as candidate molecular targets for subsequent molecular docking studies

Toxicity Prediction

The toxicity profiles of CPO constituents were predicted using the ProTox-3.0 web server (https://tox.charite.de/) [23]. Canonical SMILES structures of each compound were retrieved from the PubChem database and submitted individually to the platform. Acute oral toxicity was predicted and expressed

as median lethal dose (LD_{50}, mg/kg body weight). Compounds were further categorized into toxicity classes according to the Globally Harmonized System (GHS)-based classification implemented in ProTox-3.0. Compounds exhibiting predicted high toxicity were excluded from further molecular docking analyses.

Molecular Docking Procedure

Protein Preparation

Three-dimensional structures of proteins associated with atherosclerosis were obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB-PDB; https://www.rcsb.org/). Protein structures were prepared using AutoDock Tools v1.5.7 [24] by removing water molecules, co-crystallized ligands, and other non-essential heteroatoms. Polar hydrogen atoms were subsequently added, and Kollman charges were assigned to the protein structures. The prepared proteins were then saved in PDBQT format for subsequent molecular docking studies.

Ligand Structure Preparation

The chemical structures of crude palm oil (CPO) constituents were retrieved from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/) in Structure Data File (SDF) format. The ligand structures were converted into Protein Data Bank (PDB) format using Online SMILES Translator and Structure File Generator (https://cactus.nci.nih.gov/translate/). Then, ligand PDB structures were subjected to geometry optimization using Avogadro software (version 1.2.0) [25]. Energy minimization was done using the MMFF94 force field and Steepest Descent algorithm with 10 optimization steps to obtain stable molecular conformations while preserving the original structural characteristics of the ligands. The optimized structures were exported as PDB files to add polar hydrogen bond and converted into PDBQT format using AutoDock Tools v1.5.7. During ligand preparation, Gasteiger charges were assigned and rotatable bonds were preserved for subsequent docking calculations.

Grid and docking parameterization

The docking search space was defined by generating grid boxes using AutoDock Tools v1.5.7. For proteins containing co-crystallized ligands, the grid box center was assigned according to the coordinates of the native ligand-binding site. For proteins without co-crystallized ligands, the grid box was centered on the predicted active site identified through structural analysis and literature reports. Grid dimensions were adjusted to fully encompass the binding cavity and its surrounding residues to ensure adequate exploration of ligand conformations. Grid parameter files containing the center coordinates (X, Y, and Z) and grid dimensions (size X, size Y, and size Z) were prepared for each protein target and subsequently used in docking calculations. Following grid preparation, the docking protocol was established using a genetic algorithm with 50 runs and a population size of 200 individuals to explore potential ligand-protein binding conformations [26].

Running molecular docking and results interpretation

Molecular docking simulations were performed using AutoDock Tools version 1.5.7 on a workstation equipped with an AMD Ryzen 5-5500U processor (12 cores) and AMD Radeon Graphics. The docking procedure was conducted to predict the binding affinity of crude palm oil constituents toward selected atherosclerosis-related proteins. Docking poses were visualized using BIOVIA Discovery Studio Visualizer for two-dimensional interaction mapping [27] and PyMOL [28] for three-dimensional structural analysis. In addition to binding affinity and inhibition constant predictions, protein–ligand interactions were comprehensively evaluated to identify key hydrogen bonds, hydrophobic contacts, π-interactions, and other non-covalent interactions that may influence the stability and biological relevance of the complexes.

RESULTS AND DISCUSSION

Toxicity Prediction and Identification of Potential Anti-Atherosclerotic Targets

A total of 19 compounds previously reported in crude palm oil (CPO) were subjected to *in silico* toxicity prediction using the ProTox platform. The compounds comprised carotenoids, phytosterols, fatty acids, vitamin E derivatives, and phenolic compounds. Most compounds were classified within toxicity classes IV–VI, indicating relatively low acute toxicity, whereas oleic acid exhibited a predicted LD_{50} value of 48 mg/kg and was categorized as toxicity class II, suggesting a substantially higher toxicity risk than the remaining

compounds (Table 1). Consequently, oleic acid was excluded from subsequent molecular docking analyses to prioritize compounds with more favorable safety profiles.

Table 1. Predicted Toxicity of CPO Compounds

No.	Compound Name	LD_{50} (mg/kg)	Category (Toxicity Level)
1	Alpha-Carotene	1,510	IV
2	Beta-Carotene	1,510	IV
3	Beta-Sitosterol	890	IV
4	Campesterol	890	IV
5	Ferulic Acid	1,772	IV
6	Gallic Acid	2,000	IV
7	Linoleic Acid	10,000	VI
8	Monoglyceride	8,250	VI
9	Oleic Acid	48	II
10	Palmitic Acid	900	IV
11	Protocatechuic Acid	2,000	IV
12	Protocatechuic Aldehyde	2,195	V
13	Salicylic Acid	2,200	V
14	Squalene	5,000	V
15	Stigmasterol	890	IV
16	Tocopherol	5,000	V
17	Tocotrienol	500	IV
18	Triglyceride	6,400	VI
19	Vanillic Acid	2,000	IV

To identify molecular targets associated with atherosclerosis, a protein–protein interaction (PPI) network was constructed using the STRING database. The resulting network comprised 21 proteins implicated in key pathogenic processes, including lipid metabolism, cholesterol transport, inflammation, and endothelial dysfunction (Figure 1). Several proteins, notably APOE, APOA1, ABCA1, SCARB1, VCAM1, and INS, exhibited high connectivity within the network, accompanied by a significant PPI enrichment value ($p < 1 \times 10^{-16}$), suggesting their central roles in coordinating multiple biological processes relevant to atherogenesis. Highly connected nodes, or hub proteins, are often considered promising therapeutic targets because their modulation may influence multiple interconnected signaling pathways. Although RSAD2 displayed a lower degree of connectivity, it remained functionally linked to key inflammatory mediators, including IL1B and TNF, indicating a potential involvement in immune and inflammatory responses within the vascular microenvironment. Overall, the PPI network highlights the complex and interconnected molecular landscape of atherosclerosis, underscoring the coordinated contributions of dysregulated lipid homeostasis, chronic inflammation, and vascular dysfunction to disease progression.

Molecular Docking Reveals alpha-Carotene and beta-Carotene as the Most Promising Bioactive Compounds

Molecular docking of CPO-derived compounds against the 21 candidate proteins revealed considerable variability in binding affinity values (Table 2). Among all compounds evaluated, α-carotene and β-carotene consistently demonstrated the strongest binding affinities across multiple targets, particularly SCARB1, RSAD2, and CETP. These findings suggest that carotenoids may represent the principal anti-atherosclerotic constituents of CPO. Interestingly, the binding affinities of α-carotene and β-carotene were substantially lower than those observed for most phenolic compounds, fatty acids, tocopherols, tocotrienols, and phytosterols. The highly hydrophobic structures of both carotenoids likely facilitate extensive interactions within lipid-binding pockets and hydrophobic channels present in proteins involved in cholesterol transport and metabolism.

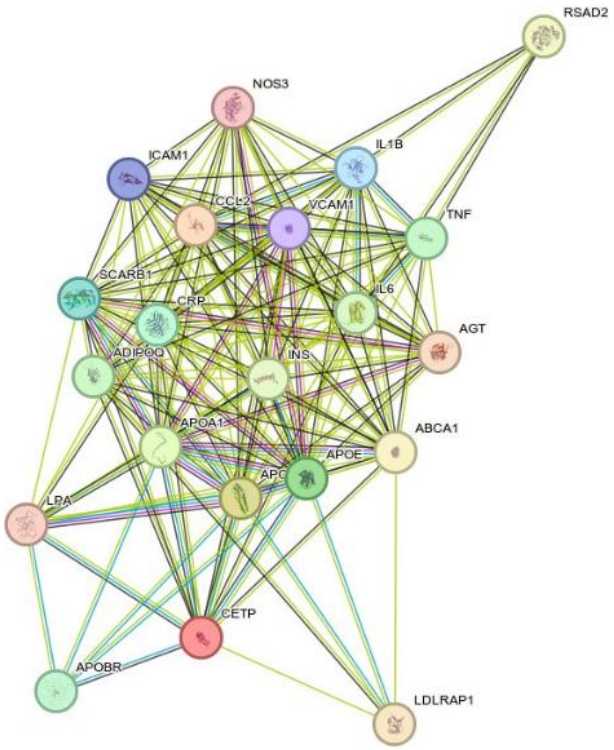

Figure 1. Predicted protein-protein interaction (PPI) network of atherosclerosis-related proteins generated using the STRING database (https://string-db.org/).

Table 2. Predicted binding affinity results (kcal/mol) of compounds identified in crude palm oil against proteins involved in the atherosclerosis pathway

No	Compound Name	Predicted Binding Affinity Values (kcal/mol)										
		ABCA1	ADIPOQ	AGT	ApoA	ApoA1	ApoB	ApoBR	ApoE	CCL2	CETP	CRP
1	Alpha-Carotene	-9.16	-9.12	-8.41	-6.07	-7.22	-9.26	-7.12	-8.12	-8.35	-12.91	-9.36
2	Beta-Carotene	-9.46	-8.66	-7.38	-6.17	-6.99	-8.76	-7.13	-7.88	-8.11	-12.42	-9.60
3	Beta-Sitosterol	-9.67	-7.34	-7.73	-6.22	-6.65	-8.56	-6.20	-7.32	-8.03	-10.30	-8.83
4	Campesterol	-9.66	-7.79	-8.05	-6.51	-6.80	-8.66	-6.29	-7.62	-7.62	-10.15	-9.10
5	Ferulic Acid	-8.02	-5.72	-5.68	-5.95	-3.74	-4.56	-2.98	-5.07	-6.23	-6.22	-6.13
6	Gallic Acid	-9.35	-4.91	-5.07	-5.66	-3.15	-5.04	-2.99	-4.67	-5.14	-5.73	-5.75
7	Linoleic Acid	-7.19	-3.01	-4.74	-4.05	-2.36	-3.98	-1.79	-4.33	-4.85	-5.09	-4.81
8	Monoglyceride	-5.36	-3.75	-3.19	-2.69	-2.05	-2.69	-2.54	-2.92	-2.84	-4.29	-3.72
9	Palmitic Acid	-7.33	-3.75	-4.6	-4.03	-1.75	-3.80	-1.42	-4.30	-4.27	-4.44	-3.98
10	Protocatechuic Acid	-8.64	-5.95	-5.18	-6.33	-3.19	-5.64	-2.79	-5.51	-5.50	-5.70	-6.05
11	Protocatechuic Aldehyde	-6.40	-5.45	-4.94	-4.81	-3.54	-4.79	-4.44	-4.97	-4.87	-6.14	-5.30
12	Salicylic Acid	-8.22	-4.94	-5.04	-5.94	-3.33	-5.53	-2.69	-5.04	-4.98	-5.60	-5.47
13	Squalene	-6.86	-4.84	-5.34	-3.65	-3.83	-6.90	-4.68	-4.26	-5.27	-8.27	-5.76
14	Stigmasterol	-9.96	-7.70	-8.47	-6.20	-6.70	-9.39	-6.38	-7.53	-8.22	-10.62	-8.95
15	Tocopherol	-8.25	-5.73	-6.43	-4.99	-4.44	-6.72	-4.28	-5.82	-6.37	-8.23	-6.65
16	Tocotrienol	-8.92	-6.28	-7.44	-6.55	-4.80	-8.73	-6.22	-5.43	-6.75	-8.80	-7.75
17	Triglyceride	-5.72	-4.00	-3.88	-3.81	-1.69	-2.79	-2.84	-3.01	-3.75	-4.61	-4.14
18	Vanillic Acid	-7.32	-5.13	-5.10	-5.80	-3.02	-5.49	-2.72	-5.13	-4.91	-5.95	-6.52
19	Co-crystallized Ligand	UA	UA	UA	-8.1	UA	UA	UA	UA	UA	UA	UA

UA= co-crystallized ligand unavailable

Table 2. Predicted binding affinity results (kcal/mol) of compounds identified in crude palm oil against proteins involved in the atherosclerosis pathway (continued)

No		Predicted Binding Affinity Values (kcal/mol)

	Compound Name	ICAM1	IL1B	IL6	INS	LDLRAP1	NOS3	RSAD2	SCRB1	TNF	VCAM1
1	Alpha-Carotene	-7.59	-8.61	-9.46	-7.2	-10.18	-9.57	-13.1	-16.34	-8.22	-8.79
2	Beta-Carotene	-7.3	-8.84	-9.33	-7.62	-9.71	-9.85	-13.14	-16.11	-7.81	-8.23
3	Beta-Sitosterol	-7.53	-7.84	-7.97	-6.7	-8.7	-11.54	-11.45	-11.8	-7.53	-7.94
4	Campesterol	-6.87	-7.97	-7.19	-6.76	-9.06	-11.41	-11.1	-11.91	-7.26	-7.71
5	Ferulic Acid	-5.56	-6.5	-6.72	-4.87	-5.71	-5.99	-6.14	-5.09	-4.07	-5.05
6	Gallic Acid	-5.01	-5.5	-6.18	-3.75	-5	-5.51	-6.06	-4.33	-4.24	-4.01
7	Linoleic Acid	-4.54	-5.7	-7.02	-3.31	-5.09	-6.51	-7.17	-5.63	-3.46	-2.65
8	Monoglyceride	-2.41	-2.54	-2.79	-1.96	-1.87	-3.9	-2.81	-2.11	-1.76	-3.05
9	Palmitic Acid	-3.94	-4.89	-4.79	-2.36	-4.93	-6.24	-6.33	-5.09	-2.94	-2.75
10	Protocatechuic Acid	-4.66	-5.77	-6.62	-4.04	-5.31	-5.74	-6.08	-4.35	-4.66	-4.41
11	Protocatechuic Aldehyde	-4.47	-4.9	-4.68	-3.91	-4.72	-7.16	-5.36	-4.68	-4.38	-4.4
12	Salicylic Acid	-4.76	-5.32	-6.23	-3.88	-4.99	-5.68	-5.98	-4.09	-4.39	-4.41
13	Squalene	-4.04	-5.56	-5.24	-4.41	-5.91	-8.15	-9.84	-10.09	-4.05	-4.44
14	Stigmasterol	-7.69	-7.89	-7.53	-6.85	-8.82	-11.69	-11.23	-11.98	-7.58	-7.81
15	Tocopherol	-5.58	-7.57	-6.01	-5.34	-7.32	-8.87	-11.07	-9.78	-5.79	-6.21
16	Tocotrienol	-6.79	-6.66	-7.14	-5.53	-7.08	-8.76	-10.45	-9.4	-5.6	-5.92
17	Triglyceride	-3.02	-3.52	-3.66	-2.84	-2.46	-4.47	-3.29	-2.41	-2.24	-3.47
18	Vanillic Acid	-4.85	-5.38	-6.32	-4.14	-5.14	-5.95	-6.4	-4.26	-3.93	-4.7
19	Co-crystallized Ligand	UA	UA	UA	UA	UA	-10.21	UA	UA	-6.44	UA

UA= co-crystallized ligand unavailable

Interaction of alpha-Carotene and beta-Carotene with SCARB1

Among all the targets investigated, SCARB1 displayed the strongest predicted interaction with both carotenoids. α-Carotene and β-carotene exhibited binding affinities of −16.34 and −16.11 kcal/mol, respectively, corresponding to predicted inhibition constants in the picomolar range. These values suggest exceptionally stable protein–ligand complexes and indicate a high likelihood of biological relevance. SCARB1 (SR-B1) is a multifunctional receptor that mediates selective uptake of HDL-derived cholesterol and regulates reverse cholesterol transport, a critical anti-atherogenic process. In addition to its role in cholesterol homeostasis, SCARB1 contributes to efferocytosis and resolution of vascular inflammation. However, endothelial SCARB1 may also facilitate LDL transcytosis, thereby contributing to early atherosclerotic lesion formation. Interaction analysis revealed that α-carotene primarily formed alkyl and π-alkyl interactions with residues ILE139, LEU140, MET159, ALA162, LEU187, PHE199, ALA338, LEU340, and LEU390. Similarly, β-carotene interacted with LEU140, ALA144, MET159, ALA162, LEU187, ILE191, PHE199, PHE208, LEU211, ARG335, ALA338, LEU340, and PHE416 (Figure 2). Several of these residues, including LEU140, PHE199, LEU211, and ARG335, have previously been implicated in ligand recognition and cholesterol transport [29]. Therefore, occupation of this region by carotenoids may interfere with ligand accessibility or receptor-mediated lipid trafficking. Although the exact biological consequences require experimental validation, these findings suggest that α-carotene and β-carotene may modulate SCARB1-mediated cholesterol transport and inflammatory responses during atherosclerosis progression.

Interaction of α-Carotene and β-Carotene with RSAD2

The second most favorable target identified was RSAD2 (viperin), with binding affinities of −13.10 kcal/mol for α-carotene and −13.14 kcal/mol for β-carotene. RSAD2 is an interferon-inducible radical S-adenosylmethionine enzyme that regulates innate and adaptive immunities, mitochondrial metabolism, oxidative stress, and inflammatory signaling [30,31]. Recent evidence indicates that RSAD2 is highly expressed in human atherosclerotic plaques and contributes to vascular inflammation. Based on Figure 3, docking analysis revealed that α-carotene interacted with residues HIS78, CYS83, PHE91, ALA94, VAL155, LYS246, and TYR301, whereas β-carotene primarily interacted with CYS83, CYS90, ALA94, and VAL155. Notably, CYS83 and CYS90 participate in coordination of the catalytic [4Fe–4S] cluster that is essential for RSAD2 conformational stability [32]. The localization of these carotenoids binding around this region may influence RSAD2-mediated redox and metabolic processes [33]. Oxidative stress is a major driver of endothelial dysfunction and atherosclerotic plaque development. Because iron-sulfur clusters can participate in redox reactions and reactive oxygen species generation [34], modulation of RSAD2 activity by carotenoids may contribute to attenuation of oxidative stress within vascular tissues. This mechanism may aligns closely

with the well-established antioxidant properties of carotenoids and provides a plausible molecular explanation for their protective effects against vascular injury.

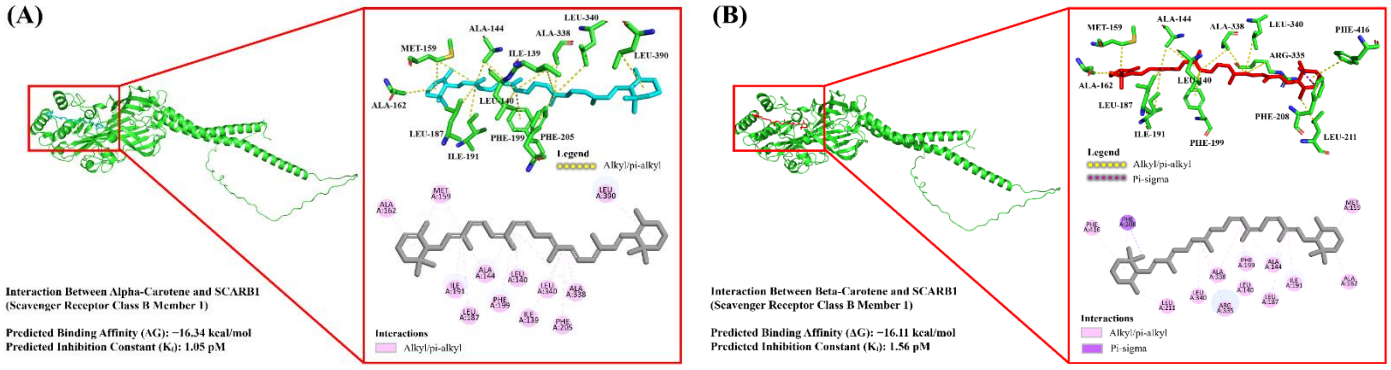

Figure 2. Interactions of α-carotene **(A)** and β-carotene **(B)** with SCARB1 (Scavenger Receptor Class B Member 1).

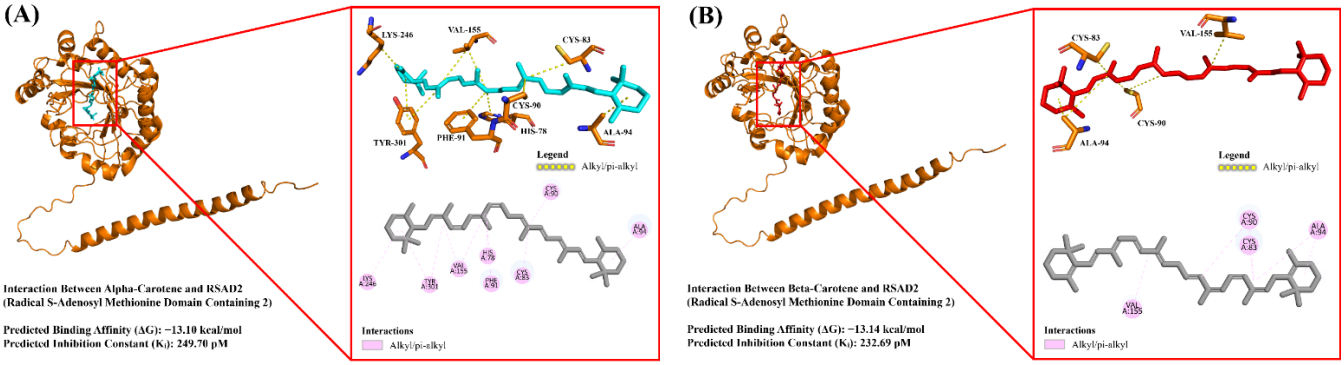

Figure 3. Interactions of α-carotene **(A)** and β-carotene **(B)** with RSAD2 (Radical S-Adenosyl Methionine Domain Containing 2).

Interaction of α-Carotene and β-Carotene with CETP

CETP was identified as the third major target of α-carotene and β-carotene, exhibiting binding affinities of −12.91 and −12.42 kcal/mol, respectively. CETP plays a central role in lipoprotein remodeling by facilitating the exchange of cholesteryl esters and triglycerides between HDL and apoB-containing lipoproteins. Elevated CETP activity is frequently associated with reduced HDL levels and increased venous thromboembolism risk [35,36]. According to Figure 4, both carotenoids occupied highly hydrophobic regions within the CETP structure and formed extensive alkyl, π-alkyl, and π-sigma interactions. Several interacting residues, including LEU283, VAL323, VAL344, and LEU425, are located within the P1 lipid-transfer region, whereas ALA195 is positioned within the P2 region [37]. Particularly noteworthy is the interaction between β-carotene and PHE265, a residue located within the neck region of CETP that is crucial for maintaining the integrity of the hydrophobic tunnel responsible for cholesteryl ester transfer and contribute to this local high energy barrier. Previous structural studies have demonstrated that disruption of this hydrophobic tunnel impairs lipid transfer efficiency [38]. Therefore, binding of β-carotene to PHE265 may destabilize CETP-mediated lipid transport, potentially reducing LDL and VLDL formation. Such an effect could contribute to an improved lipoprotein profile and reduced atherosclerotic burden.

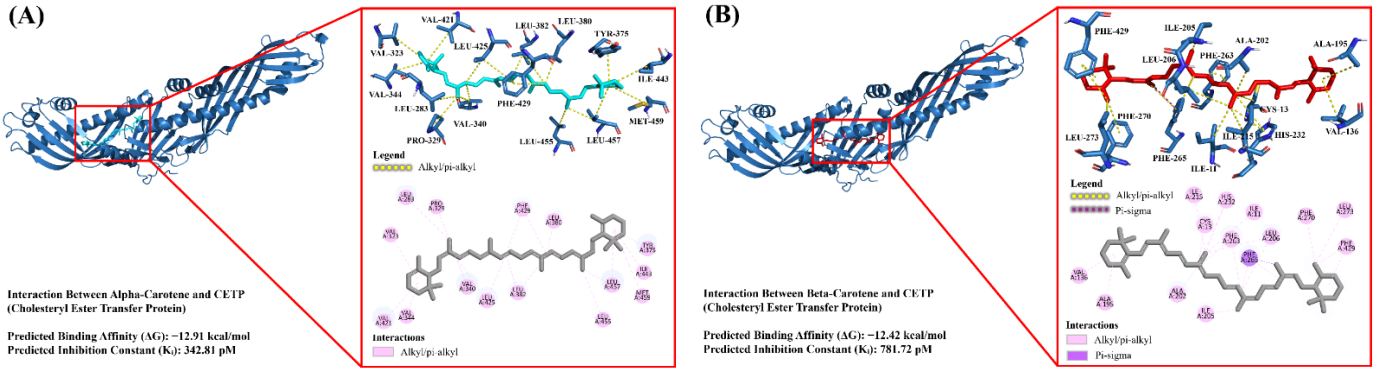

Figure 4. Interactions of α-carotene **(A)** and β-carotene **(B)** with CETP (Cholesteryl Ester Transfer Protein).

Relationship Between Antioxidant Activity and Molecular Docking Findings

The antioxidant activity observed for CPO is mechanistically consistent with the molecular docking results obtained in this study. Atherosclerosis is widely recognized as an oxidative-inflammatory disease in which excessive reactive oxygen species (ROS) promote LDL oxidation, endothelial dysfunction, inflammatory activation, foam cell formation, and plaque progression. Consequently, compounds capable of simultaneously reducing oxidative stress and modulating key atherosclerosis-related proteins may exert synergistic protective effects. Among the identified CPO constituents, α-carotene and β-carotene demonstrated dual functionality. First, as potent carotenoid antioxidants, they can directly scavenge free radicals and suppress lipid peroxidation. Second, docking analysis revealed strong interactions with proteins involved in cholesterol transport (SCARB1 and CETP) and inflammatory-redox signaling (RSAD2). These findings suggest that the anti-atherosclerotic potential of carotenoids extends beyond simple antioxidant activity and may involve direct regulation of molecular pathways controlling lipid metabolism, vascular inflammation, and oxidative stress. The possible pathway and clinical outcomes have been depicted in Figure 5.

CONCLUSION

This study explored the potential anti-atherosclerotic properties of bioactive compounds identified in crude palm oil (CPO) through toxicity prediction, protein-protein interaction analysis, and molecular docking approaches. The PPI network highlighted 21 proteins associated with lipid metabolism, cholesterol transport, inflammation, and endothelial dysfunction, indicating the complex molecular mechanisms underlying atherosclerosis. Among the evaluated compounds, α-carotene and β-carotene exhibited relatively favorable binding affinities toward several targets, particularly SCARB1, RSAD2, and CETP, suggesting that these compounds may interact with pathways involved in cholesterol homeostasis, inflammatory regulation, and lipoprotein metabolism. These observations may be associated with the antioxidant activity of carotenoids, which may contribute to reducing oxidative stress, a key factor in the initiation and progression of atherosclerosis. Collectively, the findings suggest that selected CPO constituents, especially α-carotene and β-carotene, may possess biological activities relevant to the prevention or management of atherosclerosis. However, further experimental and clinical investigations are needed to determine whether these predicted interactions might translate into meaningful biological effects under physiological conditions.

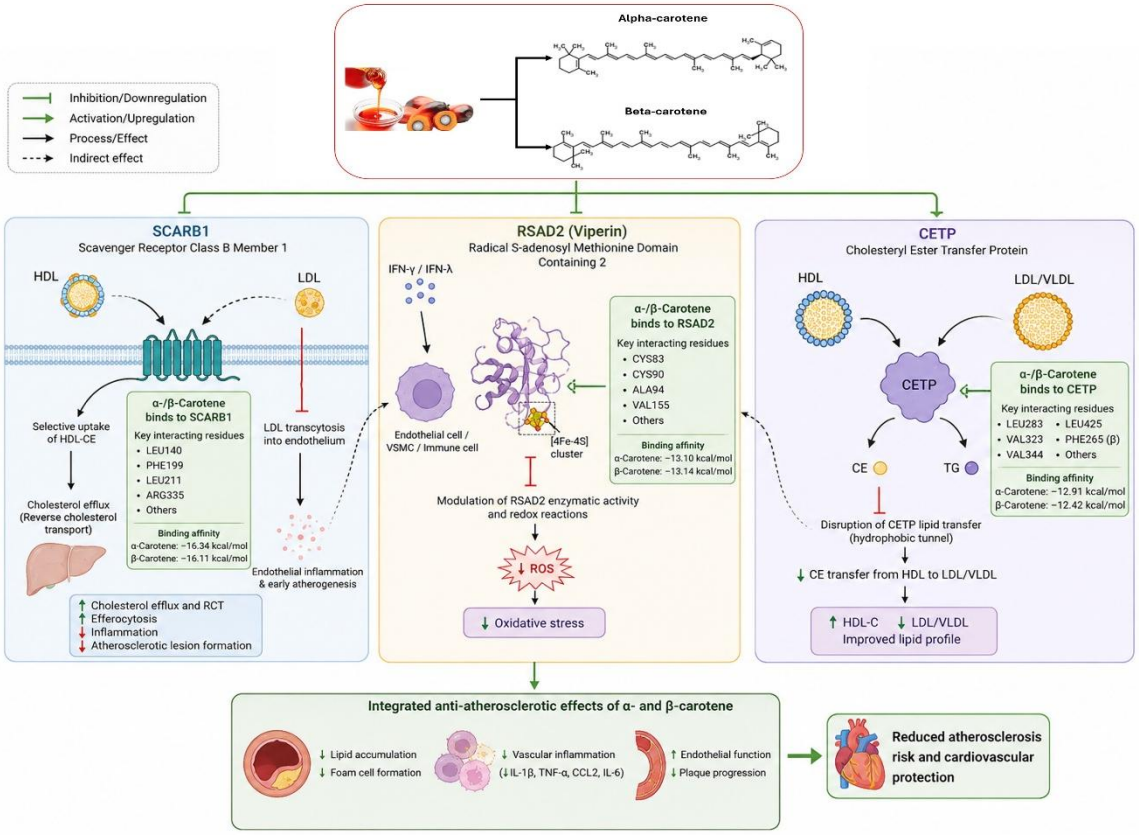

Figure 5. Possible anti-atherosclerotic mechanism of α-carotene and β-carotene derived from CPO

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