Original Paper

Discovery of Novel Inhibitors of HMG-CoA Reductase Using Bioactive Compounds Isolated From Cochlospermum Species Through Computational Methods: Virtual Screening and Algorithm Validation Study

Toba Isaac Olatoye[1,2], BSc, MSc

[1]Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria
[2]Schools and Colleges, Kwara State Teaching Service Commission, Ilorin, Nigeria

Corresponding Author:

Toba Isaac Olatoye, BSc, MSc
Department of Biochemistry
Faculty of Life Sciences, University of Ilorin
P.M.B. 1515, Ilorin, Kwara State
Ilorin, 240003
Nigeria
Phone: 234 07030262928
Email: isaactoba22@gmail.com

Related Articles:
Preprint (bioRxiv): https://www.biorxiv.org/content/10.1101/2025.01.19.633828v1
Peer-Review Report by Shailee Rasania, Sylvester Sakilay, Sayan Mitra, Randa Salah, Gomaa Mahmoud, Julie Moonga: https://med.jmirx.org/2025/1/e74084
Authors' Response to Peer-Review Reports: https://med.jmirx.org/2025/1/e78093

Abstract

Background: Cholesterol biosynthesis is a critical pathway in cellular metabolism, with 3-hydroxy-3-methylglutaryl coenzyme-A reductase (HMGR) catalyzing its committed step. HMGR inhibition has been widely explored as a therapeutic target for managing hypercholesterolemia, and statins are the most commonly used competitive inhibitors. However, the search for novel, natural HMGR inhibitors remains a vital area of research, due to the adverse effects associated with long-term statin use. *Cochlospermum planchonii* and *Cochlospermum tinctorium* are West African medicinal plants traditionally used to treat metabolic disorders, including dyslipidemia. Despite their usefulness, the specific bioactive compounds responsible for these effects are currently poorly characterized, justifying further investigations.

Objective: This study investigates the potential of phytochemicals from *Cochlospermum planchonii* and *Cochlospermum tinctorium* as natural inhibitors of human HMGR using molecular docking techniques.

Methods: A total of 84 phytochemicals from 2 species of *Cochlospermum* as reported in literature, were evaluated as potential inhibitors of HMGR. Using DataWarrior software, their drug-likeness and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties were screened in accordance with Lipinski's Rule of Five. The 32 compounds that met the criteria were docked on PyRx against the HMG-binding site of HMGR, alongside atorvastatin (native ligand) and 6 known statins, which served as control ligands.

Results: Docking analysis of their two best binding modes showed that 10 (31.3%) out of the 32 screened phytochemicals demonstrated strong binding affinities and interactions with the HMG-binding pocket (residues 682-694) of HMGR, with binding energy (ΔG) scores ranging from –4.6 to –6.0 kcal/mol, comparable to or exceeding those of statins (–4.6 to –5.7 kcal/mol). Their docking scores (–13.272 to –32.103) also compared favorably with those of statins (–25.939 to –36.584). Interestingly, 3-O-methylellagic acid (ID_13915428) demonstrated the strongest interaction, forming 26 binding interactions with the HMG-binding pocket residues, more than any compound, including statins. One-way ANOVA of the mean and SEM of the binding affinity scores for the phytochemicals and statins (9 replicates each) indicated a statistically significant difference at $P<.05$ (total sample size n=153; actual $P=.0001$).

Conclusions: This study is the first to virtually screen and identify specific bioactive compounds isolated from *Cochlospermum planchonii* and *Cochlospermum tinctorium* with potential cholesterol-lowering effects in humans. The findings not only support the traditional use of these plants in West Africa to manage dyslipidemia and other ailments, but also present the phytochemicals as promising drug candidates for further optimization as natural inhibitors of HMGR. However, while this study provides valuable computational insights into the molecular interactions of the compounds with HMGR, further advanced computational, in vitro, and in vivo studies are still necessary to validate their inhibitory potential and therapeutic applications.

JMIRx Bio 2025;3:e71675; doi: 10.2196/71675

Keywords: HMGR; statins; hypercholesterolemia; cochlospermum; phytochemicals; molecular docking; 3-hydroxy-3-methylglutaryl coenzyme-A reductase

Introduction

Cholesterol is a vital component of cellular membranes and serves as a precursor for the biosynthesis of steroid hormones, bile acids, and vitamin D. However, elevated levels of cholesterol, especially low-density lipoprotein (LDL) cholesterol, are strongly associated with the development of atherosclerosis and cardiovascular diseases (CVDs), which are among the leading causes of morbidity and mortality worldwide [1]. Although lifestyle changes in individuals such as exercise, healthy diets, and drug therapies particularly statins, have been touted as effective in the prevention and management of hypercholesterolemia including its attendant cardiovascular complications [1,2]. Nevertheless, the challenge is yet far from over, as these conditions still remain major global concerns, especially in high-income countries like the United States, where about 48% of adults are currently affected [3].

HMG-CoA (3-hydroxy-3-methylglutaryl-coenzyme A) reductase (HMGR), the rate-limiting enzyme in the mevalonate pathway, catalyzes the 4-electron reductive deacylation of HMG-CoA to mevalonate, a crucial precursor of cholesterol biosynthesis in human [4]. Statins, a class of synthetic drugs with inhibition constant (Ki) values in the nanomolar range, are competitive inhibitors of HMGR widely used to lower serum cholesterol levels in human [5]. These drugs occupy the catalytic portion of the enzyme where the substrate, HMG-CoA, binds, thus blocking its access to the active site (Figure 1). Near the carboxyl terminus of human HMGR, several catalytically relevant amino acid residues representing the HMG-binding pocket are disordered in the enzyme-statin complex. If these residues were not flexible, they would sterically hinder the binding of statins [5]. All statins have an HMG-like moiety, with rigid, hydrophobic groups that are covalently linked to them and may be present in inactive lactone form. In vivo, these drugs are enzymatically hydrolyzed to their active hydroxy-acid forms [6]. In addition to lowering cholesterol, statins seem to have other functions, including the nitric oxide–mediated promotion of the growth of new blood vessels [7], stimulation of bone formation [8], protection against oxidative modification of LDL, and anti-inflammatory effects with a reduction in C-reactive protein levels [9]. Nevertheless, the use of statins is often limited by their side effects such as myopathy, liver and kidney dysfunction, and an increased risk of diabetes [10-12]. These limitations have necessitated the search for alternative cholesterol-lowering agents, especially those from natural sources, which may offer safer and more effective therapeutic needs.

Figure 1. (A) Active site of human 3-hydroxy-3-methylglutaryl coenzyme-A reductase in complex with 3-hydroxyl-3-methylglutaric acid (HMG), coenzyme A (CoA), and nicotinamide adenine dinucleotide phosphate ($NADP^+$). (B) Binding of rosuvastatin to 3-hydroxy-3-methylglutaryl coenzyme-A reductase [5]. A: Ala; C: Cys; D: Asp; E: Glu; F: Phe; G: Gly; H: His; I: Ile; K: Lys; L: Leu; M: Met; N: Asn; P: Pro; Q: Gln; R: Arg; S: Ser; T: Thr; V: Val; W: Trp; Y: Tyr. Adapted from Istvan and Deisenhofer [5], with permission from International Union of Crystallography.

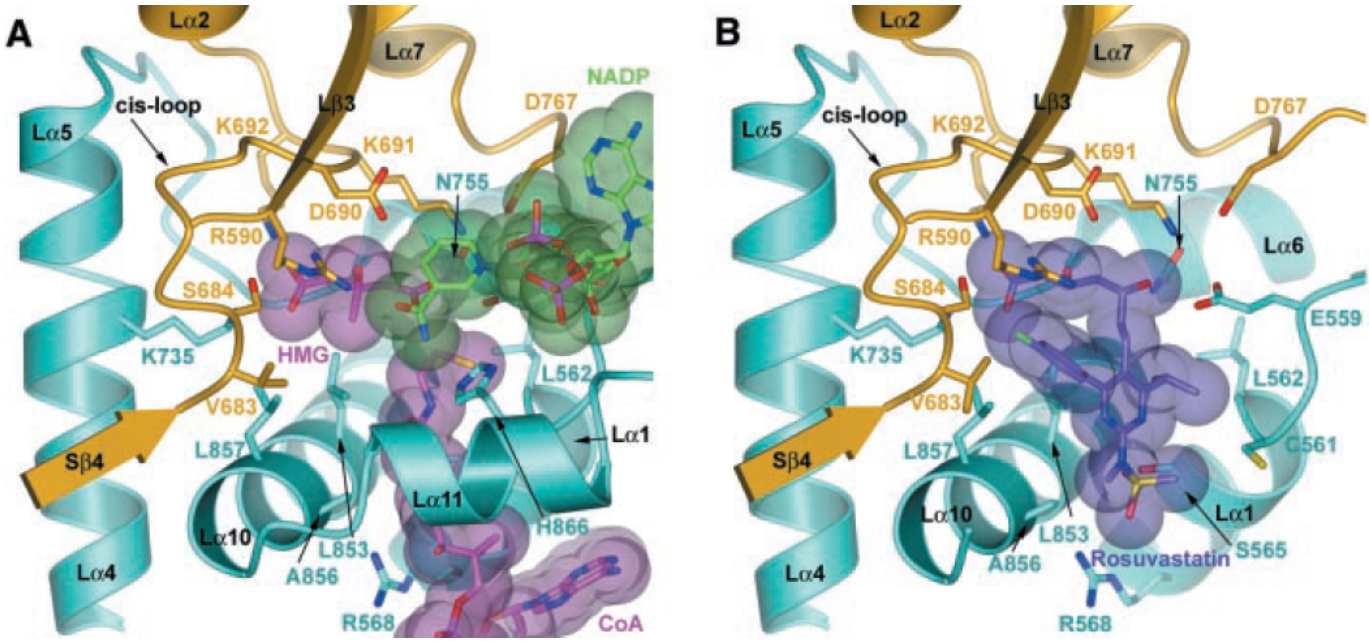
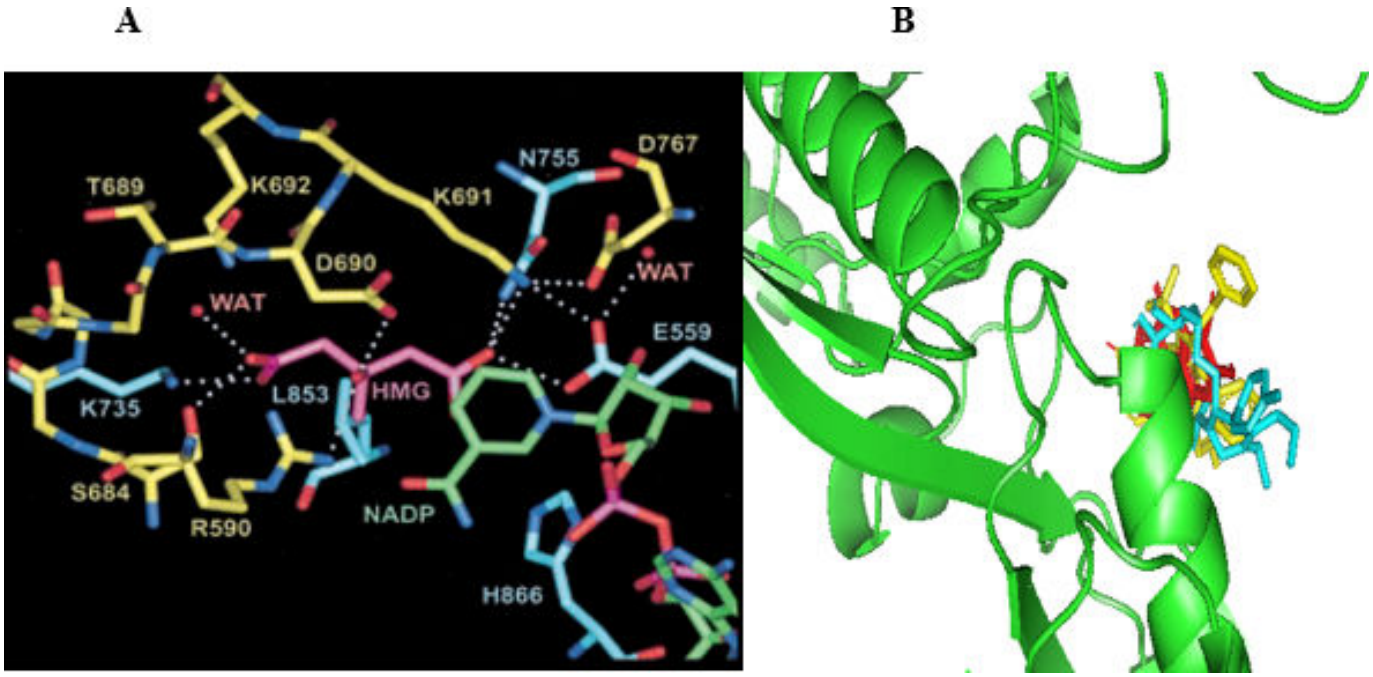


Figure 2. (A) Single-letter abbreviations of residues involved in HMG-binding based on the crystal structure of human 3-hydroxy-3-methylglutaryl coenzyme-A reductase [4]. (B) 3D representation showing the binding modes of cocrystallized atorvastatin (yellow), cerivastatin (cyan), and 3-O-methylellagic acid (red), at the HMG-binding site of human 3-hydroxy-3-methylglutaryl coenzyme-A reductase. A: Ala; C: Cys; D: Asp; E: Glu; F: Phe; G: Gly; H: His; I: Ile; K: Lys; L: Leu; M: Met; N: Asn; P: Pro; Q: Gln; R: Arg; S: Ser; T: Thr; V: Val; W: Trp; Y: Tyr. Adapted from Ensouf [13] and Istvan et al [4], with permission from The American Association for the Advancement of Science.



The 3D crystal structure of human HMGR (Protein Data Bank [PDB] ID: 1HWK) is a tetramer (subunits A: PRO442–HIS861; B: SER463–GLY860; C: LEU462–GLY860; D: SER463–GLY860) that contains the catalytic domains of HMGR in complex with 4 atorvastatin molecules at the interface of 2 adjacent monomers [5,14]. Structurally, these domains are divided into three subdomains: an "N-domain" (residues 460-527) connecting the catalytic portion of the enzyme to the membrane domain; a large "L-domain" (residues 528-590 and 694-861); and a small "S-domain" (residues 592-682). In the monomer, the amino acid residues of the L- and S-domains form the 2 active sites: (1) the HMG-binding pocket characterized by a narrow cis-loop (residues 682-694) and formed between the S- and L-domains; and (2) the nicotinamide adenine dinucleotide phosphate (NADPH)-binding site (containing residues

592-682 of the S-domain), which is also inserted into the L-domain (Figures 1 and 2) [4,5]. As all statins share HMG-like moieties, which enable them to compete with HMG-CoA by sterically preventing its binding at the cis-loop, then it is imperative to computationally explore this mode of binding and mechanism of inhibition, in order to determine whether the phytochemicals of interest (sharing similar polar side groups as the HMG-like moieties of statins but with much less hydrophobic rings), will exhibit similar binding interactions at this narrow binding site.

Cochlospermum planchonii (*C planchonii*) and *Cochlospermum tinctorium* (*C tinctorium)*, 2 species of *Cochlospermum*, are plants extensively used in West African herbal medicine to manage several ailments [15-19]. They are known for their rich phytochemical constituents such as tannins, saponins, carotenoids, triterpenoids, flavonoids, and other polyphenolic compounds, which exhibit various pharmacological activities including antimalarial, antidiabetic, antioxidant, anti-inflammatory, antimicrobial, and enzyme-inhibitory properties [16,20-27]. Other studies have also demonstrated the antihyperlipidemic and cholesterol-lowering potential of their extracts (root, rhizomes, and leaf) [17,28], suggesting they contain bioactive compounds capable of managing lipid disorders. As far as the literature is concerned, no specific compounds isolated from *C planchonii* and *C tinctorium* have been directly studied or linked as potential inhibitors of HMGR. However, their phytochemicals, mostly reported to possess antioxidant, antimicrobial, anti-inflammatory, and enzyme-inhibitory activities, are thought to be significant contributors to the plants' lipid-lowering ability [16,17,28,29].

The aim of this study is to explore the efficacy of these phytochemicals as potential inhibitors of human HMGR and as promising alternatives to statins, using molecular docking tools. Molecular docking is a computational technique used to predict the preferred orientation of a small molecule (ligand) when it binds to a target protein (enzyme), allowing researchers to assess the binding mode and affinity, as well as the chemical interactions between the ligand and the enzyme in a complex [30]. Therefore, adopting this approach helps in evaluating the mechanism of phytochemical interactions with HMGR, their mode of binding and affinity, their fitness at the active site, as well as the stability of the HMGR-phytochemical complexes formed, in a context that is relevant to HMGR inhibition.

Methods

Phytochemical Selection

The selection of phytochemicals for this study was guided by a comprehensive review of existing literature. A Google Scholar search was conducted to identify peer-reviewed articles reporting on the phytochemical constituents and pharmacological activities of various extracts from *C planchonii* and *C tinctorium*. This search was conducted using key terms including "phytochemicals from *C. planchonii*," "phytochemicals from *C. tinctorium*," "HPLC analysis of *C. planchonii* and *C. tinctorium*," "GC-MS analysis of *C. planchonii* and *C. tinctorium*," and "bioactive compounds of *C. planchonii* and *C. tinctorium*." Articles were included if they (1) reported the use of high-performance liquid chromatography (HPLC) or gas chromatography mass-spectrometry (GC-MS) techniques in the phytochemical profiling of *C planchonii* and *C tinctorium*; (2) provided compounds with identifiable names; and (3) described pharmacological activities relevant to hypercholesterolemia or metabolic disorders. In total, 16 articles were evaluated, of which 4 met the inclusion criteria and provided the sufficient details used in the identification of the compounds [15-17,31]. A total of 84 phytochemicals were compiled, with 32 from *C planchonii* and 52 from *C tinctorium* (Tables S1-S3 in Multimedia Appendix 1). The selected compounds were included for this computational study based on the following criteria: (1) reported bioactivities, (2) structural integrity, (3) acceptable molecular weight for molecular docking, and (4) availability of their 2D structure data in the PubChem database. Their 2D structures were retrieved in structure data file (SDF) format from PubChem database on August 5, 2024 [32], and subsequently concatenated using Open Babel software [33], before being used for virtual screening.

Virtual Screening

DataWarrior is excellent for managing and screening large libraries of compounds based on their chemical properties [34,35]. The software was used to narrow down the large pool of 84 potential drug candidates, ensuring that only the most promising ones make it to the docking step. This approach helps save computational resources and time by focusing on most viable candidates. The phytocompounds were subjected to virtual screening to determine their drug-likeness and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties, in accordance with the Lipinski's Rule of Five. According to the Rule of Five, compounds are considered likely to be well absorbed into the systemic circulation when they possess octanol-water partition coefficient (CLogP) value ≤5, molecular weight ≤500, the number of hydrogen bond donors ≤5, the number of hydrogen bond acceptors ≤10, and the topological polar surface area ≤160 Å [36,37]. These properties were calculated for the concatenated compounds after importing them on DataWarrior using the "calculate compound properties from chemical structure" tab of the software. The 32 compounds meeting the criteria were selected and saved in SDF format. Other parameters screened for were mutagenicity, carcinogenicity, reproductive effectiveness, ligand efficiency, drug-likeness, and irritancy (Table 1). These parameters allow screening out compounds that do not meet the physicochemical criteria for drug-like behavior. The 2D structures of statins were also downloaded from PubChem database [32] and subjected to the same screening to serve as reference (Table 2).

Table 1. Drug-likeness and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of top-ranked phytochemicals of *Cochlospermum planchonii* and *Cochlospermum tinctorium*.

Serial number	Pubchem ID	Compound name	MW[a] (g/mol)	HA[b]	HD[c]	ClogP[d]	TPSA[e]	DL[f]	LE[g]	RE[h]	Mutagenic	Tumorigenic	Irritant
1	73641	Arjunolic acid	488.71	5	4	4.286	97.99	–5.555	0.162	None	None	None	None
2	12305768	Alphitolic acid	472.71	4	3	5.519	77.76	–21.780	0.077	None	None	None	None
3	5281855	Ellagic acid	302.19	8	4	1.277	133.52	–1.598	0.142	None	None	None	None
4	72277	Epigallocatechin	306.26	7	6	1.163	130.61	0.315	0.258	None	None	None	None
5	13915428	3-O-methylellagic acid	316.22	8	3	1.553	122.52	–1.390	0.111	None	None	None	None
6	5280417	3,7-di-O-methylquercetin	330.29	7	3	2.194	105.45	–0.105	0.130	None	None	None	None
7	44446550	3,4'-O-dimethylquercetin	332.31	7	3	1.662	105.45	0.503	0.077	None	None	None	None
8	9064	Catechin	290.27	6	5	1.509	110.38	0.315	0.329	None	None	None	None
9	535203	3-(Azepan-1-yl)-1,2-benzothiazole 1,1-dioxide	264.35	4	0	2.478	58.12	–1.176	0.249	None	None	None	None
10	101202074	(2E,4E,6E,8E,10E,12E)-13-[(1S)-1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl]-2,6,11-trimethyltrideca-2,4,6,8,10,12-hexaenoic acid	396.53	4	2	6.038	74.60	0.101	0.047	None	None	None	None

[a]MW: molecular weight.
[b]HA: hydrogen acceptor.
[c]HD: hydrogen donor.
[d]ClogP: Octanol-water partition coefficient.
[e]TPSA: topological polar surface area.
[f]DL: drug-likeness.
[g]LE: ligand efficiency.
[h]RE: reproductive effectiveness.

Table 2. Drug-likeness and ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties of statins.

Serial number	Pubchem ID	Compound name	MW[a] (g/mol)	HA[b]	HD[c]	ClogP[d]	TPSA[e]	DL[f]	LE[g]	RE[h]	Mutagenic	Tumorigenic	Irritant
1	Protein Data Bank ID: 117/obj01	Atorvastatin (cocrystallized control)	558.65	7	4	5.622	111.79	4.451	—[i]	High	None	None	None
2	60823	Atorvastatin	558.65	7	4	5.622	111.79	4.451	0.141	High	None	None	None
3	64715	Mevastatin (Compactin)	390.52	5	1	3.626	72.83	0.578	0.205	None	None	None	None
4	446155	Fluvastatin	411.47	5	3	3.978	82.69	1.804	0.153	High	None	None	None
5	446156	Cerivastatin	459.56	6	3	4.320	99.88	–0.283	0.139	None	None	None	None

Serial number	Pubchem ID	Compound name	MW[a] (g/mol)	HA[b]	HD[c]	ClogP[d]	TPSA[e]	DL[f]	LE[g]	RE[h]	Mutagenic	Tumorigenic	Irritant
6	446157	Rosuvastatin	481.54	9	3	2.100	149.3	3.454	0.139	None	None	None	None
7	54454	Simvastatin	418.57	5	1	4.461	72.83	0.668	0.195	None	None	None	None

[a]MW: molecular weight.
[b]HA: hydrogen acceptor.
[c]HD: hydrogen donor.
[d]ClogP: Octanol-water partition coefficient.
[e]TPSA: topological polar surface area.
[f]DL: drug-likeness.
[g]LE: ligand efficiency.
[h]RE: reproductive effectiveness.
[i]Not applicable.

As shown in Tables 1 and 2, the drug-likeness score is a crucial parameter used in determining whether a compound is likely to be an effective drug. A positive score indicates that a compound possesses structural features similar to known drugs, while a negative score suggests that such compound has structural features that are less common in known drugs. Good drug-like compounds usually have scores greater than zero [35,36]. Ligand efficiency (LE) is a metric used to evaluate the binding efficiency of compounds relative to their size. A higher LE score indicates that a compound achieves its binding affinity with fewer atoms making it more efficient, while a lower LE score suggests that a compound relies on a larger structure to achieve its binding, which might be less desirable [38]. When screening for toxic compounds, those that may bind to unintended off-target sites, which could lead to adverse effects such as genetic mutations, cancer development, or cause irritation to tissues like skin, eyes, or mucous membrane, were eliminated. Reproductive effectiveness parameter was used to predict the potential impact of a compound on reproductive health, including infertility and harm to fetal development [35].

Drug Target Preparation

The 3D crystal structure of human HMGR (PDB ID: 1HWK) complexed with atorvastatin (PDB ID: 117) was retrieved in PDB format August 5, 2024, from PDB [14]. The drug target was prepared by removing redundant subunits (B, C, D), Adenosine diphosphate, heteroatoms, and water molecules using PyMOL visualization tool [39]. The unique ligand atorvastatin (obj01/117), which served as one of the control ligands was extracted from the catalytic subunit A, in addition to the 6 other statins downloaded from PubChem. Both target and ligand were saved in PDB and SDF formats, respectively. Using PyMOL allows one to visualize and predict the grid co-ordinates around the HMG-binding pocket, while Discovery Studio visualizer [40] helps in identifying and characterizing the residues at the binding site.

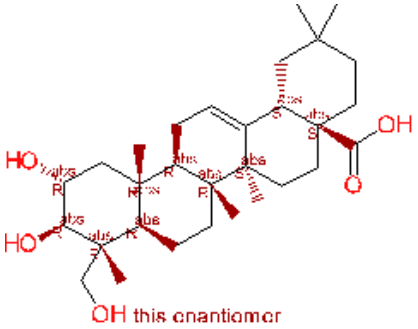
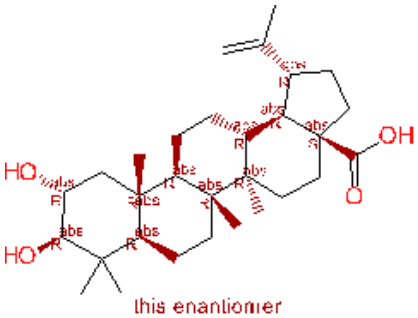
Molecular Docking Analysis

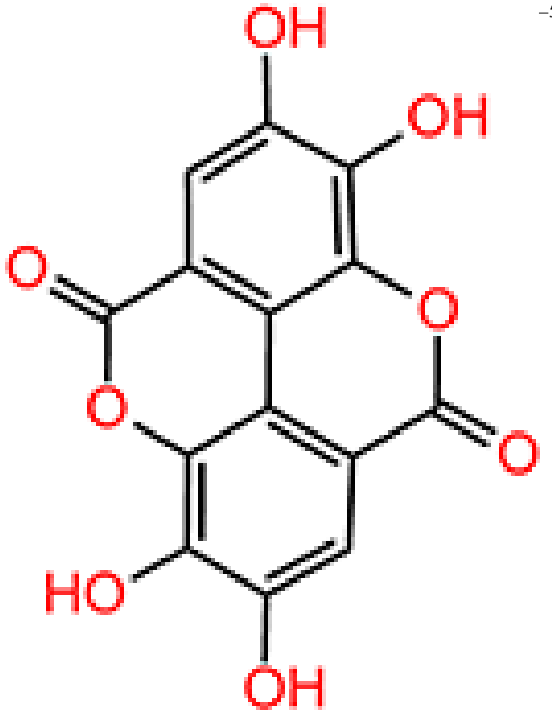
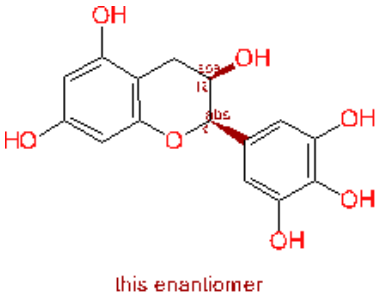
PyRx virtual screening tool [41] was used for the molecular docking. The prepared drug target HMGR was loaded on PyRx in PDB format, hydrogen atoms were added to ensure the protein is correctly protonated and made as macromolecule, after which the screened phytochemicals were imported in SDF format. These compounds were subjected to energy minimization using the optimization algorithm tool of PyRx, and the required force field was set at "ghemical," adjusting the positions of atoms in the phytochemicals in order to reduce their overall energy and steric clashes, thus attaining stable conformations. The compounds were converted to PDBQT format for compatibility with the docking algorithm Autodock Vina. Docking was performed specifically at the HMG-binding pocket (residues 682-694) of the protein. The 3D docking grid box which encloses this region, where the compounds will bind was centered at co-ordinates (X: 22.2175, Y: –3.5559, Z: 5.8150) with grid box dimensions of 21.0454 × 28.2041 × 28.7731 Å, along the same axes, respectively. This type of docking is semirigid, where the structure of receptor (HMGR) remains rigid while the phytochemicals and statins have some degree of flexibility at the binding pocket. In the molecular docking, the PyRx AutoDock Vina Wizard exhaustive search docking function was used because of its balance between computational efficiency and accuracy. To ensure the feasibility of the study protocol and accuracy of the docking algorithm, 6 statins (atorvastatin, mevastatin/compactin, fluvastatin, cerivastatin, rosuvastatin, and simvastatin) were downloaded from PubChem database in addition to the native ligand (cocrystallized obj01/117 extracted from the drug target 1HWK). Before docking the phytochemicals, each statin was redocked into the HMG-binding site. The resulting poses, binding interactions, and binding energies were compared with those in the literature, especially the original crystallographic data (PDB IDs: 1HWK, 1HWI, 1HWL, 1HWJ, 1HW8, and 1HW9) [14] reported by Istvan and Deisenhofer [5]. The consistency between the docked results and published experimental data validated the efficiency and accuracy of the Autodock Vina docking algorithm. After this validation, the docking of the 32 hit (screened) phytochemicals was performed. Their results were exported as PDBQT files and visualized using PyMOL and Discovery Studio to evaluate the best poses (binding modes), hydrogen bonding, hydrophobic interactions, and molecular fit within the binding pocket. Their binding energy scores were saved

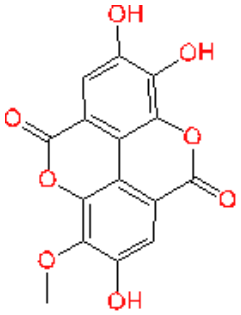
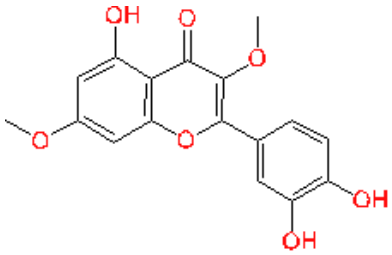
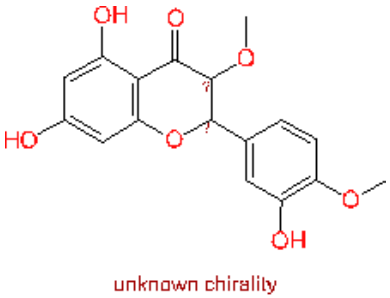
in excel format for statistical analysis. The docking process was repeated for all 84 phytochemicals without screening, to determine whether potential inhibitors, which might have been previously screened out, could be identified as drug candidates. To generate the docking scores for the compounds and statins, another round of docking was performed using the “Dock structure into protein cavity” tab on DataWarrior.

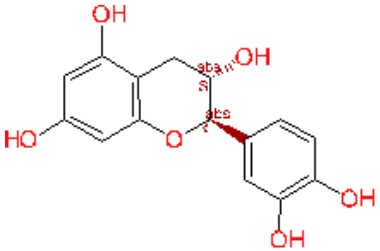
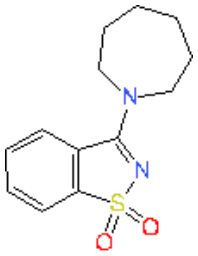
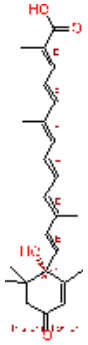
The docking score and binding energy score are two key metrics used in this molecular docking study (Tables 3 and 4). The docking score was a value generated by DataWarrior software to represent the quality of the ligand’s fit into the receptor’s binding site and is derived using a scoring function based on factors such as hydrogen bonding, van der Waal’s, hydrophobic, and electrostatic interactions [35]. The docking score was mainly used to rank the different compounds in terms of how well they bind to the HMG-binding site of HMGR and to compare the quality and fitness of their binding with those of statins. Higher docking scores (more negative) generally indicate a better fit between the compounds and HMGR, and vice versa. However, the docking score is not an absolute energy value.

Table 3. Molecular docking results of the top-ranked phytochemicals' binding at 3-hydroxy-3-methylglutaric acid–binding pocket of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (Protein Data Bank ID: 1HWK).

Serial number[a]	PubChem ID	Compound name	Molecular structure (2D)	Binding energy ΔG (kcal/mol)	Ki[b] (μM)	RMSD[c]	Docking score	HMG binding pocket residues	Other amino acid residues
1	73641	Arjunolic acid	 MF[d]: $C_{30}H_{48}O_5$	−6.0	39.7	0.0	−20.320	LYS691, LYS692, ASN686, VAL683	ASN658
2	12305768	Alphitolic acid	 MF: $C_{30}H_{48}O_4$	−5.5	92.4	0.0	−19.805	LYS691, LYS692, VAL683	GLU665, ASN658

Serial number[a]	PubChem ID	Compound name	Molecular structure (2D)	Binding energy ΔG (kcal/mol)	Ki[b] (μM)	RMSD[c]	Docking score	HMG binding pocket residues	Other amino acid residues
3	5281855	Ellagic acid	 **MF: $C_{14}H_6O_8$**	–5.1	181.7	0.0	–20.728	SER684, ARG590, LYS692, ASP690	—[e]
4	72277	Epigallocatechin	 this enantiomer **MF: $C_{15}H_{14}O_7$**	–5.1	181.7	0.0	–26.477	VAL683, ARG590, ASP690, SER684, ASN686, LYS692	SER661, GLU665

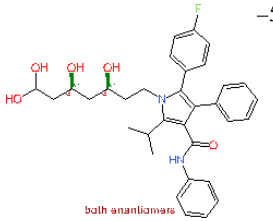
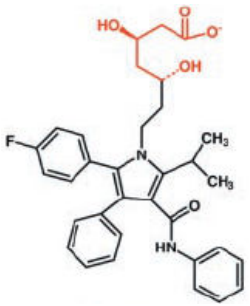
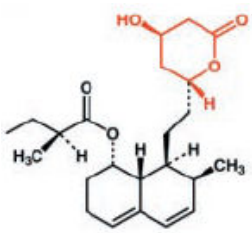
Serial number[a]	PubChem ID	Compound name	Molecular structure (2D)	Binding energy ΔG (kcal/mol)	Ki[b] (μM)	RMSD[c]	Docking score	HMG binding pocket residues	Other amino acid residues
5[f]	13915428	3-O-methyl ellagic acid	 **MF: $C_{15}H_8O_8$**	–5.0	215.2	0.0	–21.684	VAL683, ARG590, SER684, ASN686, ASP690, LYS692, LYS691	—
6[g]	5280417	3,7-Di-O-methylquercetin	 **MF: $C_{17}H_{14}O_7$**	–5.0	215.2	0.0	–32.103	LYS691, ARG590, SER684, ASN686	MET655, MET657
7[h]	44446550	3,4'-O-Dimethylquercetin	 **MF: $C_{17}H_{16}O_7$**	–4.9	254.8	0.0	–29.303	LYS692, ASP690, LYS691, ARG590	ASN658, SER661, GLU665

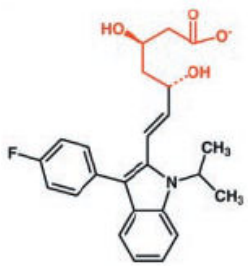
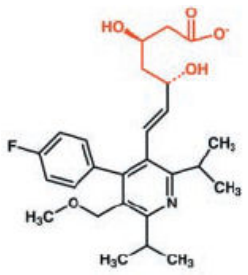
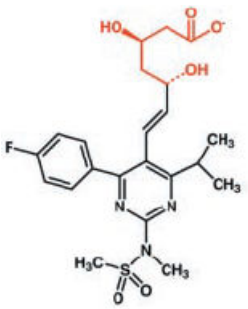
Serial number[a]	PubChem ID	Compound name	Molecular structure (2D)	Binding energy ΔG (kcal/mol)	Ki[b] (μM)	RMSD[c]	Docking score	HMG binding pocket residues	Other amino acid residues
8	9064	Catechin	 this enantiomer **MF: $C_{15}H_{14}O_6$**	–4.9	254.8	0.0	–26.213	LYS692, ASP690, VAL683, SER684, ASN686, ARG590	ASN658, SER661, GLU665
9	535203	3-(Azepan-1-yl)-1,2-benzothiazole-1,1-dioxide	 **MF: $C_{13}H_{16}N_2O_2S$**	–4.7	357.3	0.0	–13.272	LYS692, SER684, ARG590, ASP690, LYS691, VAL683	MET657
10	101202074	Cochloxanthin (2E,4E,6E,8E,10E,12E)–13-[(1S)-1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl]–2,6,11-trimethyltri-deca-2,4,6,8,10,12-hexaenoic acid		–4.6	422.6	0.0	–25.083	VAL683, ASP690, LYS691, LYS692	GLU665

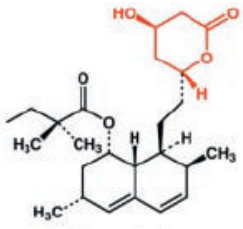
Serial number[a]	PubChem ID	Compound name	Molecular structure (2D)	Binding energy ΔG (kcal/mol)	Ki[b] (μM)	RMSD[c]	Docking score	HMG binding pocket residues	Other amino acid residues
			MF: $C_{25}H_{32}O_4$						

[a]The interaction of the ligands with the catalytic residues of 3-hydroxy-3-methylglutaryl coenzyme-A reductase, as presented in the table, are curated from their two best poses (1 and 2), while their binding energy scores are derived from binding pose 1.
[b]Ki: inhibition constant.
[c]RMSD: root mean square deviation.
[d]MF: molecular formula.
[e]Not applicable.
[f]Compound 5: C-3➞O-CH_3.
[g]Compound 6: C-3➞O-CH_3, C-7➞O-CH_3.
[h]Compound 7: C-3➞O-CH_3, C-4➞O-CH_3.

Table 4. Molecular docking results of statins' binding at 3-hydroxy-3-methylglutaric acid–binding pocket of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (Protein Data Bank ID: 1HWK).

Serial number[a]	PubChem ID	Compound name	Molecular structure (2D)	Binding energy ΔG (kcal/mol)	Ki[b] (μM)	RMSD[c]	Docking score	HMG binding pocket residues	Other amino acid residues
1	Protein Data Bank ID: 117/Obj01	Atorvastatin (cocrystallized control)	 **MF:[d] $C_{33}H_{35}FN_2O_5$**	–5.3	129.6	0.0	–31.329	LYS692, ASP690, ARG590, SER684, VAL683	SER661, LYS662, ASN658
2	60823	Atorvastatin	 **MF: $C_{33}H_{35}FN_2O_5$**	–5.1	181.7	0.0	–33.050	LYS691, ARG590, LYS692, ASN686, SER684, VAL683	ALA769, ASN658, SER661
3	64715	Mevastatin (Compactin)	 **MF: $C_{23}H_{34}O_5$**	–4.9	254.8	0.0	–27.147	ASP690, LYS691, SER684, ARG590, VAL683, LYS692	SER661

Serial number[a]	PubChem ID	Compound name	Molecular structure (2D)	Binding energy ΔG (kcal/mol)	Ki[b] (μM)	RMSD[c]	Docking score	HMG binding pocket residues	Other amino acid residues
4	446155	Fluvastatin	 **MF: $C_{24}H_{26}FNO_4$**	–5.3	129.6	0.0	–33.559	LYS691, ASP690, ARG590, ALA682, VAL683, SER684	MET657
5	446156	Cerivastatin	 **MF: $C_{26}H_{34}FNO_5$**	–4.6	422.6	0.0	–36.584	LYS692, ARG590, SER684, ASP690, LYS691, VAL683	GLU665, MET657, ASN658
6	446157	Rosuvastatin	 **MF: $C_{22}H_{28}FN_3O_6S$**	–4.9	254.8	0.0	–31.207	ARG590, SER684, VAL683, LYS692	SER661, ASN658

Serial number[a]	PubChem ID	Compound name	Molecular structure (2D)	Binding energy ΔG (kcal/mol)	Ki[b] (μM)	RMSD[c]	Docking score	HMG binding pocket residues	Other amino acid residues
7	54454	Simvastatin		–5.7	66.0	0.0	–25.939	ASP690, ARG590, SER684, LYS691, VAL683	—[e]

MF: $C_{25}H_{38}O_5$

[a]The interaction of the statins with the catalytic residues of 3-hydroxy-3-methylglutaryl coenzyme-A reductase, as presented in the table, are curated from their two best poses (1 and 2), while their binding energy scores are derived from binding pose 1.
[b]Ki: inhibition constant.
[c]RMSD: root mean square deviation.
[d]MF: molecular formula.
[e]Not applicable.

In contrast, the binding energy score, represented as the free energy of binding (ΔG) and measured in kcal/mol, refers to the binding affinity and strength of the interaction between a ligand and its target. It was generated by the AutoDock Vina algorithm in PyRx. The binding energy score predicts how strongly a compound will bind to the HMGR under physiological conditions, with higher (more negative) values indicating a stronger binding and more thermodynamically favorable formation of complexes [42]. Unlike the docking score, the binding energy score was directly correlated with inhibition constant (Ki) value using the formula $\{Ki = e^{\Delta G/RT}\}$, where R is molar gas constant (1.987 cal/mol/K), and T is standard temperature in Kelvin (298K). Therefore, the selection of compounds was focused on those with stronger interactions and more effective binding energies rather than on the ones with good docking scores, in addition to using their drug-likeness and ADMET properties.

Statistical Analysis

All binding affinity scores for test compounds and control ligands were expressed as the means of 9 determinations each representing 9 different binding modes and SE of the mean. Statistical evaluation of data was performed using one-way ANOVA on Graphpad Prism (version 8.0; Graphpad Software Inc.). Significance levels were tested at $P<.05$.

Ethical Considerations

This study did not require ethical approval because it involved only computational analyses and did not include any human participants, identifiable personal data, or animal experiments, in accordance with institutional and international guidelines.

Results

To investigate the mechanism of binding and inhibition of the bioactive compounds isolated from *C planchonii* and *C tinctorium* on human HMGR activity, statins and each compound were docked against the HMG-binding pocket of the enzyme. The docking study results revealed that 10 lead compounds, each at 9 different binding poses, exhibited strong binding affinities, with binding energy (ΔG) scores ranging from –4.6 to –6.0 kcal/mol (Figure 3; Table 3). These phytochemicals also interacted well with the relevant amino acid residues at the HMG-binding pocket of the enzyme (Figure 4 and Figures S1-S5 in Multimedia Appendix 2) when compared with the interactions of statins (Figure S6 in Multimedia Appendix 3). Their ΔG scores were comparable to or exceeded those of the control ligands (–4.6 to –5.7 kcal/mol; Table 4). Their docking scores (–13.272 to –32.103) also compared favorably with those of statins (–25.939 to –36.584). One of the lead compounds, 3-O-methylellagic acid (ID_13915428) demonstrated stronger and more substantial binding interactions with the HMG-binding pocket residues of the drug target than any compound, including statins, in addition to exhibiting high binding energy (Table S4 in Multimedia Appendix 4).

Figure 3. Binding potential of statins (red) and top-ranked phytochemicals (blue) at 3-hydroxy-3-methylglutaric acid–binding pocket of human 3-hydroxy-3-methylglutaryl-coenzyme A reductase (Protein Data Bank ID: 1HWK).

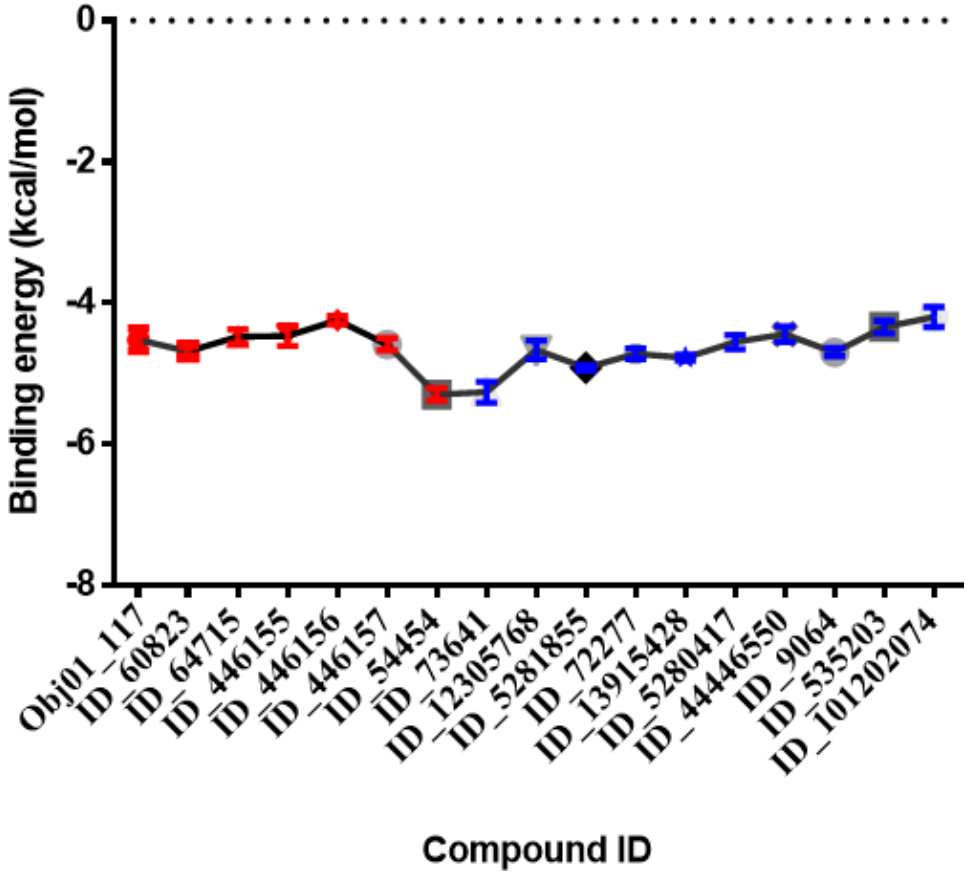
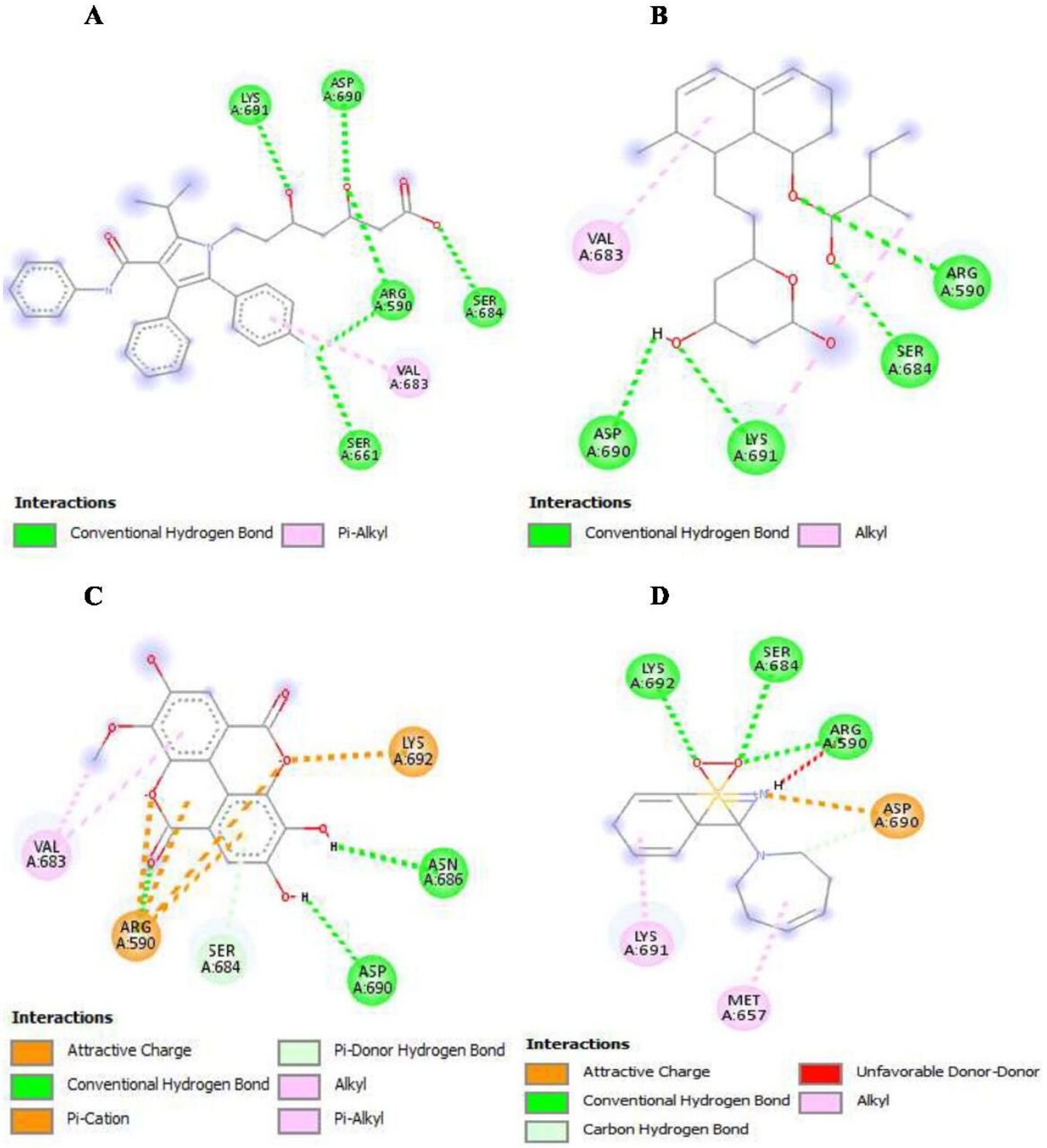


Figure 4. 2D representation showing the binding interactions of (A) Protein Data Bank ID_117/objt01 from literature, (B) ID_64715 pose 1,(C) ID_13915428 pose 1, and (D) ID_535203 pose 1, with 3-hydroxy-3-methylglutaric acid–binding pocket residues of human 3-hydroxy-3-methylglutaryl-coenzyme A reductase.



Discussion

Principal Findings

The molecular docking analysis of the two best binding modes of the 10 top-ranked phytochemicals demonstrated their cholesterol-lowering potential, as they clearly showed strong biochemical interactions and high binding affinities with the relevant amino acid residues that constitute the HMG-binding pocket (residues 682-694) of human HMGR (Tables S4-S13 in , Multimedia Appendix 4; Figures 3 and 4), comparable to or better than statins (Tables S14-S20 in Multimedia Appendix 5). This suggests that the phytochemicals could hinder the binding of the substrate HMG-CoA through competitive inhibition, in a similar binding mechanism as statins.

As shown in Table 3, the 10 top-ranked phytochemicals identified in this study, in no particular order, comprise 2 hydrolysable tannins (ellagitannins): ellagic acid (ID_5281855) and 3-O-methylellagic acid (ID_13915428); 4 flavonoids: catechin (ID_9064), epigallocatechin (ID_72277), 3,7-Di-O-methylquercetin (ID_5280417), and 3,4'-O-Dimethylquercetin (ID_44446550); 2 triterpenoid saponins: arjunolic acid (ID_73641) and alphitolic acid (ID_12305768); 1 carotenoid: cochloxanthin (ID_101202074); and 1 benzothiazole derivative, 3-(Azepan-1-yl)–1,2-benzothiazole-1,1-dioxide (ID_535203).

Comparison to Prior Work

Several polar interactions with the cis-loop residues (Arg^{590}, Ser^{684}, Asn^{686}, Asp^{690}, Lys^{691}, Lys^{692}) of HMGR, are formed by the hydroxyl (-OH) groups of the aromatic rings, carbonyl groups (C=O), and lactone ring oxygen atoms of the ellagitannins. Their bulky rings also establish several electrostatic and hydrophobic contacts with residues Val^{683}, Arg^{590}, Ser^{684}, Asp^{690}, Lys^{691}, and Lys^{692} (Multimedia Appendices 2 and 4). No interactions of these polyphenols were observed with other residues within the HMGR-binding site. Among all the compounds, including statins, 3-O-methylellagic acid (ID_13915428) exhibits the greatest number (26) of binding interactions, indicating that this polyphenolic compound could be a viable drug candidate for HMGR inhibition. A recent in vivo and in vitro study by Lee et al [43] demonstrated that ellagic acid inhibits HMGR by activating AMP-activated protein kinase (AMPK), leading to the phosphorylation and subsequent inactivation of the enzyme. This study, which included rats fed with a high-cholesterol diet, revealed that the administration of ellagic acid (4 mg/kg/d, orally) resulted in significant reductions in serum total cholesterol, LDL-C, and triglyceride levels. Ellagic acid was also found to downregulate the gene expression of sterol regulatory element-binding protein-2 (SREBP-2) and its target protein HMGR, thereby reducing cholesterol biosynthesis in the liver [43]. In addition to its roles in cholesterol metabolism, ellagic acid and its derivatives also exhibit antioxidant and anti-inflammatory properties, which contribute to their protective effects against cardiovascular diseases [44].

The 4 flavonoids identified, belonging to the catechin and quercetin classes of polyphenols (ID_9064, ID_72277, ID_5280417, and ID_44446550), demonstrated their potential to mimic the binding of statins by forming polar hydrogen bonds with cis-loop residues (Arg^{590}, Ser^{684}, Asn^{686}, Asp^{690}, Lys^{691}, and Lys^{692}) and other residues (Asn^{658} and Glu^{665}). They also formed several electrostatic and nonpolar hydrophobic interactions with Val^{683} and other residues, including Met^{655}, Met^{657}, and Ser^{661}, at the HMGR-active site. This capability is attributed to their basic flavan-ring structure with multiple polar -OH, C=O, pyran ring oxygen atoms, and methoxy (-OCH_3) groups (Multimedia Appendices 2 and 4). An in vitro experiment showed that catechin isolates from *Uncaria gambir,* an Indonesian plant, exhibit strong inhibitory activities against HMGR with 97.46% efficacy, compared to 85.74% for simvastatin, a performance suggesting it could stand out as a promising therapy for hypercholesterolemia treatment [45]. Surprisingly, epigallocatechin gallate has been shown to potently and reversibly inhibit HMGR in vitro by competing with its cofactor NADPH and binding at the cofactor site instead of the HMG-binding pocket [46]. However, this present study suggests that epigallocatechin gallate may possess both capabilities. Quercetin dihydrate and gallate supplements have also been reported to lower plasma and hepatic cholesterol levels in rats fed with a cholesterol-rich diet. The results of the study concluded that quercetin dihydrate significantly reduced hepatic HMGR activity compared to normal control groups [47]. Furthermore, other several studies have elucidated the ability of quercetin to drastically reduce HMGR activity, inhibit fatty acid and triacylglycerol synthesis in hepatocytes, and alleviate endothelial dysfunction associated with age-related cardiovascular diseases [48-50].

Alphitolic acid and arjunolic acid are the two pentacyclic triterpenoids examined in this study. They generally exhibited fewer binding interactions with the HMG-binding site of HMGR, possibly due to their bulky and less polar triterpene core structure. However, the -OH and carboxylic (-COOH) groups present at both ends of their side chains formed polar hydrogen interactions with relevant residues such as Asp^{690}, Lys^{691}, Lys^{692}, Asn^{658}, and Glu^{665}. In addition, their pentacyclic rings engaged in non-polar hydrophobic interactions with important residues including Val^{683} and Lys^{691} (Multimedia Appendices 2 and 4). Direct studies on the inhibition of HMGR by alphitolic acid and arjunolic acid are currently lacking. However, several studies have shown that other structurally related triterpenoids possess direct inhibitory effects on HMGR. For example, 3α,26-dihydroxytirucalla-7,24-dien-21-oic acid (ARM-2) and 3β-hydroxylanosta-9,24-dien-21-oic acid (RA-5), isolated from *Protorhus longifolia*, demonstrated potent HMGR inhibition with IC_{50} values lower than lovastatin and simvastatin [51]. Similarly, Shen et al [52] reported that the doses of 25 and 125 μg/mL of oleanolic acid, a pentacyclic triterpenoid found in *Cassia mimosoides*, showed inhibitory effects on HMGR that were comparable to those of standard pravastatin groups. A patent report by Wöhrle et al [53] also identified several polyhydroxylated pentacyclic triterpenes as novel HMGR inhibitors, highlighting the therapeutic potential of this class of compounds. Moreover, arjunolic acid has been reported to protect against atorvastatin-induced oxidative stress and apoptosis in renal and hepatic tissues [54]. Its role in activating AMPK and suppressing neuroinflammation in animal models further suggests it may exert an indirect regulatory effect on HMGR inhibition [55].

Cochloxanthin is a carotenoid pigment found in certain plants, including *Cochlospermum* species. This compound showed polar hydrogen interactions between its polar side chain (-OH and -COOH groups) and a few HMG-binding residues, such as Asp^{690}, Lys^{692}, and others Glu^{665}. Additionally, hydrophobic bonds were formed between the carbon atoms of its long polyene chain and relevant residues, including Val^{683} and Lys^{691} (Multimedia Appendices 2 and 4). These relatively few binding interactions likely occurred due to the compound's linear long-chain skeleton,

which may not fit properly into the narrow HMG-binding pocket of the enzyme. Metibemu et al [56] in their in-silico study, investigated several carotenoids isolated from *Spondias mombin* and suggested that these compounds possess strong HMGR inhibitory effects, along with antilipidemic and anticancer properties, but there was no direct link established with cochloxanthin. Similarly, in vitro studies by Alvi et al [57] reported that lycopene, a red carotenoid predominantly found in tomatoes, demonstrated significant inhibitory effects on HMGR, with an IC_{50} value of 36 ng/mL, which surpassed that of pravastatin (IC_{50}=42 ng/mL). Their molecular docking analyses also revealed that lycopene binds effectively to the hydrophobic portion of the HMGR active site, showing a competitive inhibition [57]. In addition, Moreno et al [58] in their own study involving rat liver tissues showed that the administration of 70 mg/kg β-carotene (a precursor of vitamin A) led to a 50% reduction in hepatic HMGR mRNA expression. The authors suggested the role of β-carotene in modulating cholesterol biosynthesis at post-transcriptional level [58].

3-(Azepan-1-yl)-1,2-benzothiazole-1,1-dioxide is a heterocyclic sulfonamide derivative with a benzothiazole scaffold and an azepane ring structure, isolated from *C tinctorium*. Interestingly, this compound revealed promising polar interactions between the sulfonyl functional group (SO_2) of its benzothiazole ring and nitrogen atom with HMG-binding residues including Arg^{590}, Ser^{684}, Asp^{690}, and Lys^{692}. Additionally, its benzene and azepane rings formed several catalytically important hydrophobic contacts with residues Val^{683}, Lys^{691}, Asp^{690}, and Met^{657} (Multimedia Appendices 2 and 4). These interactions suggest the compound may serve as a novel, natural inhibitor of human HMGR. Currently, there is no information available on the effect of this compound on HMGR activities. Nevertheless, a molecular docking study by Ikpa and Tochukwu [59] demonstrated that this compound exhibited higher antiulcer potential than omeprazole by binding strongly to the H^+/K^+-ATPase receptor, a key drug target for proton pump inhibitors. The authors suggested that the compound may have superior gastric proton pump inhibitory potential compared to omeprazole, justifying its traditional use for relieving ulcer in patients.

Strengths and Limitations

This study has several strengths. Firstly, it is the first computational study to virtually screen and identify specific bioactive compounds isolated from two indigenous *Cochlospermum* species as potential inhibitors of human HMGR, through a structured and comprehensive literature review. Secondly, the study integrates several open-source and cost-effective software applications known for their high accuracy and reproducibility, such as PyMOL, PyRx, Open Babel, DataWarrior, and Discovery Studio, in the phytochemical screening and molecular docking analysis. This approach enhances the strength of the findings without the need for immediate wet-lab resources in the discovery of potential drug candidates, thus saving time and cost in the early stages of drug discovery. Lastly, the use of a validated human HMGR structure, with docking focusing on its HMG-binding pocket (cis-loop), a critical region responsible for its catalytic activity, ensures the biological relevance of the docking results. In addition, the inclusion of known statin inhibitors and the native ligand as controls provides a robust benchmark for comparing and assessing the inhibitory potential of the phytochemicals of interest.

However, there are some limitations. Due to resource constraints, this study did not include molecular dynamics simulation (MDS), a computational technique that could have provided additional insights into the dynamic behavior, conformational flexibility, and stability of the HMGR-phytochemical complexes over specific time. Also, the docking approach used was semirigid, where the crystal structure of HMGR is kept rigid and only the statins and phytochemicals have conformational flexibility. This method may not fully account for induced-fit effects, which could potentially lead to an underestimation of the compounds binding affinities and specificities, or a misinterpretation of their binding interactions.

In order to address these limitations, a pragmatic alternative was taken. The accuracy of the PyRx Autodock Vina docking algorithm was validated by cross-checking its docked statin results against the previously reported wet-lab experimental data of statins from the literature, before docking the phytochemicals. The consistency between the docking results and validated data from the literature supports the efficiency, reliability, and accuracy of the computational tools utilized in this study.

Future Directions

Although the literature review approach adopted in this study was crucial in the identification of bioactive compounds isolated from *C planchonii* and *C tinctorium*, however, it did not meet the full PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) criteria for a systematic review, due to limitations in the coverage of database, the lack of a second reviewer, and absence of a registered protocol. Therefore, a more extensive systematic review of phytochemicals from all species of *cochlospermum* is recommended for future studies, as this would help in the identification of potential drug candidates for HMGR inhibition that were not evaluated in this study.

Building on this study's findings, future work should include MDS to better capture the dynamic behavior, stability, and conformational changes of the HMGR-phytochemical complexes over time. These simulations can help in validating the docking results and reveal the compounds potential to maintain stable interactions with HMGR under physiological conditions. Furthermore, introducing other computational techniques such as quantitative structure-activity relationship (QSAR) modeling and free energy calculations (eg, MM/PBSA or MM/GBSA) would also improve the predictive accuracy of the binding affinities of the phytochemicals.

To complement these computational techniques, the 10 top-ranked phytochemicals identified in this study should be subjected to in vitro enzymatic assays and cell-based

experiments in order to evaluate their actual inhibitory effects on HMGR activity. These efforts should also be followed by in vivo pharmacokinetic and toxicological studies which are necessary to determine the safety profile and therapeutic viability of these natural compounds. In the end, these combined computational and experimental approaches will be essential in translating the results of this study into meaningful advances in drug discovery.

Conclusions

This study has identified several bioactive compounds isolated from *C planchonii* and *C tinctorium* with potential to inhibit the activity of HMGR. The molecular docking results showed that compounds such as ellagic acid and its derivative, flavonoids, triterpenoids, carotenoids, and a benzothiazole derivative, exhibited significant biochemical interactions with the cis-loop residues of the enzyme, in addition to their high binding affinities. This demonstrates the ability of these phytochemicals of interest to potentially serve as natural and safer alternatives for hypercholesterolemia treatment, addressing the limitations posed by synthetic statins.

The findings are also consistent with previous studies that support the cholesterol-lowering and cardioprotective effects of these compounds, either directly or indirectly, through mechanisms such as AMPK activation, HMGR downregulation, and antioxidant properties. Although this study provides valuable computational insights into the molecular interactions of the compounds with HMGR, further advanced computational, in vitro, and in vivo studies are still necessary to validate their inhibitory potential and therapeutic applications.

Acknowledgments

This study would not have been possible without the grace of God. The author has no funding to declare.

Data Availability

All data generated or analyzed during this study are included in this published article and its supplementary information files in Multimedia Appendix 6.

Authors' Contributions

TIO: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Validation, Visualization, Writing - original draft, Writing - review & editing.

Conflicts of Interest

None declared.

Multimedia Appendix 1

Phytochemicals identified from *Cochlospermum planchonii* and *Cochlospermum tinctorium*.
[PDF File (Adobe File), 300 KB-Multimedia Appendix 1]

Multimedia Appendix 2

The best two binding interactions of top-ranked phytochemicals with 3-hydroxy-3-methylglutaric acid–binding pocket residues of human 3-hydroxy-3-methylglutaryl coenzyme-A reductase.
[PDF File (Adobe File), 506 KB-Multimedia Appendix 2]

Multimedia Appendix 3

The best two binding interactions of statins with 3-hydroxy-3-methylglutaric acid–binding pocket residues of human 3-hydroxy-3-methylglutaryl coenzyme-A reductase.
[PDF File (Adobe File), 417 KB-Multimedia Appendix 3]

Multimedia Appendix 4

Interaction profiles of the top-ranked phytochemicals at 3-hydroxy-3-methylglutaric acid–binding site of human 3-hydroxy-3-methylglutaryl coenzyme-A reductase.
[PDF File (Adobe File), 183 KB-Multimedia Appendix 4]

Multimedia Appendix 5

Interaction profiles of statins at 3-hydroxy-3-methylglutaric acid–binding site of human 3-hydroxy-3-methylglutaryl coenzyme-A reductase.
[PDF File (Adobe File), 158 KB-Multimedia Appendix 5]

Multimedia Appendix 6

Manuscript raw data files and analysis.
[RAR File (RAR archive File), 22492 KB-Multimedia Appendix 6]

References

1. Goldstein JL, Brown MS. A century of cholesterol and coronaries: from plaques to genes to statins. Cell. Mar 26, 2015;161(1):161-172. [doi: 10.1016/j.cell.2015.01.036] [Medline: 25815993]
2. Ference BA, Ginsberg HN, Graham I, et al. Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. Eur Heart J. Aug 21, 2017;38(32):2459-2472. [doi: 10.1093/eurheartj/ehx144] [Medline: 28444290]
3. Virani SS, Newby LK, Arnold SV, et al. 2023 AHA/ACC/ACCP/ASPC/NLA/PCNA Guideline for the management of patients with chronic coronary disease: a report of the American Heart Association/American College of Cardiology Joint Committee on Clinical Practice Guidelines. Circulation. Aug 29, 2023;148(9):e9-e119. [doi: 10.1161/CIR.0000000000001168] [Medline: 37471501]
4. Istvan ES, Palnitkar M, Buchanan SK, Deisenhofer J. Crystal structure of the catalytic portion of human HMG-CoA reductase: insights into regulation of activity and catalysis. EMBO J. Mar 1, 2000;19(5):819-830. [doi: 10.1093/emboj/19.5.819] [Medline: 10698924]
5. Istvan ES, Deisenhofer J. Structural mechanism for statin inhibition of HMG-CoA reductase. Science. May 11, 2001;292(5519):1160-1164. [doi: 10.1126/science.1059344] [Medline: 11349148]
6. Corsini A, Maggi FM, Catapano AL. Pharmacology of competitive inhibitors of HMG-CoA reductase. Pharmacol Res. Jan 1995;31(1):9-27. [doi: 10.1016/1043-6618(95)80042-5] [Medline: 7784310]
7. Kureishi Y, Luo Z, Shiojima I, et al. The HMG-CoA reductase inhibitor simvastatin activates the protein kinase Akt and promotes angiogenesis in normocholesterolemic animals. Nat Med. Sep 2000;6(9):1004-1010. [doi: 10.1038/79510] [Medline: 10973320]
8. Mundy G, Garrett R, Harris S, et al. Stimulation of bone formation in vitro and in rodents by statins. Science. Dec 3, 1999;286(5446):1946-1949. [doi: 10.1126/science.286.5446.1946] [Medline: 10583956]
9. Davignon J, Laaksonen R. Low-density lipoprotein-independent effects of statins. Curr Opin Lipidol. Dec 1999;10(6):543-560. [doi: 10.1097/00041433-199912000-00010]
10. Taylor F, Huffman MD, Macedo AF, et al. Statins for the primary prevention of cardiovascular disease. Cochrane Database Syst Rev. Jan 31, 2013;2013(1):CD004816. [doi: 10.1002/14651858.CD004816.pub5] [Medline: 23440795]
11. Collins R, Reith C, Emberson J, et al. Interpretation of the evidence for the efficacy and safety of statin therapy. The Lancet. Nov 2016;388(10059):2532-2561. [doi: 10.1016/S0140-6736(16)31357-5]
12. Cho Y, Choe E, Lee Y ho, et al. Risk of diabetes in patients treated with HMG-CoA reductase inhibitors. Metab Clin Exp. Apr 2015;64(4):482-488. [doi: 10.1016/j.metabol.2014.09.008]
13. Esnouf RM. Further additions to *MolScript* version 1.4, including reading and contouring of electron-density maps. Acta Crystallogr D Biol Crystallogr. Apr 1999;55(4):938-940. [doi: 10.1107/S0907444998017363]
14. Complex of the catalytic portion of human HMG-COA reductase with atorvastatin. Protein Data Bank. URL: https://www.rcsb.org/structure/1HWK [Accessed 2024-08-05]
15. Lamien-Meda A, Kiendrebeogo M, Compaoré M, et al. Quality assessment and antiplasmodial activity of West African Cochlospermum species. Phytochemistry. Nov 2015;119:51-61. [doi: 10.1016/j.phytochem.2015.09.006] [Medline: 26429632]
16. Dall'Acqua S, Kumar G, Sinan KI, et al. An insight into Cochlospermum planchonii extracts obtained by traditional and green extraction methods: relation between chemical compositions and biological properties by multivariate analysis. Ind Crops Prod. May 2020;147:112226. [doi: 10.1016/j.indcrop.2020.112226]
17. Ahmad MH, Jatau AI, Khalid GM, Alshargi OY. Traditional uses, phytochemistry, and pharmacological activities of Cochlospermum tinctorium A. Rich (Cochlospermaceae): a review. Futur J Pharm Sci. Dec 2021;7(1):20. [doi: 10.1186/s43094-020-00168-1]
18. Haidara M, Bourdy G, De Tommasi N, et al. Medicinal plants used in Mali for the treatment of malaria and liver diseases. Nat Prod Commun. Mar 2016;11(3):339-352. [doi: 10.1177/1934578x1601100309] [Medline: 27169180]
19. Johnson-Fulton SB, Watson LE. Comparing medicinal uses of Cochlospermaceae throughout Its geographic range with insights from molecular phylogenetics. Diversity (Basel). 2018;10(4):123. [doi: 10.3390/d10040123]
20. Ballin NZ, Traore M, Tinto H, et al. Antiplasmodial compounds from Cochlospermum tinctorium. J Nat Prod. Sep 2002;65(9):1325-1327. [doi: 10.1021/np020008h] [Medline: 12350157]
21. Habtemariam S. α-Glucosidase inhibitory activity of Kaempferol-3- O -rutinoside. Nat Prod Commun. Feb 2011;6(2):201-203. [doi: 10.1177/1934578X1100600211] [Medline: 21425674]
22. Benoit-Vical F, Valentin A, Mallie M, Bessiere JM. Antiplasmodial activity of Cochlospermum planchonii and C. tinctorium tubercle essential oils. J Essent Oil Res. 2001;13(1):65-67. [doi: 10.1080/10412905.2001.9699609]

23. Tijjani MB, Bello IA, Aliyu AB, et al. Phytochemical and antibacterial studies of root extract of Cochlospermum tinctorium A. Rich. (Cochlospermaceae). Research J of Medicinal Plant. Jan 1, 2009;3(1):16-22. [doi: 10.3923/rjmp.2009.16.22]
24. Etuk EU, Agaie BM, Ladan MJ, Garba I. The modulatory effect of Cochlospermum tinctorium a rich aqueous root extract on liver damage induced by carbon tetrachloride in rats. Afr J Pharm Pharmacol. 2009;3(4):151-157. [doi: 10.5897/AJPP.9000271]
25. Nergard CS, Diallo D, Inngjerdingen K, et al. Medicinal use of Cochlospermum tinctorium in Mali Anti-ulcer-, radical scavenging- and immunomodulating activities of polymers in the aqueous extract of the roots. J Ethnopharmacol. Jan 4, 2005;96(1-2):255-269. [doi: 10.1016/j.jep.2004.09.018] [Medline: 15588678]
26. Musa AA. Cytotoxicity activity and phytochemical screening of Cochlospermum tinctorium Perr Ex A. Rich rhizome. J App Pharm Sci. Jul 28, 2012;2(7):155-159. [doi: 10.7324/JAPS.2012.2723]
27. Nafiu M, Akanji MA, Yakubu MT. Effect of aqueous extract of Cochlospermum planchonii rhizome on some kidney and liver functional indicies of albino rats. Afr J Tradit Complement Altern Med. 2011;8(1):22-26. [doi: 10.4314/ajtcam.v8i1.60488] [Medline: 22238479]
28. Nafiu MO, Salawu MO, Idowu AO, Akanji MA. Anti-hyperlipidemic activity of polyphenol-rich extract of Cochlospermum Planchonii roots in Triton x-100 induced rats. Fountain J Nat Appl Sci. 2020;9(1):1-10. [doi: 10.53704/fujnas.v9i1.258]
29. Da-Costa-Rocha I, Bonnlaender B, Sievers H, Pischel I, Heinrich M. Hibiscus sabdariffa L. – a phytochemical and pharmacological review. Food Chem. Dec 15, 2014;165:424-443. [doi: 10.1016/j.foodchem.2014.05.002] [Medline: 25038696]
30. Morris GM, Huey R, Lindstrom W, et al. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. J Comput Chem. Dec 2009;30(16):2785-2791. [doi: 10.1002/jcc.21256] [Medline: 19399780]
31. Yahaya MF, Dimas K, Yelwa JM, Abel A. GC-MS Analysis, antioxidant, and antimicrobial studies of ethanolic extract of Cochlespermum and Anchus officinalis L. J Interdiscipl Cycle Res. 2020. URL: https://www.academia.edu/42727852/GC_MS_Analysis_Antioxidant_and_Antimicrobial_Studies_of_ethanolic_extract_of_Cochlespermum_and_Anchus_officinalis_L [Accessed 2025-07-04]
32. PubChem. URL: https://pubchem.ncbi.nlm.nih.gov/ [Accessed 2024-08-05]
33. Open Babel. URL: https://openbabel.org/ [Accessed 2024-08-05]
34. DataWarrior. openmolecules.org. URL: https://www.openmolecules.org/datawarrior/ [Accessed 2024-08-05]
35. Sander T, Freyss J, von Korff M, Rufener C. DataWarrior: an open-source program for chemistry aware data visualization and analysis. J Chem Inf Model. Feb 23, 2015;55(2):460-473. [doi: 10.1021/ci500588j] [Medline: 25558886]
36. Lipinski CA. Lead- and drug-like compounds: the rule-of-five revolution. Drug Discov Today Technol. Dec 2004;1(4):337-341. [doi: 10.1016/j.ddtec.2004.11.007] [Medline: 24981612]
37. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. Jan 1997;23(1-3):3-25. [doi: 10.1016/S0169-409X(96)00423-1]
38. Hopkins AL, Keserü GM, Leeson PD, Rees DC, Reynolds CH. The role of ligand efficiency metrics in drug discovery. Nat Rev Drug Discov. Feb 2014;13(2):105-121. [doi: 10.1038/nrd4163] [Medline: 24481311]
39. PyMOL. URL: https://www.pymol.org/ [Accessed 2024-08-05]
40. Dassault Systemes. URL: https://www.3ds.com/ [Accessed 2024-08-05]
41. PyRx – Python Prescription Virtual Screening Tool. URL: https://pyrx.sourceforge.io/ [Accessed 2024-08-05]
42. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. Jan 30, 2010;31(2):455-461. [doi: 10.1002/jcc.21334] [Medline: 19499576]
43. Lee KH, Jeong ES, Jang G, et al. Unripe Rubus coreanus Miquel extract containing ellagic acid regulates AMPK, SREBP-2, HMGCR, and INSIG-1 signaling and cholesterol metabolism in vitro and in vivo. Nutrients. Feb 26, 2020;12(3):610. [doi: 10.3390/nu12030610] [Medline: 32110925]
44. Salvamani S, Gunasekaran B, Shukor MY, Shaharuddin NA, Sabullah MK, Ahmad SA. Anti-HMG-CoA reductase, antioxidant, and anti-inflammatory activities of Amaranthus viridis leaf extract as a potential treatment for hypercholesterolemia. Evid Based Complement Alternat Med. 2016;2016(1):8090841. [doi: 10.1155/2016/8090841] [Medline: 27051453]
45. Yunarto N, Sulistyowati I, Finolawati A, Elya B, Sauriasari R. HMG-COA Reductase inhibitory activity of extract and catechin isolate from Uncaria Gambir as a treatment for hypercholesterolemia. J Southwest Jiaotong Uni. 2021;56(6):490-499. [doi: 10.35741/issn.0258-2724.56.6.43]

46. Cuccioloni M, Mozzicafreddo M, Spina M, et al. Epigallocatechin-3-gallate potently inhibits the in vitro activity of hydroxy-3-methyl-glutaryl-CoA reductase. J Lipid Res. May 2011;52(5):897-907. [doi: 10.1194/jlr.M011817] [Medline: 21357570]
47. Bok SH, Park SY, Park YB, et al. Quercetin dihydrate and gallate supplements lower plasma and hepatic lipids and change activities of hepatic antioxidant enzymes in high cholesterol-fed rats. Int J Vitam Nutr Res. May 2002;72(3):161-169. [doi: 10.1024/0300-9831.72.3.161] [Medline: 12098884]
48. Khamis AA, Salama AF, Kenawy ME, Mohamed TM. Regulation of hepatic hydroxy methyl glutarate – CoA reductase for controlling hypercholesterolemia in rats. Biomed Pharmacother. Nov 2017;95:1242-1250. [doi: 10.1016/j.biopha.2017.09.071] [Medline: 28938515]
49. Gnoni GV, Paglialonga G, Siculella L. Quercetin inhibits fatty acid and triacylglycerol synthesis in rat-liver cells. Eur J Clin Invest. Sep 2009;39(9):761-768. [doi: 10.1111/j.1365-2362.2009.02167.x] [Medline: 19508303]
50. Dagher O, Mury P, Thorin-Trescases N, Noly PE, Thorin E, Carrier M. Therapeutic potential of quercetin to alleviate endothelial dysfunction in age-related cardiovascular diseases. Front Cardiovasc Med. 2021;8:658400. [doi: 10.3389/fcvm.2021.658400] [Medline: 33860002]
51. Ndlovu M, Serem JC, Selepe MA, et al. Triterpenoids from Protorhus longifolia exhibit hypocholesterolemic potential via regulation of cholesterol biosynthesis and stimulation of low-density lipoprotein uptake in HepG2 cells. ACS Omega. Aug 29, 2023;8(34):30906-30916. [doi: 10.1021/acsomega.3c01995] [Medline: 37663489]
52. Shen C, Huang L, Xiang H, et al. Inhibitory effects on the HMG-CoA Reductase in the chemical constituents of the *Cassia mimosoides* Linn. Rev Rom Med Lab. Dec 1, 2016;24(4):413-422. [doi: 10.1515/rrlm-2016-0041]
53. Wöhrle I, J M, Köpcke B, T K, Bitzer J, Reinhardt K, inventors; Polyhydroxylated pentacyclic triterpene acids as HMG-coa reductase inhibitors. May 14, 2015. URL: https://patents.google.com/patent/US20150133552A1/en [Accessed 2025-07-04]
54. Pal S, Sarkar A, Pal PB, Sil PC. Protective effect of arjunolic acid against atorvastatin induced hepatic and renal pathophysiology via MAPK, mitochondria and ER dependent pathways. Biochimie. May 2015;112:20-34. [doi: 10.1016/j.biochi.2015.02.016] [Medline: 25736991]
55. Yang Y, Lai Y, Tong X, Li Z, Cheng Y, Tian LW. Arjunolic acid ameliorates lipopolysaccharide-induced depressive behavior by inhibiting neuroinflammation via microglial SIRT1/AMPK/Notch1 signaling pathway. J Ethnopharmacol. Aug 2024;330:118225. [doi: 10.1016/j.jep.2024.118225]
56. Metibemu DS, Akinloye OA, Akamo AJ, Okoye JO, Omotuyi IO. In-silico HMG-CoA reductase-inhibitory and in-vivo anti-lipidaemic/anticancer effects of carotenoids from Spondias mombin. J Pharm Pharmacol. Sep 7, 2021;73(10):1377-1386. [doi: 10.1093/jpp/rgab103] [Medline: 34343336]
57. Alvi SS, Iqbal D, Ahmad S, Khan MS. Molecular rationale delineating the role of lycopene as a potent HMG-CoA reductase inhibitor: in vitro and in silico study. Nat Prod Res. Sep 2016;30(18):2111-2114. [doi: 10.1080/14786419.2015.1108977] [Medline: 26548547]
58. Moreno FS, Rossiello MR, Manjeshwar S, et al. Effect of β-carotene on the expression of 3-hydroxy-3-methylglutaryl coenzyme A reductase in rat liver. Cancer Lett. Sep 1995;96(2):201-208. [doi: 10.1016/0304-3835(95)03933-N]
59. Ikpa CBC, Tochukwu OM. In-silico molecular studies of the phytochemicals in ethanolic extract of Chromolaena Odorata against H+/K+-ATPase enzyme for Proton Pump inhibitor. JIST. 2024;12(5):5. [doi: 10.62110/sciencein.jist.2024.v12.801]

Abbreviations

ADMET: absorption, distribution, metabolism, excretion, and toxicity
AMPK: AMP-activated protein kinase
CVD: cardiovascular disease
GC-MS: gas chromatography-mass spectrometry
HMG: 3-hydroxy-3-methylglutaric acid
HMG-CoA: 3-hydroxy-3-methylglutaryl coenzyme-A
HMGR: 3-hydroxy-3-methylglutaryl coenzyme-A reductase
HPLC: high-performance liquid chromatography
LDL-C: low-density lipoprotein cholesterol
LE: ligand efficiency
MDS: molecular dynamics simulation
MM/GBSA: molecular mechanics generalized Born surface area
MM/PBSA: molecular mechanics Poisson-Boltzmann surface area
NADPH: nicotinamide adenine dinucleotide phosphate
PDB: Protein Data Bank
PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses

SDF: structure data file

Edited by Amy Schwartz; peer-reviewed by Shailee Rasania, Sylvester Sakilay, Sayan Mitra, Randa Salah, Gomaa Mahmoud, Julie Moonga; submitted 23.01.2025; final revised version received 18.05.2025; accepted 23.05.2025; published 10.07.2025

Please cite as:
Olatoye TI
Discovery of Novel Inhibitors of HMG-CoA Reductase Using Bioactive Compounds Isolated From Cochlospermum Species Through Computational Methods: Virtual Screening and Algorithm Validation Study
JMIRx Bio 2025;3:e71675
URL: https://bio.jmirx.org/2025/1/e71675
doi: 10.2196/71675