ORIGINAL ARTICLE

Computational Analysis of Bioactive Compounds From *Diospyros ebenum, Oldenlandia umbellata* and *Momordica charantia* as Promising Inhibitors of HMG-CoA reductase Involved in Hypercholesterolemia

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ABSTRACT

Introduction: Obesity and high cholesterol are significant global health issues linked to various diseases, including atherosclerosis, osteoarthritis, coronary artery disease, and cerebrovascular diseases. These conditions can lead to severe consequences, such as restricted blood flow, which heightens the risk of coronary heart disease and mortality. Cholesterol, a fatty substance found in all body cells, is essential for hormone production, vitamin D synthesis, and digestion. The enzyme HMG-CoA reductase plays a critical role in cholesterol production, and its inhibition is the primary target of statins, the widely used cholesterol-lowering medications. While statins effectively manage cholesterol levels, their prolonged and excessive use may result in adverse health effects. **Methods:** This study aimed to identify natural compounds from plants such as *Momordica charantia* (bitter melon), *Diospyros ebenum* (Ceylon ebony), and *Oldenlandia umbellata* (chay root) that could inhibit HMG-CoA reductase. **Results:** In silico analysis demonstrated that phytochemicals from *M. charantia, D. ebenum,* and *O. umbellata* exhibited non-toxicity and favorable pharmacological properties. Docking studies indicated that compounds like Ebenone, Ursolic acid, Caffeic acid, p-coumaric acid, Kuguacin A, Momordicin, Ascorbic acid, Syringaldehyde, Luteolin, and Zeatin riboside effectively bind to HMG-CoA reductase, suggesting their potential to inhibit its activity and reduce cholesterol accumulation. **Conclusion:** This study provides insights for experimental analysis, indicating that these plants may serve as potential sources of anti-cholesterol agents. This revision enhances clarity, maintains a professional tone, and ensures the information flows logically.
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Keywords: *Diospyros ebenum*, HMG-CoA reductase, *Momordica charantia*, Obesity, *Oldenlandia umbellata*

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INTRODUCTION

Hypercholesterolemia is a metabolic disorder characterized by elevated levels of cholesterol in the blood. Cholesterol plays a vital role in cellular functions and is a structural component of cell membranes. It is involved in the synthesis of bile acids, steroid hormones, and vitamin D, contributing to membrane stability and fluidity. Cholesterol levels in the blood are regulated by a balance between endogenous production and dietary intake, maintaining cholesterol homeostasis. Although all cells and tissues generate cholesterol, it is predominantly found in the digestive system, reproductive system, and liver. However, elevated levels can lead to the accumulation of cholesterol and other deposits on artery walls, a condition known as atherosclerosis. This plaque buildup can reduce blood flow through the arteries, resulting in serious conditions such as heart attacks, strokes, and chest pain (1,2).

Maintaining cholesterol homeostasis is crucial in combating high cholesterol levels. A key aspect of cholesterol regulation is the balance of positive and negative feedback mechanisms in cholesterol synthesis and metabolism. Understanding these mechanisms is essential for developing strategies to manage cholesterol levels effectively. A critical enzyme in endogenous cholesterol synthesis is 3-hydroxy-3-methylglutaryl CoA reductase (HMGR), which plays a rate-limiting and regulatory role by converting HMG CoA to mevalonate, with NADPH as a reducing agent. Inhibiting HMGR activity can reduce cholesterol synthesis and prevent its accumulation. Statins, which are HMGR inhibitors,

are widely prescribed for managing high cholesterol. The six statins currently available include pitavastatin, atorvastatin, rosuvastatin, pravastatin, simvastatin, and fluvastatin (3,4). While statins are generally well tolerated and effective, certain studies have reported potential adverse effects, such as rash, insomnia, headache, abdominal pain, rhabdomyolysis, and acute liver failure. These rare but serious side effects highlight the need for ongoing research into alternative cholesterol management strategies (5,6).

In recent years, computational approaches have gained prominence in drug discovery, especially focusing on natural plant-based compounds. These compounds offer various benefits, including antioxidant, anti-inflammatory, immunomodulatory, and antimicrobial properties (7). In silico predictions and analyses provide an efficient and cost-effective way to identify potential bioactive compounds from natural sources. Investigating the bioactive constituents of medicinal plants, such as *Momordica charantia* (*M. charantia*), *Diospyros ebenum* (*D. ebenum*), and *Oldenlandia umbellata* (*O. umbellata*), may reveal new HMGR inhibitors with potential benefits for managing hypercholesterolemia.

Diospyros ebenum Roxb., a member of the Ebenaceae family, is well-known in traditional Indian medicine for its medicinal properties. Various parts of this plant, including its fruits, bark, leaves, and dried flowers, have been used to treat different ailments. The bark's astringent properties help with gastrointestinal issues like diarrhea and dyspepsia, while the leaves' diuretic, laxative, carminative, and styptic properties make them useful for digestive and wound-healing conditions. Additionally, the dried flowers have been used to treat urinary and skin infections (8). Studies on the ethanolic extract of *D. ebenum* (EDE) have revealed its hepatoprotective and antidiabetic effects, reducing fasting plasma glucose levels and hepatic transaminase enzyme levels. Moreover, it has shown potential in protecting the liver from diabetes-related damage (9). Although previous research has explored the anti-diabetic and anti-obesity properties of various Diospyros extracts, there is limited investigation into their potential anticholesterolemic effects. This study aims to fill this knowledge gap by conducting docking analyses to assess the interactions of these phytocompounds with HMGR, a key enzyme in cholesterol biosynthesis.

Oldenlandia umbellata L. (synonym: Hedyotis umbellata (L.) Lam.), belonging to the Rubiaceae family and distributed across Asia, including India, holds significant medicinal importance (10). In the Indian Siddha treatment system, *O. umbellata* has been extensively used to manage diverse health conditions like bronchitis, asthma, tuberculosis, and hemoptysis. Additionally, the plant's leaves are commonly used as a decoction to treat venomous bites. Scientific studies have demonstrated the pharmacological effects of *O. umbellata*, including antibacterial, anti-inflammatory, antipyretic, hepatoprotective, antioxidant, and antitussive activities (11). Despite its extensive historical use, comprehensive research on its impact on obesity and cholesterol management is limited, with studies primarily focusing on its potential to prevent cholesterol synthesis.

Momordica charantia L., commonly known as bitter gourd, belongs to the Cucurbitaceae family and is cultivated as a vegetable and medicinal crop in India, China, and Southeast Asia. It is highly regarded for its nutritional and medicinal properties (12,13). Traditional medicine practices worldwide have utilized *M. charantia* for treating conditions such as diabetes, cancer, and inflammation-related diseases due to its bioactive compounds (14). The plant has a long history of traditional use in managing diabetes (15), with mechanisms including the healing of injured β-cells, increased insulin levels and sensitivity, inhibition of glucose absorption by blocking glucosidase, and reduction in disaccharide activity (16). *M. charantia* is also effective in addressing hyperlipidemia. Studies in rats fed a high-fat diet have shown that bitter gourd juice and atorvastatin significantly reduce blood levels of total cholesterol, low-density lipoprotein cholesterol, and triglycerides. Furthermore, bitter melon extract has beneficial effects on obesity-related indicators such as body weight reduction and decreased fat accumulation (17). Additionally, *M. charantia* exhibits the downregulation of HMGR expression, which is elevated in cholesterol synthesis (18). Experimental findings suggest that *M. charantia* compounds inhibit HMGR. To explore these interactions further and discover potential novel lead compounds for drug development, an in silico study was conducted to identify promising candidates with therapeutic potential.

The primary objective of this research was to gain insights into the potential of bioactive compounds derived from *D. ebenum*, *O. umbellata*, and *M. charantia* in binding to HMGR, a critical enzyme involved in cholesterol biosynthesis. This investigation sought to identify promising compounds that could have implications for drug development targeting cholesterol regulation.

MATERIALS AND METHODS

Target and Ligand Identification

HMGR plays a crucial role as the rate-limiting enzyme in the biosynthesis of cholesterol, making it a significant therapeutic target confirmed by the World Health Organization (WHO). In this study, we utilized the Protein Data Bank (PDB), an open-access online structure database, to obtain the sequence and structural information of the protein (19). Subsequently, through an extensive investigation of literature studies and the natural phytocompounds database, we identified a diverse list of bioactive and phytochemical components

present in three medicinal plants, namely M.charantia, *D.ebenum*, and *O.umbellata*. The fundamental details, including monoisotopic mass, molecular formula, and other pertinent information related to these small molecules, were acquired from PubChem (20).

Evaluation of pharmacokinetics

The prediction of Absorption, Distribution, Metabolism, and Excretion (ADME) properties, along with pharmacokinetic and drug-likeness qualities, plays a pivotal role in drug development. In this study, we employed SWISSADME, a valuable computational tool, to assess the physicochemical characteristics associated with ADME and other critical biochemical properties of phytocompounds. Input for SWISSADME requires the ligands to be provided in Simplified Molecular Input Line Entry System (SMILES) format which helps to facilitate the evaluation of their potential as drug candidates (21).

Evaluating the toxicity of the compounds

The assessment of toxicity is a critical aspect of drug development to ensure that lead molecules are safe for human use. OSIRIS is a widely used computational tool that enables the prediction and evaluation of various toxicological properties, including mutagenicity, tumorigenicity, and reproductive effects (22). The color-coded results provided by OSIRIS help to quickly identify potential toxic risks associated with phytomolecules, while drug likeness and drug score values offer additional insights into their suitability for further development.

Binding site analysis in target

The binding sites of a target protein is essential for studying ligand-protein interactions and their potential effects. This study aimed to investigate the binding sites of the target protein by analyzing structural data available in the Protein Data Bank (PDB) database. The obtained information on binding sites was used to gain insights into the potential binding regions for ligands with the target. Additionally, a literature review was conducted to determine the binding site of the cholesterol-lowering drug statin, which helped identify the specific amino acid residues involved in the active binding site that promotes the inhibition of HMGR

Docking studies

Molecular docking is an important computational technique used in drug discovery to predict ligand-protein interactions. In this study, the LibDock tool in Discovery Studio was employed for molecular docking and visualization studies. 2D docking interactions were generated to identify the receptor residues that interact with the bound ligand. Upon docking the bioactive substances with HMGR, it was observed that most compounds shared common binding interactions with the amino acid residues critical for statin binding.

RESULTS

Target selection

In this study, HMG CoA Reductase (HMGR), a key enzyme in cholesterol biosynthesis, was investigated for its structural insights and interaction with mevastatin. Basic information about HMGR was sourced from UniProt, facilitating a comprehensive understanding of its functional characteristics. The structural data for the HMGR-mevastatin complex, represented in Figure 1 (PDB ID: 1HW8), was obtained from the Protein Data Bank (PDB). This structural information served as a crucial foundation for subsequent analyses, enabling a detailed exploration of the binding sites and interactions between HMGR and mevastatin in the context of cholesterol metabolism.

Figure 1: The complex of compactin (mevastatin) and the catalytic portion of human HMG-CoA reductase

Binding Sites Analysis

The efficiency of the ligands in producing an inhibitory effect depends on the site of interaction with HMG CoA reductase, thus a detailed study was carried out to identify the active site of HMGR and the nature of interactions between statins and that of HMGR. The amino acid residues of in the active site of HMGR contains Glu 559, Val 683, Met 657, Lys 691, Ala 751, Arg 590, Asp 690, Leu 857, Lys 735, Ser 684, His 752, Lys 692, Asn 686, His 861, Cys 561, Ser 565, Ser 661, Leu 562, Leu 853, Asn 755, Lys 691, Met 657, Lys 691 (23).

Ligand identification

In this study, 222 bioactive compounds from *M. charantia*, 14 compounds from *D. ebenum*, and 16 compounds from *O. umbellata* have been obtained

from IMPPAT database and extensive literature review. Bioactive compounds discovered in *M. charantia*, *D.ebenum* and *O.umbellata* have been identified as having significant potential bioactivities which included proteins, lipids, carbohydrates, alkaloids, sterols, terpenoids, polypeptides, flavonoids, and saponins etc. Previous studies on phytochemicals have demonstrated the bioactive substances and their associated action (10,24,25). They serve as the main cue for the development of drugs. To determine the most promising candidates, the identified bioactive components underwent additional evaluation for toxicity and drug-likeness.

Pharmacokinetic analysis

Drug development encompasses multiple stages of screening and optimization to identify promising lead molecules with favorable pharmacokinetic properties. A critical aspect of this process involves evaluating the absorption, distribution, metabolism, and excretion, of potential compounds. To aid in this analysis, SwissADME, a widely utilized web tool, provides a comprehensive approach for quantitatively assessing the bioavailability and suitability for oral administration of candidate drugs based on physicochemical, pharmacokinetic, and drug-like parameters. Based on the assessment of various parameters, including molecular weight, drug-likeness, H-bond acceptors, H-bond donors, TPSA value, log-p value, Lipinski's violation, Veber rule, and intestinal absorption, a total of 79 compounds were found to meet the pharmacokinetic criteria. Out of these, 69 compounds were derived from *M. charantia*, 3 from *D. ebenum*, and 7 from *O. umbellata*. These compounds demonstrated adherence to drug likeness guidelines, suggesting their suitability for oral medication (Table I).

Analysis of toxicity

The OSIRIS tool is a valuable resource in drug discovery that plays an important role in predicting and evaluating the drug-likeness of potential compounds and drug-related properties, including mutagenicity, reproductive effects, and tumorigenicity of potential drug candidates. OSIRIS contributes significantly to the safety assessment of drug candidates, facilitating the development of safe and effective therapeutics that may help in the discovery of innovative medicines.

After assessing the toxicity profiles, 27 compounds from *M. charantia*, 2 compounds from *D. ebenum*, and 1 compound from *O. umbellata* that is also present in *D. ebenum* demonstrated no adverse effects on mutagenicity, reproductive effects, and tumorigenicity, making them potential candidates for developing novel therapies to reduce cholesterol accumulation.

Analysis of ligand interaction with target

Molecular docking was performed using the Libdock software of Discovery Studio, and the number of interactions and hydrogen bonds between the ligand and the protein were assessed to evaluate the docking data. Additionally, dock scores were utilized to gauge the effectiveness of the docking process. A comparison between drug-protein interactions and ligand-protein interactions was carried out to determine the efficient binding of bioactive compounds.

In the molecular docking study targeting HMGR, all 29 molecules from the three sources exhibited highly efficient binding at the target location except for Biochanin a and Riboflavin which showed unfavorable bonds (Table II).

The compounds Ebenone from *D.ebenum* and the compound Ursolic acid from both *O.umbellata* and *D.ebenum* exhibit favourable binding interactions with the target, specifically the same amino acid where statins are typically bound. Ebenone is a novel compound, and there is limited research available on its properties and effects. Further studies are required to explore the full potential and efficacy of these compounds.

Particularly, these molecules formed stable interactions with HMGR through hydrogen bonds, which is a key characteristic observed in the binding of statins. Hydrogen bond interactions further reinforces the stability and specificity of the ligand-protein complex. Moreover, the molecular docking simulations yielded good LibDock scores for all the tested molecules. The favorable LibDock scores indicate strong binding affinities and stable conformations at the active site of HMGR, further supporting their potential as HMGR inhibitors (26).

The collective results of this study suggest *D.ebenum* and *O.umbellata* as novel sources for potential inhibitors for HMGR. We further propose that the process of gaining a full understanding needs experimental studies with these compounds to create methods for personalized therapy and natural remedies for the treatment of hypercholesterolemia and prevention of obesity and other interconnected disorders.

DISCUSSION

Hypercholesterolemia, a condition characterized by elevated lipid, cholesterol, and triglyceride levels, is a major health concern associated with various lifestyle factors, including unhealthy dietary habits, chronic stress, and obesity (27). This study delves into the potential therapeutic implications of specific compounds in addressing hypercholesterolemia, focusing on their interactions with HMGR, a key enzyme in cholesterol synthesis. HMGR catalyzes the conversion of HMG-CoA to mevalonate, a key step in cholesterol synthesis crucial for producing sterols and isoprenoids. Statins, widely prescribed cholesterol-lowering drugs, inhibit HMGR by binding to its active site, blocking HMG-CoA access, and reducing mevalonate formation. This

Table I: Pharmacokinetics and Toxicity profile of the compounds *Momodica charantia*

Molecule	Molecular weight	Rotatable bonds	H-bond acceptors	H-bond donors	Consensus Log P	Bioavailability Score	Drug-like-ness	drug score
caffeic acid	180.16	2	4	3	0.93	0.56	1.62	0.89
p-coumaric acid	164.16	2	3	2	1.26	0.85	0.58	0.79
ferulic acid	194.18	3	4	2	1.36	0.85	1.12	0.84
t-cinnamic acid	148.16	2	2	1	1.79	0.85	-1.1	0.6
biochanin a	284.26	2	5	2	2.44	0.55	1.02	0.77
kuguacins A	470.68	5	4	2	4.88	0.55	-4.69	0.2
momordicine	472.7	5	4	3	4.9	0.55	-4.26	0.2
4-Methoxybenzoic acid	152.15	2	1	1	1.49	0.85	-1.99	0.54
(+)-cis-Sabinol	152.23	1	1	1	2.11	0.55	-4.55	0.47
l-Menthol	156.27	1	1	1	2.59	0.55	-10.47	0.46
(3S,4S)-4-ethenyl-4-methyl-3-prop-1-en-2-ylcyclohexene	162.27	2	1	0	3.66	0.55	-21.34	0.41
4-Hydroxycinnamic acid	164.16	2	1	2	1.26	0.85	0.58	0.79
3-(4-Hydroxyphenyl)propionic acid	166.17	3	1	2	1.31	0.85	-1.56	0.57
Ascorbic acid	176.12	2	1	4	-1.42	0.56	0.02	0.74
Syringaldehyde	182.17	3	1	1	0.93	0.55	-1.57	0.56
D-Galacturonic Acid	194.14	1	1	5	-2.12	0.56	-1.29	0.6
(+)-delta-Cadinene	204.35	1	1	0	4.14	0.55	-6.35	0.35
beta-Caryophyllene	204.35	0	1	0	4.24	0.55	-6.48	0.31
Humulene	204.35	0	1	0	4.26	0.55	-4.72	0.28
(S,1Z,6Z)-8-Isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene	204.35	1	1	0	4.3	0.55	-10.2	0.28
(1S,6R,7R)-1-methyl-3-methylidene-8-propan-2-yltricyclo[4.4.0.02,7]decane	204.35	1	1	0	4.4	0.55	-9.61	0.38
Epi-Cubebol	220.35	1	1	1	3.22	0.55	1.39	0.77
Epi-Cubenol	222.37	1	1	1	3.5	0.55	-4.36	0.42
Luteolin	286.24	1	1	4	1.73	0.55	1.9	0.84
Retinol	286.45	5	1	1	1.31	0.85	-3.49	0.28
Zeatin riboside	351.36	6	2	5	-0.56	0.55	-3.71	0.46
Riboflavin	376.36	5	2	5	-0.19	0.55	6.24	0.87
Diospyros ebenum								
Ebenone	360.36	1	5	3	3.67	0.55	-2.36	0.27
Ursolic acid	456.7	1	3	2	5.88	0.85	-3.66	0.17
Oldenlandia umbellata								
Ursolic acid	456.7	1	3	2	5.88	0.85	-3.66	0.17

decreases liver cholesterol synthesis, lowering LDL and triglyceride levels while raising HDL levels. Given statins' side effects, interest is growing in natural HMGR inhibitors. Exploring natural compounds as HMGR inhibitors is promising due to their potential for fewer side effects compared to statins. Natural inhibitors can bind to HMGR's active site, similarly preventing HMG-CoA from accessing the enzyme and thereby reducing mevalonate and cholesterol synthesis. Understanding these mechanisms can aid in developing safer alternatives for cholesterol management and offer insights into the diverse bioactivities of natural products (28).

Various plant species are being studied for their phytochemical properties due to their potential health benefits. Notable examples include Catharanthus roseus (vinblastine) and Quillaja saponaria (QS-21) for cancer treatment, Coptis teeta (anti-AChE alkaloids) for neuroprotection, and Cyamopsis tetragonoloba (antioxidants) for oxidative stress. Additionally, Amomum subulatum (antimicrobial carvacrol) and Cucurbita pepo (cucurbitacins) offer antimicrobial and therapeutic applications. Other plants under investigation include carrots (polyacetylenes), wheat and legumes (lectins), grapes and berries (polyphenols), cabbage and

Table II: Molecular docking of HMGR with *Momordica charantia*, *Diospyros ebenum*, and *Oldenlandia umbellata* compounds

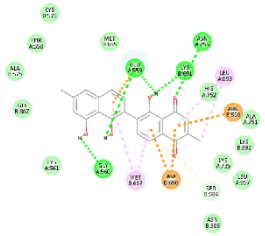
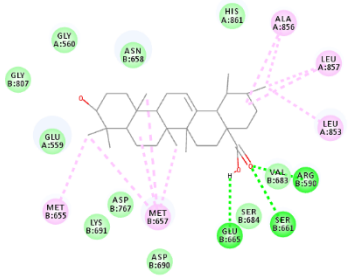
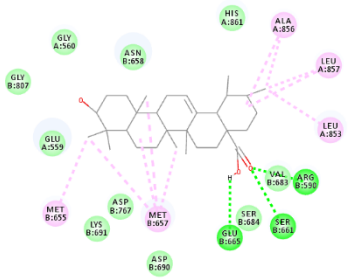
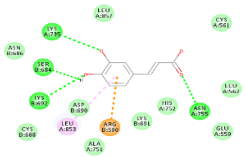
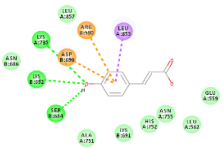
Diospyros ebenum							
Molecule	Absolute energy	Relative energy	Libdock score	H-bond interaction	Other bond interaction	Carbon hydrogen bond	2d interaction
Ebenone	64.4061	0.0479435	80.0502	5 OH-GLY 560, OH-GLU 559, OH-LYS 691, O-ASN 755, OH-GLU 559	8 GLU 559, MET 657, ASP 690, ARG 590, LEU 853	1 SER 684	
Ursolic acid	66.2887	0.541166	78.9017	3 OH-GLU 665, O-ARG 590, O-SER 661	10 MET 655, MET 657, LEU 853, LEU 857, ALA 856		
Oldenlandia umbellata							
Ursolic acid	66.2887	0.541166	78.9017	3 OH-GLU 665, O-ARG 590, O-SER 661	10 MET 655, MET 657, LEU 853, LEU 857, ALA 856		
Momordica charantia							
Caffeic acid	24.0182	0	74.642	4 O-LYS 735, OH-SER 684, O-LYS 692, O-ASN 755	2 ARG 590, LEU 853		
p-coumaric acid	23.3505	0.247215	70.7424	3 O-LYS 735, O-LYS 692, OH-SER 684	3 ARG 590, ASP 690, LEU 853		

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Table II: Molecular docking of HMGR with *Momordica charantia*, *Diospyros ebenum*, and *Oldenlandia umbellata* compounds

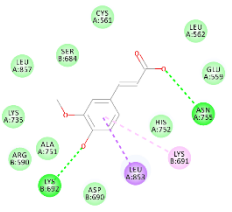
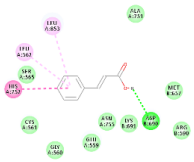
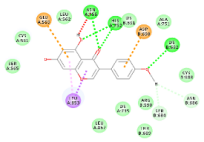
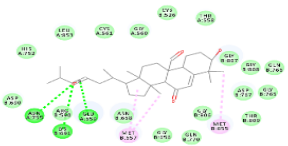
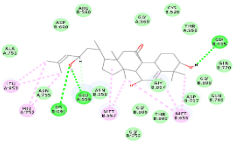
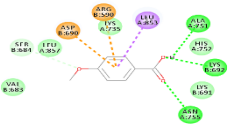
Momordica charantia							
Molecule	Absolute energy	Relative energy	Libdock score	H-bond interaction	Other bond interaction	Carbon hydrogen bond	2d interaction
Ferulic acid	33.1759	0	76.4685	2 O-LYS 692, O-ASN 755	2 LYS 691, LEU 853		
t-cinnamic acid	22.6002	0.178034	57.986	1 OH-690	3 LEU 853, LEU 562, HIS 752		
Biochanin a	43.8758	0	87.7612	4 O-HIS 752, O-ASN 755, O-HIS 752, O-LYS 692	4 GLU 559, LEU 853, LYS 690,	2 H-SAN 686, H-SER 684	
Kuguacin A	61.3937	3.93461	115.55	3 OH-GLU 559, O-LYS 691, O-ASN 755	3 MET 657, MET 655		
Momordicin	67.1493	5.87252	107.49	3 O-LYS 691, OH-GLU 559, OH-GLY 765	9 LEU 853, HIS 752, MET 657, MET 655	1 GLU 559	
4-Methoxybenzoic acid	21.2562	0.038975	62.0998	3 O-LYS 692, OH-ALA 751, O-ASN 755	3 LEU 853, ARG 590, ASP 690	1 O-SER 684	

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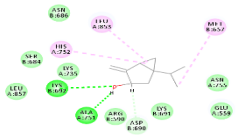
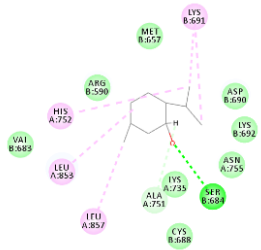
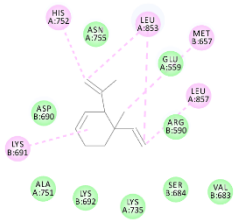
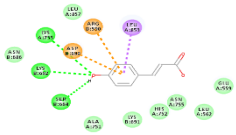
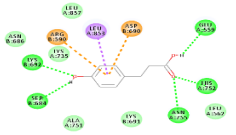
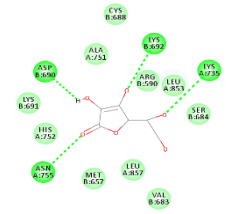
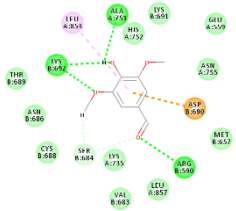
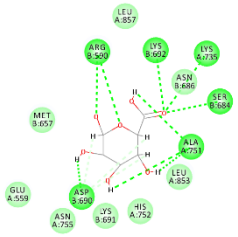
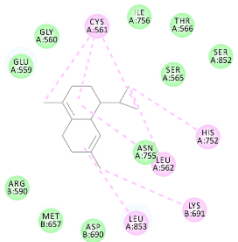
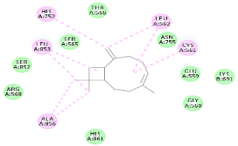
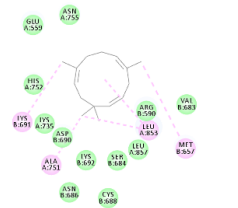
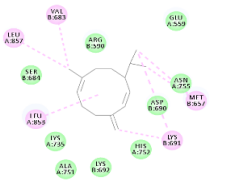
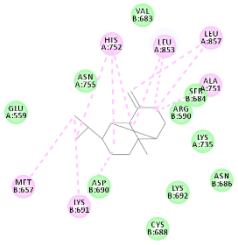
Table II: Molecular docking of HMGR with *Momordica charantia*, *Diospyros ebenum*, and *Oldenlandia umbellata* compounds

Momordica charantia							
Molecule	Absolute energy	Relative energy	Libdock score	H-bond interaction	Other bond interaction	Carbon hydrogen bond	2d interaction
(+)-cis-Sabinol	93.9909	8.49632	58.254	2 O-LYS 692, OH-ALA 751	3 LEU 853, HIS 752, MET 657	1 H-ASP 690	
l-Menthol	12.4711	5.16852	58.0773	1 O-SER 684	5 LYS 691, HIS 752, LEU 853, LEU 857	1 H-ALA 751	
(3S,4S)-4-ethenyl-4-methyl-3-prop-1-en-2-ylcyclohexene	27.8582	5.68011	57.7687	6 LYS 691, LEU 857, MET 657, LEU 853, HIS 752			
4-Hydroxy-cinnamic acid	23.3505	0.247215	70.7424	3 OH-SER 684, O-LYS 692, O-LYS 735	3 LYS 690, ARG 590, LEU 853		
3-(4-Hydroxy-phenyl) propionic acid	16.4541	2.22105	71.8841	5 O-LYS 692, OH-SER 684, O-HIS 752, O-ASN 755, OH-GLU 559	3 ARG 590, LEU 853, ASP 690		
Ascorbic acid	19.7937	3.11279	69.7326	4 OH-ASP 690, O-ASN 755, O-LYS 692, O-LYS 735			
Syringaldehyde	32.7212	2.4461	66.1476	3 O-ARG 590, O-LYS 692, OH-ALA 751, O-LYS 692	2 LEU 853, ASP 690	2 H-SER 684, O-SER 684	

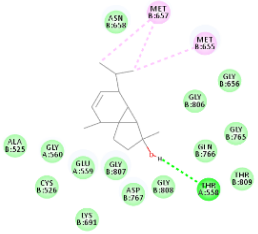
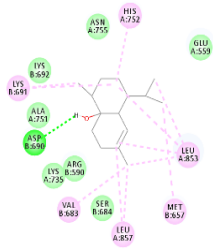
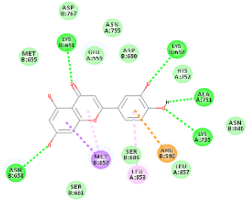
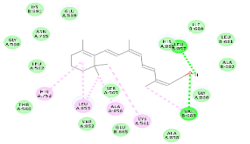
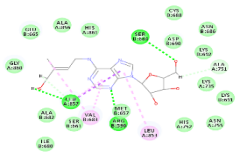
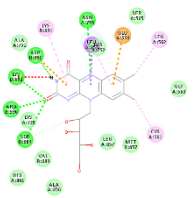
CONTINUE

Table II: Molecular docking of HMGR with *Momordica charantia*, *Diospyros ebenum*, and *Oldenlandia umbellata* compounds

Momordica charantia							
Molecule	Absolute energy	Relative energy	Libdock score	H-bond interaction	Other bond interaction	Carbon hydrogen bond	2d interaction
D-Galacturonic Acid	19.7073	6.14671	73.1905	9 OH-ASP 690, H-ALA 751, OH-ALA 751, H-ALA 751, O-SER 684, O-LYS 735, O-LYS 692, O-ARG 590, O-ARG 590		3 H-ASP 690, H-ASP 690, H-ASP 690	
(+)-delta-Cadinene	25.8705	8.52445	63.7888		8 CYS 561, HIS 752, LEU 562, LYS 691, LEU 853		
Beta-Caryophyllene	15.1638	0	60.7815		8 HIS 752, LEU 853, ALA 856, LEU 562, CYS 561		
Humulene	18.4285	0	63.0847		5 LYS 691, ALA 751, LEU 853, MET 657		
(S,1Z,6Z)-8-Isopropyl-1-methyl-5-methylenecyclodeca-1,6-diene	23.0216	0.501382	65.9582		7 VAL 683, LEU 857, LEU 853, LYS 691, MET 657		
(1S,6R,7R)-1-methyl-3-methylidene-8-propan-2-yltricyclo[4.4.0.02,7]decane	31.4013	13.3186	66.7777		11 MET 657, LYS 691, HIS 752, LEU 853, LEU 857, ALA 751		

CONTINUE

Table II: Molecular docking of HMGR with *Momordica charantia*, *Diospyros ebenum*, and *Oldenlandia umbellata* compounds

Momordica charantia							
Molecule	Absolute energy	Relative energy	Libdock score	H-bond interaction	Other bond interaction	Carbon hydrogen bond	2d interaction
Epi-Cubebol	16.0897	3.42817	69.0911	1 OH-THR 558	3 MET 657, MET 655		
Epi-Cubenol	13.5938	0	68.4086	1 OH-ASP 690	11 HIS 752, LYS 691, VAL 683, LEU 857, MET 657, LEU 853		
Luteolin	34.4952	0.0431269	89.9471	5 O-LYS 691, O-ASN 658, O-LYS 692, OH-ALA 751, O-LYS 735	4 MET 657, LEU 853, ARG 590		
Retinol	52.1557	10.0089	87.6134	2 O-VAL 683, OH-LEU 857	6 HIS 752, LEU 853, ALA 856, CYS 561, VAL 683		
Zeatin riboside	67.4758	3.29735	104.623	3 OH-LEU 857, O-SER 684, N-ARG 590	5 LEU 857, VAL 683, LEU 853	2 H-ALA 751, H-LEU 857	
Riboflavin	49.6873	5.85909	115.437	5 N-ASN 755, NH-ASP 690, O-LYS 692, O-ARG 590, O-SSER 684	7 LYS 691, ASP 690, LEU 853, GLU 559, LEU 562, CYS 561, LEU 853	1 O-SER 684	

broccoli (glucosinolates), and various herbs (terpenes), contributing to cancer treatment, immune support, and overall health. (29, 30).

In the current study, *Diospyros ebenum* (*D. ebenum*), *Oldenlandia umbellata* (*O. umbellata*), and *Momordica charantia* (*M. charantia*) were chosen due to their established medicinal use and the presence of bioactive compounds with potential cholesterol-lowering effects. *D. ebenum* has been traditionally used for its hepatoprotective and anti-inflammatory properties, which suggest its possible role in cholesterol management. *O. umbellata*, known for its antioxidant and anti-inflammatory benefits, has been historically used to treat various ailments, indicating it may contain compounds that can inhibit cholesterol biosynthesis. *M. charantia*, widely recognized for its efficacy in managing metabolic disorders, has shown promise in reducing cholesterol levels and inhibiting HMGR, the enzyme responsible for cholesterol synthesis. Therefore, in silico studies were performed to assess the bioactive compounds from these plants as potential ligands for inhibiting HMGR, focusing on their effectiveness through binding affinity, formation of hydrogen bonds, and substantial dock scores, indicative of their potential as effective HMGR inhibitors. Further exploration of these compounds through a comprehensive literature review revealed their diverse bioactivities.

Based on the results of this study, several important conclusions can be drawn regarding the potential of bioactive compounds from *Diospyros ebenum*, *Oldenlandia umbellata*, and *Momordica charantia* as HMGR inhibitors. The analysis identified 79 compounds meeting pharmacokinetic criteria, demonstrating their suitability for oral administration and drug development. Especially, 27 compounds from *M. charantia*, 2 from *D. ebenum*, and 1 from *O. umbellata* showed no adverse effects in toxicity assessments, making them promising candidates for further investigation. Among the compounds, Ebenone from *D. ebenum* and Ursolic acid from both *O. umbellata* and *D. ebenum* emerged as particularly noteworthy. These compounds exhibited favorable binding interactions with HMGR, specifically targeting the same amino acid residues as statins, which are known for their cholesterol-lowering effects. Their ability to form stable hydrogen bonds with HMGR underscores their potential as effective inhibitors.

The strong docking scores and favorable binding interactions suggest that these compounds could serve as lead candidates for developing novel therapies aimed at managing hypercholesterolemia. Ebenone, despite being a novel compound with limited existing research, showed significant promise due to its effective binding profile. Ursolic acid on the other hand, known for its broad range of bioactivities, further supports its inclusion as a key compound in this study. One significant finding revolves around Ursolic acid demonstrated multifaceted benefits, including increased irisin production, conversion of white adipose tissue into beige fat, and a weight reduction in obese mice. Additionally, it exhibited promise in preventing insulin resistance, hyperinsulinemia, and effectively lowered lipid levels, offering potential avenues for managing obesity and metabolic disorders (31).

The study also reveals several compounds from *M. charantia* with antiobesity properties. While some compounds have been extensively investigated in vivo studies, others like Kuguacin A, Momordicin, Syringaldehyde, Luteolin, and Zeatin riboside remained unexplored. For instance, Kuguacin A exhibited potential in managing diabetes by improving insulin sensitivity and regulating blood glucose levels (32). Momordicin demonstrated hypoglycemic effects (33), and Syringaldehyde, a natural compound with antioxidant and anti-inflammatory properties, presented antimicrobial, anti-bacterial and anti-tumorigenic effects, suggesting therapeutic promise (34,35). Luteolin, a flavonoid, showcased various beneficial properties, including antioxidant, anti-tumor, anti-inflammatory, and anti-diabetic effects (36). Zeatin riboside demonstrated therapeutic potential as a mammalian immunomodulatory agent with anti-inflammatory activity (37). Caffeic acid, known for its antioxidant, immunomodulatory, antimicrobial, neuroprotective, and anti-inflammatory activities, also indicated potential in reducing triglycerides and total cholesterol levels (38). p-Coumaric acid exhibited promising effects in reducing intracellular lipid accumulation and total triglyceride content and it's also known for its anti-inflammatory and antioxidant properties (39,40). Ascorbic acid demonstrated the ability to reduce lipid accumulation in hepatocytes, indicating its potential as a safe and effective dietary supplement for addressing hypertriglyceridemia (41).

The results of this study suggest that the compounds Ebenone, Ursolic acid, Caffeic acid, p-coumaric acid, Kuguacin A, Momordicin, Ascorbic acid, Syringaldehyde, Luteolin, and Zeatin riboside possess potential as HMGR inhibitors for cholesterol management. These compounds could bind to the same active site on HMGR, preventing the enzyme from interacting with its natural substrate, HMG-CoA. By forming multiple hydrogen bonds and strong interactions with key amino acid residues in the active site, these compounds could stabilize the enzyme in an inactive form, thereby effectively reducing the conversion of HMG-CoA to mevalonate. This inhibition would lead to lower levels of cholesterol being produced in the liver. These findings provide valuable insights for the future development and optimization of novel natural-based HMGR inhibitors for the treatment of hypercholesterolemia and related cardiovascular disorders.

In conclusion, this study not only highlights the

potential therapeutic effects of Ursolic acid and various compounds from *M. charantia* on hypercholesterolemia but also emphasizes the need for further experimental investigations to validate their efficacy and safety. The diverse bioactivities of these compounds open avenues for developing personalized therapies and natural remedies for hypercholesterolemia and related metabolic disorders.

CONCLUSION

This study highlights the promising potential of bioactive compounds from *Diospyros ebenum*, *Oldenlandia umbellata*, and *Momordica charantia* as inhibitors of HMGR, a critical enzyme in cholesterol biosynthesis. By employing in silico techniques, we identified 27 from *M. charantia*, 2 from *D. ebenum*, and 1 from *O. umbellata* demonstrating potential pharmacokinetic properties. The molecular docking studies revealed that Ebenone, Ursolic acid, Caffeic acid, p-coumaric acid, Kuguacin A, Momordicin, Ascorbic acid, Syringaldehyde, Luteolin, and Zeatin riboside. Natural HMGR inhibitors may offer advantages such as reduced side effects compared to synthetic statins, presenting a promising alternative or complement to current cholesterol-lowering therapies. Further investigation into these compounds could lead to the development of new dietary supplements or pharmaceutical products, enhancing cholesterol management and potentially lowering the risk of heart attacks, strokes, and other cardiovascular conditions.

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