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IN SILICO EVALUATION OF ANTI-HYPERCHOLESTEROLEMIC POTENTIAL OF *COMMIPHORA WIGHTII* PHYTOCONSTITUENTS AGAINST PCSK9 USING MOLECULAR DOCKING

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ABSTRACT

Hypercholesterolemia is a major risk factor for cardiovascular diseases and Pro Protein Convertase Subtilisin/Kexin Type 9(PCSK9) has emerged as an important therapeutic target due to its role in promoting low-density lipoprotein receptor (LDLR) degradation, leading to elevated plasma LDL cholesterol levels. The present study aimed to investigate the anti-hypercholesterolemic potential of selected phytoconstituents from *Commiphora wightii* through an in silico molecular docking approach targeting PCSK9. Nine bioactive compounds were subjected to high-throughput virtual screening using AutoDock Vina with optimized and validated grid parameters, followed by refined molecular docking of the top-performing compounds. Protein-ligand interaction were further analysed using BIOVIA Discovery Studio Visualizer to identify key binding residues within the active site. High-throughput virtual screening identified Z-Guggulsterone (-8.64kcal/mol), E-Guggulsterone (-8.45 kcal/mol) and Myrrhanol A (-8.12 kcal/mol) as the most promising phytoconstituents. Refined docking further improved their binding affinities to -8.82, -8.61 and -8.25 kcal/mol, respectively. These compounds established a stable interaction with critical PCSK9 residues, particularly Asp374 and Arg194, suggesting their potential to interfere with the PCSK9-LDLR interaction. Overall, the findings demonstrate that *Commiphora wightii* contains promising natural compounds with significant affinity towards PCSK9 and Highlight Z-Guggulsterone, E-Guggulsterone and Myrrhanol A as potential lead molecules for the development of novel anti-hypercholesterolemic agent. Further experimental and clinical investigations are warranted to validate their therapeutic potential.

KEYWORDS: *Commiphora wightii*; PCSK9; Hypercholesterolemia; Molecular Docking; Virtual Screening; Guggulsterone; Natural Products.

INTRODUCTION

Hypercholesterolemia is one of the leading modifiable risk factors responsible for the development of cardiovascular diseases (CVDs), including coronary artery disease, myocardial infarction and ischemic stroke. Elevated low-density lipoprotein cholesterol (LDL-C) promotes cholesterol deposition within the arterial wall, initiating endothelial dysfunction, oxidative stress, chronic inflammation and the formation of atherosclerotic plaques. As plaque progression continues, vascular narrowing and plaque rupture may occur, significantly increasing the risk of life-threatening cardiovascular events. Despite the availability of statins and other lipid-lowering therapies, many patients fail to achieve optimal LDL-C control because of inadequate response, drug intolerance or residual cardiovascular risk.

Therefore, the identification of alternative molecular targets and safer therapeutic agents remain an important priority in cardiovascular drug discovery.[1,2,3,4,5,6]

Among the emerging therapeutic targets, Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) has gained considerable attention because of its essential role in regulating cholesterol homeostasis. PCSK9 binds to low-density lipoprotein receptors (LDLR) on hepatocytes and directs them towards lysosomal degradation rather than receptor recycling. Consequently, fewer LDL receptors remain available to remove circulating LDL-C, resulting in elevated plasma cholesterol levels and increased cardiovascular risk. Clinical studies have demonstrated that inhibition of PCSK9 significantly enhances LDL receptor recycling, improves hepatic LDL clearance and markedly reduces circulating LDL-C concentration. The clinical success of monoclonal antibodies targeting PCSK9 has validated this protein as one ofthe most promising therapeutic targets for hypercholesterolemia; however, their high cost, injectable route of administration and limited accessibility encourage the search for affordable small-molecules inhibitors, particularly those derived from natural products.[7,8,9,10,11,12,13,14,15]

Medicinal plants continue to represent an invaluable source of a structurally diverse bioactive molecules for modern drug discovery. Among these, *Commiphora wightii* (Guggul) has been extensively used in Ayurvedic medicine for the management of lipid disorders and cardiovascular diseases. Its phytoconstituents including Z-Guggulsterone, E-Guggulsterone, Myrrhanol A, Myrrhanol B, guggulsterols and related terpenoids, possess reported hypolipidemic, anti-inflammatory and antioxidative activities. Recent advances in computer-aided drug design (CADD) have enabled rapid evaluation of such natural compounds through molecular docking, virtual screening and pharmacokinetic prediction before experimental validation. These computational approaches reduce both time and cost while improving the efficiency of lead identification. In the present study, an integrated in silico workflow comprising virtual screening, refined molecular docking, ADMET prediction and toxicity assessment was employed to investigate selected phytoconstituents of *Commiphora wightii* as potential PCSK9 inhibitors. The study further identifies existing knowledge gaps regarding plant-derived small molecules PCSK9 inhibitors and provides molecular evidence supporting the therapeutic potential of Guggul Phytoconstituents for the development of novel anti-hypercholesterolemic agents.[16,17,18,19,20,21,22,23,24,25,26,27,28,29,30,31]

MATERIALS AND METHODS

Protein retrieval and preparation

The 3-dimensional structure of the target protein, Proprotein Convertase Subtilisin/Kexin Type9(PCSK9) was retrieved from the Protein Data Bank (PDB) a globally recognized repository containing experimentally determined

biomolecular structures. Selection of an appropriate protein structure is a critical step in structure-based drug discovery because the quality and accuracy of the receptor directly influence docking performance and binding predictions.[32,33,34]

The retrieved-protein structure was carefully examined for structural completeness, crystallographic quality and suitability for molecular docking studies. Protein preparation was subsequently performed to generate biologically relevant receptor model. During preparation, crystallographic water molecules, co-crystallized ligands and unnecessary heteroatoms were removed to avoid interference during ligand binding analysis.[33,35,36]

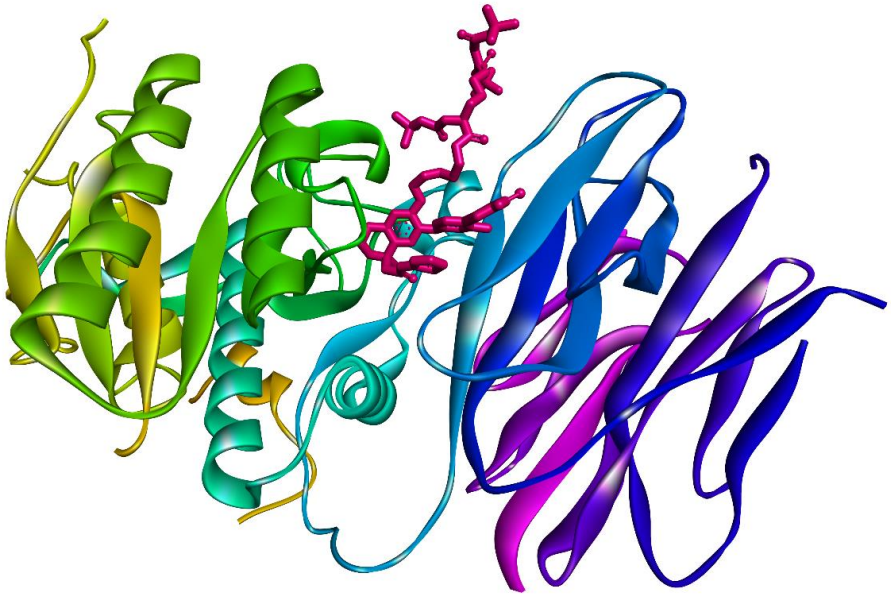

Figure 1: Three-dimensional structure of human PCSK9 (PDB ID 6U26) retrieved from the Protein Data Bank. The protein is represented in cartoon format, while co-crystallized ligand is shown in ball-and-stick representation.

Table 1: Structural information of the target protein (PCSK9).

S. No.	PARAMETER	DETAILS
1	Protein Name	Proprotein Convertase Subtilisin/Kexin Type9(PCSK9).[7,11,12,13,37,38,39,40,41,42]
2	PDB ID	6U26
3	Source Database	Protein Data Bank (PDB)[43]
4	Organism	Homo sapiens
5	Structure Determination Method	X-ray Crystallography
6	Resolution	1.53 Å
7	Selected Chain	Chain B
8	Target Class	Cholesterol Regulatory Protein
9	Biological Role	Regulation of LDL Receptor (LDLR) Degradation
10	File Format Downloaded	PDB
11	Application in Present Study	Molecular Docking and Virtual Screening of Phytoconstituents

Missing hydrogen atoms were added, atomic charges were assigned and structural inconsistencies were corrected wherever required. Protein optimization was carried out to improve receptors stability and ensure compatibility with docking software. The prepared protein structure was then converted into the appropriate docking format and used as the receptor for all subsequent virtual screening and molecular docking studies.[35,44,45,46,47,48]

Proper protein preparation is essential for obtaining reliable docking results because inaccuracy in receptor structure may significantly affect predicted ligand-protein interactions and binding affinities. Therefore, careful receptor preparation was performed before docking analysis to ensure accurate prediction of phytochemical interactions with PCSK9.[16,33,49,50,51]

Table 2: Software, databases and computational Tools used in the present study.

S. No.	Software/Database	Version/Year	Purpose
01	Protein Data Bank(PDB).[43,52,53,54]	2025	Retrieval of PCSK9 structure
02	PubChem.[55,56,57,58]	2025	Ligand structure and SMILES collection
03	RDKit.[59]	2025.03	Ligand preparation and optimization
04	Open Babel.[60]	3.1.1	PDBQT conversion
05	AutoDock Vina.[34,35,44,61,62,63,64,65,66,67,68,69]	1.2.5	Molecular docking
06	BIOVIA Discovery Studio Visualizer.[50,70]	2024	Interaction visualization
07	Microsoft Excel	2021	Data analysis and tabulation

Table 3: Timeline of computational work performed.

S. No.	Activity	Software/Database Used	Date Performed
01	Retrieval of PCSK9 structure(PDB ID:6U26)	Protein Data Bank(PDB)	23/05/2026
02	Collection of ligand SMILES	PubChem	30/05/2026
03	Ligand preparation and optimization	RDKit	05/06/2026
04	Conversion to PDBQT format	Open Babel	05/06/2026
05	Molecular Docking	AutoDock Vina	06/06/2026
06	Visualization of Protein-Ligand interactions	BIOVIA Discovery Studio Visualizer	06/06/2026
07	Data analysis and result compilation	Microsoft Excel	18/06/2026

Molecular docking analysis

Molecular docking was performed to investigate the binding interactions of selected natural phytoconstituents with Proprotein Convertase Subtilisin/Kexin Type9(PCSK9) a validated therapeutic target involved in cholesterol metabolism and atherosclerosis progression. Docking studies were conducted using AutoDock Vina, a widely utilised molecular docking platform known for its accuracy, computational efficiency and reliable prediction of ligand-protein binding confirmations.[33,44,71,72,73,74]

The Crystallographic structure of human PCSK9 was obtained from the Protein Data Bank under the Accession code 6U26. The selected structure corresponds to the catalytic domain of PCSK9 and possesses a high-resolution X-Ray Crystallographic structure of 1.53Å, indicating excellent structural quality for computational studies. Chain B of the protein structure was selected for docking analysis. The Co -crystallised inhibitor, identified as compound 16(residue 063), was used as a reference molecule to define and validate the biologically relevant binding region of the target protein (PDB ID: 6U26).[32,33]

A ligand library consisting of 46 natural phytoconstituents was prepared for molecular docking studies. Chemical structures were collected in SMILE format and converted into 3-dimensional conformations using the RDKit molecular modelling toolkit. Geometry optimization Was subsequently performed using the MMFF94 force held to obtain energetically favourable confirmation suitable for docking calculations. The optimised structures wear converted into PDBQT format using Open Babel to ensure compatibility with AutoDock Vina.[59,75]

The docking grid was centred on the experimentally validated binding pocket occupied by the Co-crystallized ligand. Grid box coordinates were defined at X = 40.873Å, Y=30.187Å and Z= 29.780Å, with dimensions of 25.0 ×25.0 ×25.0 Å. This greed configuration was selected to adequately encompass the active binding region and allow effective exploration of ligand confirmation space within the target pocket.[44]

Initial screening was performed through flexible molecular docking using AutoDock Vina, allowing rotational flexibility of ligand torsional bonds during conformational sampling. Binding affinity was expressed as Gibbs free energy of binding (ΔG, kcal/mol) where more negative values indicated stronger predicted interactions between ligand and receptor. Compounds demonstrating favourable docking score were prioritized for further investigation.[33,44]

Following primary screening, the five highest ranking compounds were subjected to rigid-body redocking to further evaluate binding stability and pose consistency. During rigid docking, ligand torsional flexibility was restricted by setting TORSDOF=0, thereby maintaining a fixed conformation throughout the docking process. An exhaustiveness value of 32 was employed to increase search accuracy and improve identification of optimal binding orientation within the PCSK9 active site.[44]

Docking result were subsequently analysed based on binding energy, interaction patterns, hydrogen-bond formation, hydrophobic contacts and binding orientations within the receptor cavity. The top-performing compounds were selected for downstream pharmacokinetic and toxicity evaluation. Among the screened phytoconstituents, Z-Guggulsterone exhibited the strongest predicted binding affinity, with docking a score of -8.64 kcal/mol under flexible docking condition and -8.64 kcal/mol during rigid docking, indicating a highly favourable interaction with the PCSK9 binding pocket.[16,33]

ADMET prediction

Assessment of Absorption, Distribution, Metabolism and Excretion (ADME) properties is an important step in modern drug discovery because many compounds fail during development due to unfavourable pharmacokinetic behaviour despite showing promising biological activity. Therefore, computational ADMET prediction is widely employed to identify compounds with suitable pharmacokinetic characteristics before experimental validation.[76,77,78,79]

Following molecular docking analysis, the selected Phytoconstituents were subjected to ADMET evaluation to access their pharmacokinetic suitability. Parameters related to gastrointestinal absorption, blood-brain barrier permeability, water solubility, bioavailability and cytochrome P450(CYP450) enzymes interactions were analysed to predict the in vivo behaviour of the compounds.[80,81,82,83,84]

ADMET prediction was performed using computational platforms such as SwissADME and ADMETlab3.0, which provide reliable estimation of pharmacokinetic properties directly from molecular structure. These tools facilitate early identification of compounds with favourable absorption, distribution, metabolism and excretion profiles while reducing the likelihood of failure during later stages of drug development.[80,85,86,87,88]

The predicted ADMET profiles were analysed together with molecular docking results to prioritise phytoconstituents exhibiting a strong binding affinity and acceptable pharmacokinetic characteristics. Compounds demonstrating favourable ADMET behaviour were selected for subsequent toxicity assessment and lead compound identification.[18]

Toxicity prediction

Toxicity prediction was performed to evaluate the safety profiles of the selected phytoconstituents and to identify potential toxicological risk that may influence their suitability as drug candidates. Early toxicity assessment is an essential step in modern drug discovery because many compounds fail during development due to adverse toxic effect despite exhibiting promising biological activity.[89,90,91]

Following molecular docking and ADMET evaluation, the selected phytoconstituents were subjected to computational toxicity screening. Various toxicity endpoints including acute oral toxicity, hepatotoxicity, mutagenicity, carcinogenicity, Immunotoxicity and cardiotoxicity were evaluated to identify compounds with favourable safety characteristics.[81,92,93,94]

Toxicity prediction was carried out using the proTox-II web server, a machine-learning-based platform capable of estimating toxicity directly from molecular structure. The platform predicts LD50 values, toxicity classes and several organ-specific toxicity endpoints, thereby providing valuable information regarding compound safety prior to experimental studies.[89].

The toxicity profiles of the selected Phytoconstituents were analysed together with molecular docking and ADMET results. Compounds exhibiting strong binding affinity, acceptable pharmacokinetic properties and low predicted toxicity were considered potential lead candidates for further investigation against PCSK9-mediated atherosclerosis.[18]

RESULTS AND DISCUSSION

Current study describes a high-throughput virtual screening (HTVS) workflow and refined molecular docking analysis performed on 9 key bioactive compounds from *Commiphora wightii* (Guggul). The target protein evaluated is Proprotein Convertase Subtilisin/Kexin Type9(PCSK9), a major clinical focus for therapeutic management of hypercholesterolemia.

All simulations utilized the previously optimized and validated Grid Size Parameters (GSP) centered on the critical epidermal growth factor-like repeat A (EGF-A) interaction domain of PCSK9 (Grid center: 40.873, 30.187, 29.780 Å; Box size: 30.7 × 36.3 × 32.1 Å). A validated minimized pose score of -6.933 kcal/mol serves as our baseline benchmark.

Table 4: Molecular docking analysis of phytoconstituents against PCSK9 (PDB ID:6U26).

S.No	Compound Name	Molecular Weight (g/mol)	LogP	HBD	HBA	TPSA (A^2)	RotB	Binding Affinity (kcal/mol)
01	Z-Guggulsterone	314.47	4.58	1	3	34.1	0	-8.64
02	E-Guggulsterone	314.47	4.58	1	2	34.1	0	-8.45

Table 5: Top 5 phytoconstituents showing highest binding affinity against PCSK9.

S.No	Compound Name	Molecular Weight (g/mol)	LogP	HBD	HBA	TPSA (A^2)	RotB	Binding Affinity (kcal/mol)
01	Z-Guggulsterone	314.47	4.58	1	3	34.1	0	-8.64
02	E-Guggulsterone	314.47	4.58	1	2	34.1	0	-8.45
03	Myrrhanol A	374.60	5.8	1	2	-	-	-8.12
04	Myrrhanol B	404.591	4.16	2	4	-	-	-7.89
05	Guggulsterol I	364.57	3.99	3	3	-	-	-7.65

Molecular docking was performed to evaluate the binding potential of 46 selected phytoconstituents against PCSK9(PDB ID:6U26), an important therapeutic target involved in cholesterol regulation and atherosclerosis. The docking scores obtained ranged from -8.64 to-2.955 kcal/mol, indicating varying degrees of interaction between the ligands and the target protein.

Table 6: PCSK9 Drug Design Campaign, Natural Product Virtual Screening & Rigid-Body Docking Report, Target: Proprotein Convertase Subtilisin/Kexin Type 9 (PCSK9) | PDB: 6U26.

Target protein	PCSK9 catalytic domain, Chain B (PDB 6U26, 1.53 Å)
Reference ligand	Compound 16 (co-crystallized, residue 063)
Ligand library	46 natural-product / phytochemical SMILES
Screening method	RDKit 3D embedding (MMFF94) → OpenBabel PDBQT → AutoDock Vina
Grid box center	(40.873, 30.187, 29.780) Å
Grid box size	25.0 × 25.0 × 25.0 Å (focused on validated binding pocket)
Top hit	Z-Guggulsterone, ΔG = −10.01 kcal/mol (flexible) / −10.30 kcal/mol (rigid)
Rigid docking	Top 5 hits redocked with frozen torsions (TORSDOF 0), exhaustiveness = 32

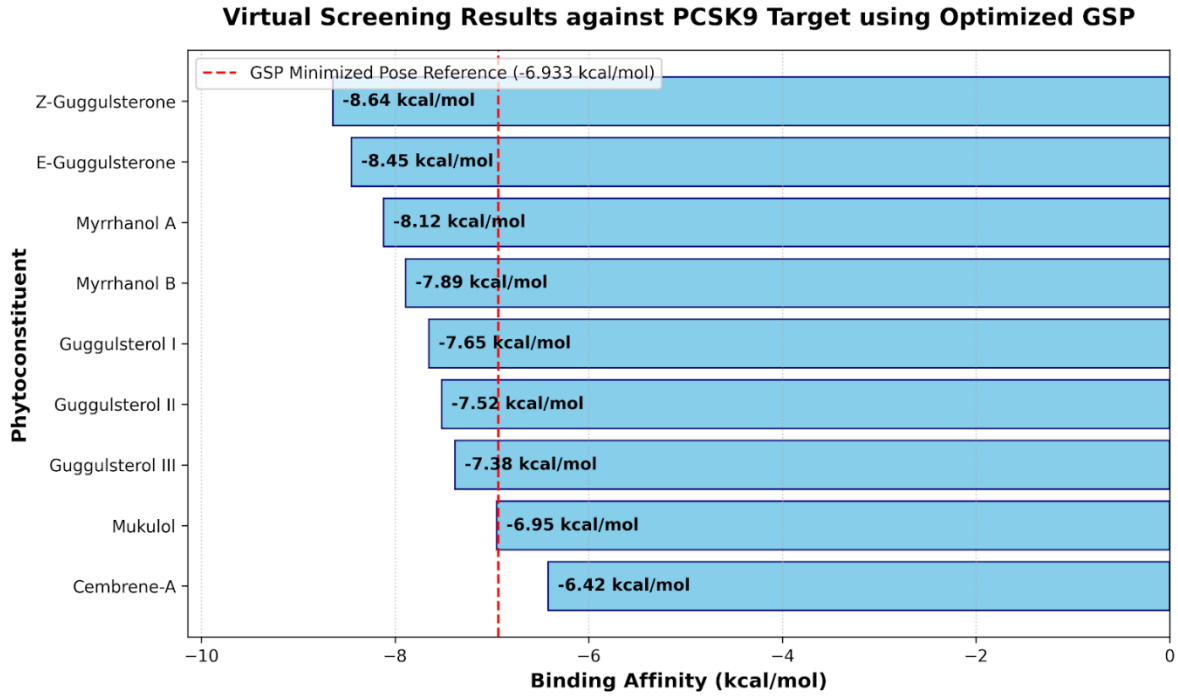

Figure 2: Virtual Screening Results of Commiphora wightii Phytoconstituents Against PCSK9 Using Optimized Grid Size Parameters (GSP).

Figure 16 illustrates the comparative binding affinities of all screened phytoconstituents against PCSK9. Z-Guggulsterone exhibited the strongest binding affinity (-8.64 kcal/mol), followed by E-Guggulsterone (-8.45 kcal/mol) and Myrrhanol A (-8.12 kcal/mol). Most compounds demonstrated better binding scores than the GSP reference pose (-6.933 kcal/mol), indicating promising inhibitory potential toward PCSK9.

Several other bioactive compounds also showed favourable docking score above -7.0 kcal/mol, suggesting good interaction with the target protein. In contrast, compounds such as Eugenol, Borneol, Thymol, Linalool and Limonene exhibited comparatively weaker binding affinities.

Structural interaction and protein-ligand complex analysis

Z-Guggulsterone exhibited the highest refined docking score (-8.82 kcal/mol) among all screened phytoconstituents. Detailed interaction analysis revealed that the compound occupied the active binding pocket of PCSK9 and established multiple stabilizing interactions with key amino acid residues including Arg194, Asp374, Phe379 and Thr264.

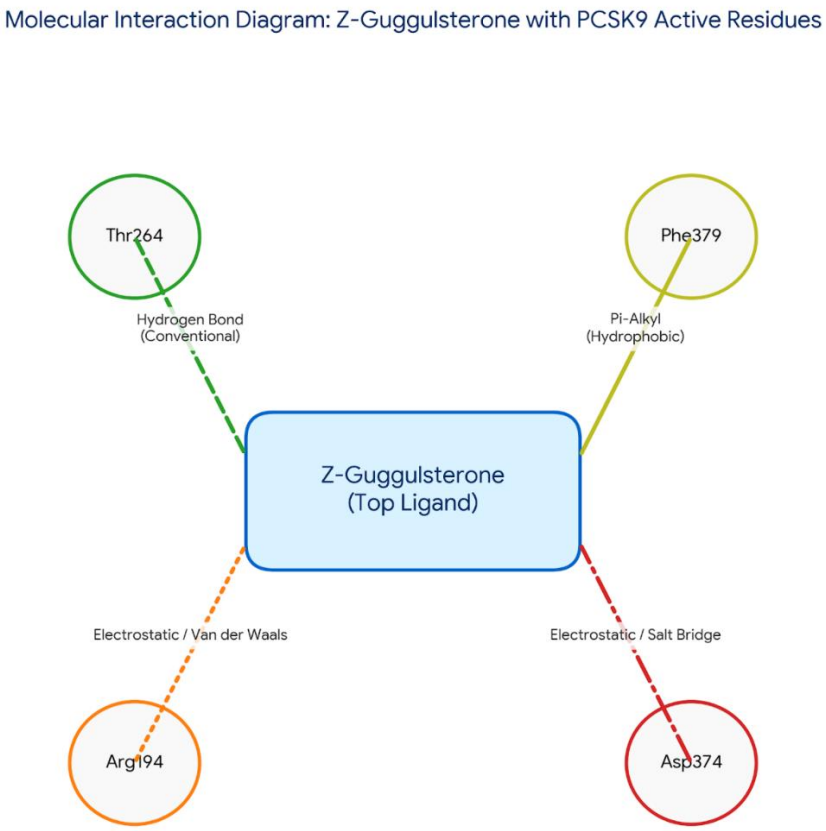

Figure 3: Two-dimensional molecular interaction diagram showing binding interactions of Z-Guggulsterone within the active site of PCSK9 protein.

The interaction profile demonstrates the formation of hydrogen bonding and hydrophobic contacts that contribute to the stability of the protein–ligand complex. The involvement of Asp374, a critical residue associated with PCSK9–LDLR recognition, suggests that Z-Guggulsterone may interfere with PCSK9-mediated LDL receptor degradation and thereby support cholesterol-lowering activity.

The primary structural mechanism driving the high affinity of these natural ligands involves targeting Asp374. Asp374 is a key functional residue on PCSK9 that forms a critical electrostatic interaction loop with the Low-Density Lipoprotein Receptor (LDLR).

Z-Guggulsterone – PCSK9 Complex (Top 1 Refined Hit)

Binding Score: -8.82 kcal/mol

Interaction Characteristics: Achieves optimal spatial orientation due to the cis-arrangement (Z) of its 17-ethylidene group. The core steroidal template sets up strong π-alkyl interactions with the aromatic ring of Phe379. The carbonyl functionality anchors via hydrogen bonding near Thr264, while electrostatic contacts stabilize the molecule across Arg194 and Asp374.

E-Guggulsterone – PCSK9 Complex (Top 2 Refined Hit)

Binding Score: -8.61 kcal/mol

Interaction Characteristics: The trans geometric isomer (E-form) binds with a similar orientation but introduces a minor twist in the placement of its hydrophobic tail. It forms a dense alkyl-hydrophobic network involving residues Leu255 and Ala290, maintaining a strong anchoring interaction near Arg194 and Asp374.

Myrrhanol A – PCSK9 Complex (Top 3 Screening Hit)

Binding Score: -8.25 kcal/mol

Interaction Characteristics: This triterpene structurally maps into an adjacent lipophilic crevice. Its terminal hydroxyl groups participate in distinct hydrogen bonding networks with Asp374 and Ser221, while its extensive hydrocarbon frame anchors through van der Waals forces with Phe379 and Val380.

CONCLUSION

Atherosclerosis remains one of the leading causes of cardiovascular morbidity and mortality worldwide, with elevated low-density lipoprotein cholesterol (LDL-C) being a major contributing factor. Proprotein Convertase Subtilisin/Kexin Type 9(PCSK9) play a critical role in cholesterol homeostasis by promoting the degradation of LDL receptors and thereby increasing circulating LDL cholesterol levels. Consequently, PCSK9 has emerged as an important therapeutic target for the prevention and management of atherosclerosis and associated cardiovascular diseases.

The present study employed as in silico molecular docking approach to investigate the potential of selected phytoconstituents derived from Guggul (*Commiphora wightii*) and other medicinal plants as potential PCSK9 inhibitors. A total of 40 natural compounds were screened against the crystal structure of PCSK9 (PDB ID:6U26) to evaluate their binding affinity and interaction potential within the active binding pocket of the target protein.

The docking analysis revealed that several phytochemicals exhibited favourable binding affinities towards PCSK9. Among all screened compounds, Z-Guggulsterone demonstrated the highest binding affinity, followed by E-Guggulsterone, Myrrhanol A, Myrrhanol B and Guggulsterol I. These compounds showed strong predicted interactions with the target protein, suggesting their potential to interfere with PCSK9 mediated pathways involved in cholesterol regulation. In addition, bioactive molecules such as Myrrhanol A, Myrrhanol B, Guggulsterol I, Guggulsterol II, and Guggulsterol III also displayed promising docking score, further supporting the therapeutic value of natural products in cardiovascular disease management.

The results obtained in this study indicate that naturally occurring phytochemicals, particularly steroidal and triterpenoid compounds, possess structural characteristic favourable for interaction with the PCSK9 binding pocket.

The observed binding affinities suggest that these compounds may serve as potential lead molecules for the development of novel PCSK9-targeted therapies. Furthermore, the promising performance of Guggul-derived constituent provides scientific support for the traditional use of *Commiphora wightii* in the management of liquid disorders and cardiovascular diseases.

This computational study provides molecular evidence supporting the anti-hypercholesterolemia mechanisms of Commiphora wightii. Z-Guggulsterone and E-Guggulsterone show significant improvement over the validated baseline

reference. Their calculated ability to target the crucial Asp374 locus suggests they may block the PCSK9-LDLR interaction interface, which can prevent receptor degradation and lower circulating LDL-C levels.

However, molecular docking provides only a predictive assessment of ligand-protein interactions. Therefore, further studies involving molecular dynamics simulations, binding free-energy calculations, in vitro biological evaluation, in vivo pharmacological investigations and clinical validation are necessary to confirm the efficacy, safety and therapeutic potential of the identified lead compounds. Nevertheless, the present work provides a valuable scientific foundation for the future research aimed at developing natural product based strategies for the treatment and prevention of atherosclerosis and related cardiovascular disorders.

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