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RESEARCH ARTICLE

Computational insights into the hypocholesterolemic activity of *Humboldtia vahliana* Wight phytoconstituents

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Abstract

PCSK9 (Proprotein convertase subtilisin/Kexin type 9) is a promising therapeutic target for the development of novel cholesterol-lowering drugs. In this study, we investigated the potential of *Humboldtia vahliana* Wight as a natural PCSK9 inhibitor through a combination of gas chromatography-mass spectrometry/mass spectrometry (GC-MS/MS) analysis and molecular docking techniques. Methanolic bark extract of *H. vahliana* were subjected to GC-MS/MS, which identified several bioactive constituents, including Phenyl methyl ester (16.19 %), 13-docosenamide (11.64 %), hexadecanoic acid (9.20 %) and D-glycero-D-ido-heptose (7.13 %). The PCSK9 inhibitory potential of these compounds was evaluated through molecular docking studies. Results revealed that the major active compounds exhibited strong binding affinities toward PCSK9, forming up to 5–6 hydrogen bonds with the target protein. This binding suggests a potential inhibitory mechanism that could down regulate PCSK9 activity. According to the docking results, d-Glycero-d-ido-heptose (-5.1 Kcal/mol) and phenyl methyl ester/ acetic acid (-6.0 Kcal/mol) are promising natural inhibitors of PCSK9 that may reduce cholesterol via modifying LDL receptor recycling. Phenyl methyl ester is a possible lead chemical for PCSK9 inhibition, according to cross docking study, which shows that it has a greater binding energy of 1.1 Kcal/mol than the small-molecule PCSK9 inhibitor (-4.9 Kcal/mol), suggesting a better fit in the receptor cavity. Overall, the findings support the therapeutic promise of *H. vahliana* as a natural source of PCSK9 inhibitors, potentially contributing to the reduction of lipid levels in the bloodstream and the development of novel hypocholesterolemic agents. The presence of several moderate-binding compounds together points to a polypharmacological effect of the extract from *H. vahliana*.

Keywords: GC-MS/MS analysis; *Humboldtia vahliana;* hypocholesterolemic agents; molecular docking techniques; PCSK9

Introduction

Cardiovascular diseases remain the leading cause of mortality worldwide, with elevated low-density lipoprotein cholesterol (LDL-C) levels being a major contributing risk factor. PCSK9 (Proprotein convertase subtilisin/Kexin type 9) is a key regulatory protein involved in cholesterol homeostasis. It promotes the degradation of LDL receptors (LDLR) on hepatocytes, thereby reducing the capacity of the liver to remove LDL-C from the bloodstream (1). Inhibiting PCSK9 can increase LDLR recycling and enhance cholesterol clearance, making it an attractive therapeutic target for hypercholesterolemia (Fig. 1).

PCSK9 and its role in cholesterol metabolism

A key modulator of plasma cholesterol levels, PCSK9 interacts with the LDLR pathway. In order to decrease the recycling of the receptor back to the plasma membrane, PCSK9 attaches itself to LDLR on the surface of hepatocytes and encourages its internalisation and destruction within lysosomes (2). As a result, ability of the liver cells to remove LDL-C from the blood is reduced, which raises the levels of LDL-C in the blood. Because of its physiological importance, PCSK9 is a key target in studies on hypercholesterolemia and cardiovascular disease. Increased PCSK9 activity raises the risk of atherosclerosis and leads to dyslipidemia (3). On the other hand, PCSK9 inhibition greatly improves LDL-C clearance via increasing LDLR availability on hepatocyte surfaces. In patients with familial hypercholesterolemia and statin intolerance, this has resulted in the development and clinical use of PCSK9-targeted therapies, such as monoclonal antibodies (e.g. alirocumab and evolocumab), which have shown strong effects on lowering LDL-C and cardiovascular risk (4). As seen in lipoprotein metabolic maps, the PCSK9 mechanistic pathway emphasises the function of protein in post-translational control of LDLR and its potential for therapeutic use in cholesterol management techniques (5).

While several monoclonal antibodies targeting PCSK9 have been developed and approved, their high cost and need for parenteral administration present challenges, especially in resource-limited settings. As a result, there is growing interest in identifying small-molecule or natural compound-based PCSK9 inhibitors with improved accessibility and affordability.

Medicinal uses of *Humboldtia vahliana*

Humboldtia vahliana, a lesser-studied medicinal plant native to specific regions of India, has been traditionally used for its therapeutic properties (6). However, its potential in modulating

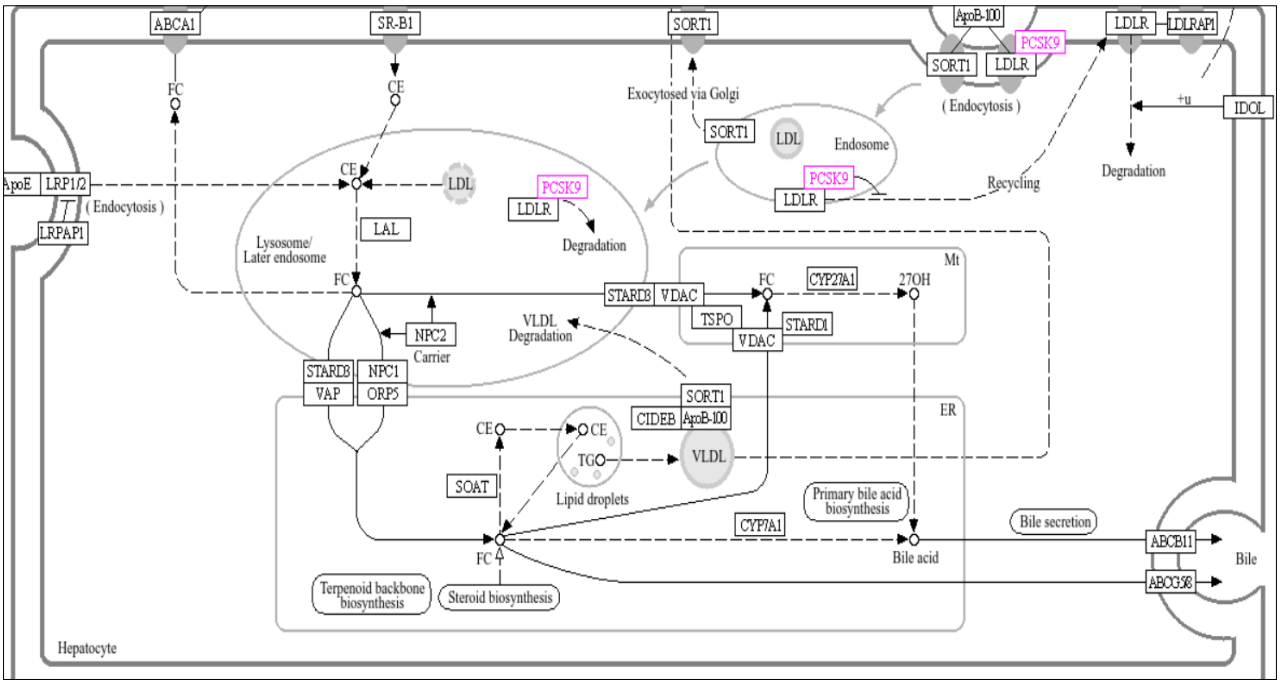


Fig. 1. PCSK9-LDLR pathway.

cholesterol metabolism via PCSK9 inhibition remains unexplored. The bioactive constituents of *H. vahliana* therapeutic properties are given in Table 1.

This study aims to investigate the bioactive constituents of methanolic bark extracts of *H. vahliana* using GC-MS/MS and to evaluate their PCSK9 inhibitory potential through molecular docking analysis.

Materials and Methods

Collection of plant materials

Fresh bark of *H. vahliana* was collected from Anamala road, Athirappilly (Latitude 10.2889° and Longitude 76.5142°) in the month of January 2025 and authenticated with the help of Flora. The material was preserved and Voucher (Vr. Sp. No. -SMCH210) submitted to Herbarium, Department of Botany, St Mary's College (Autonomous), Thrissur. The plant materials were washed with tap water to remove the soil and unwanted dust particles. Then, shade dried for 20 days and then powdered by using mechanical blender and stored in airtight bottle.

Preparation of plant extract

The bark of *H. vahliana* was shade-dried and powdered (fresh weight to dry weight ratio, 2.31:1). The powdered plant material (30 g) was packed into a Soxhlet apparatus and extracted with methanol for 72 hr. The organic extract was concentrated under controlled temperature and pressure using a water bath to obtain a gummy mass. The resulting residue was stored for further analysis (10). The extract was subsequently subjected to GC-MS/MS analysis.

Characterisation of bioactive constituents of plant extract by gas chromatography-mass spectrometry

The bioactive constituents in methanolic bark extract were determined by gas chromatography (Agilent 1010 triple quadrupole GC-MS system) equipped with HP-5MS ultra insert column mass spectrometer operated at initial column temperature of -60 °C and heated up to 350 °C at 5 °C per 5 min (11). Chromatographic conditions were a flow of 1 mL/min of high purity helium as carrier gas in splitless mode. The identification of the compounds in spectrum was done based on retention time and integral area of peaks. The similarity of compounds matched with >75 % are listed based on NIST library search.

Molecular docking

The crystal structure of PCSK9 with 6u26 was retrieved from PDB. The protein structure of PCSK9 was edited using Pymol. Binding site of PCSK9 was defined and edited using Discovery studio visualiser. PyRx (Autodock vina) was used to optimise the protein. The missing hydrogens were inserted throughout the optimisation step (12).

Kollman unified atom charges were given to the edited protein Table 2 shows the grid position and size reflecting the whole protein during the docking process. The 3D structures of the top 14 compounds identified in the GC-MS/MS analysis of *H. vahliana* were chosen for docking, while small-molecule PCSK9

Table 2. The grid position and grid size of the targeted protein

Gene	Grid position	Grid size
PCSK9	47.24 x 23.87 x 25.70	106.56 x 70.16 x 25.00

Table 1. Primary and secondary metabolites of *Humboldtia vahliana* and their therapeutic uses in different countries (7–9)

Scientific name	Primary metabolite	Secondary metabolite	Uses of plants in different countries	Preparations / therapeutic uses
Humboldtia vahliana Wight	Carbohydrates, Proteins, Amino acids, Fatty acids	Flavonoids, Tannins, Phenolic compounds, Alkaloids, Saponins	Endemic to Western Ghats (India); locally used in traditional medicine by tribal communities. The folklore practitioners belonging to 'Muthuvan' tribes of Kerala use its bark paste in wound management and leprosy	Bark and leaf extracts used for anti-inflammatory, antioxidant, antimicrobial and anticonvulsant properties; decoctions prepared for wound healing, skin ailments and fever management

inhibitor was used as positive docking controls for PCSK9. Three dimensional structures of the identified compounds were obtained from PubChem. All structures were optimised before molecular docking. Open Babel was used to add hydrogen atoms to every structure a three-dimensionalient algorithm with 200 minimisation steps was used for the optimisation of these compounds. Molecular docking was utilised to explore protein-ligand binding. The activity of these compounds was screened against the potential drug target PCSK9 using the docking tool PyRx (Autodock vina), which is a virtual screening package for computational drug discovery. Docking accuracy was set to regular. All run parameters were left at their default settings and the Autodock Vina algorithm was used to confirm all docking. The RF-score V2 scoring function was added for improved binding affinity prediction and the population size was set to 200 (13, 14).

Binding site analysis

Discovery Studio was used to visualise the binding properties of the compounds that had a lower binding energy to the targeted protein, PCSK9, than small-molecule PCSK9 inhibitor. The protein-ligand complexes were presented as 2D and 3D. CavityPlus tool was used to compare the binding position in order to determine the structure binding protein. Ki (inhibition constant for each protein-ligand complex was computed. A complex with a lower Ki will bond more tightly and effectively (15).

ADMET analysis

Due to its high binding affinity, D-Glycero- D-ido-heptose and Phenyl methyl ester were undergone ADMET analysis, by uploading the canonical SMILE format of the compound in SwissADME for identifying whether the compounds were compliant with Lipinski's rule of 5. Molecular weight (MW), topological polar surface area (TPSA), number of hydrogen-bond donors and acceptors (HBD, HBA), rotatable bonds and LogP were computed.

Results and Discussion

Gas chromatography-mass spectrometry/mass spectrometry analysis

The methanolic bark extract of *H. vahliana was* subjected to GC-MS/MS analysis, which identified 23 phytochemical constituents based on retention time, molecular weight and spectral similarity (Fig. 2). The relative abundance of each compound was quantified based on the percentage of peak area in the chromatogram. The most abundant compounds included Phenyl methyl ester/acetic acid (16.19 %), Hexadecanoic acid (9.20 %) and 13-docosenamide (11.64 %), whereas minor compounds like naphthalene (1.41 %) and 1,4-dibutyl benzene-1,4 -dicarboxylae (1.24 %) were also detected. From these 15 phytochemicals with peak area greater than 1 % were considered for further docking studies against PCSK9. The active compounds in the *H. vahliana* are shown in Table 3.

Pocket binding site analysis of PCSK9

Pocket binding analysis of the PCSK9 protein using Cavityplus tool, revealed the presence of 3 distinct classes of binding pockets based on their predicted binding affinity: 1 strong, 1 medium and 2 low-affinity pockets (Fig. 3A–D, Table 4). These pockets were characterised by their constituent amino acid residues and their potential to accommodate ligand binding.

The strong binding site is comprised of 28 amino acid residues including TYR-78, VAL-79, LEU-74, PHE-115, LEU-112, ASP-146 and HIS-116, among others. This site exhibited a high probability of stable ligand binding due to its favourable structural conformation and residue hydrophobicity (15).

The medium-affinity pocket included residues such as GLU-132, SER-127, ARG-66, HIS-65, LEU-133 and PRO-75. While slightly less favourable than the strong pocket, this site still provided a viable environment for moderate ligand interaction.

Two low-affinity pockets were identified, involving residues such as LYS-83, GLU-85, SER-89, THR-86, LEU-82 in the first low site and GLU-132, LEU-133, HIS-139, VAL-140 and LYS-136 in the second. These regions demonstrated less consistent interaction potential, likely due to solvent exposure or unfavourable geometry.

Ligand interactions preferences

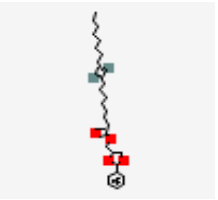
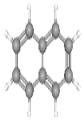
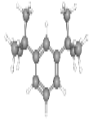
Compounds identified from the methanol extract of *H. vahliana* showed preferential binding toward different PCSK9 binding sites. Compounds such as phenyl methyl ester, d-Glycero-d-ido-heptose, hexadecanoic acid, 13-docosenamide, methyl stearate, benzene propanoic acid, oleic acid and 9-octadecenoic acid showed strong affinity for the strong and medium binding sites. These molecules form stabilising interactions within hydrophobic and charged pockets, suggesting their potential as effective PCSK9 inhibitors (16). In contrast, Decane, Undecane, Naphthalene, 3-O-Methyl-d-glucose and 1,4-Dibutyl benzene-1,4-dicarboxylate primarily interacted with low-affinity sites, indicating comparatively weaker and potentially transient binding.

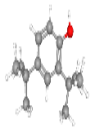
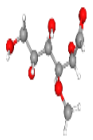
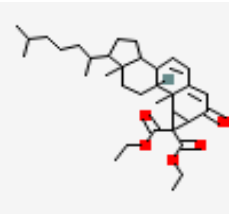
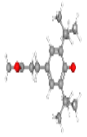
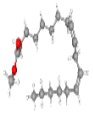
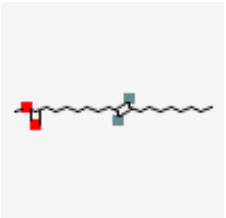
These findings suggest that specific phytochemicals from *H. vahliana* possess significant binding potential at functionally relevant PCSK9 pockets, particularly at the strong and medium affinity sites, making them promising candidates for further *in silico* and *in vitro* validation (Table 4, Fig. 3).

Molecular docking analysis of 15 selected phytochemicals from *H. vahliana*

Docking simulations were carried out using the PyRx virtual screening tool to evaluate the binding affinities of the identified compounds toward PCSK9 active/binding sites (12). The docking results were compared with small-molecule PCSK9 inhibitor, a standard drug (monoclonal antibody inhibitor) as a positive control. The binding energy (ΔG) values ranged from -6.0 to -3.4 kcal/mol. The inhibition constants (Ki) were derived using the binding energy values, indicating potential inhibitory activity of the phytochemicals on PCSK9. Compounds with lower binding energy (more negative) and lower Ki values indicate stronger binding and better inhibitory potential. The positive control small-molecule PCSK9 inhibitor has set as a reference for evaluating natural compounds obtained from *H. vahliana*. Small-molecule PCSK9 inhibitor has showed -4.9 kcal/mol binding energy with Ki :77.424 µM. Phenyl methyl ester/acetic acid showed the most favourable binding with -6.0 kcal/mol and Ki = 40 µM, surpassing the control inhibitor (Table 5). D-Glycero-d-ido-heptose (-5.5 kcal/mol, 94 µM) and hexadecanoic acid (-5.1 kcal/mol, 180 µM) displayed moderate affinity and could contribute to PCSK9 inhibition**.** 13-docosenamide with 4.9 kcal/mol binding energy and Ki 260 µM showed comparable binding to small-molecule PCSK9 inhibitor. Compounds such as Benzene propanoic acid, 3-5-bis 4-hydroxymethyl ester (-3.4 kcal/mol,

Table 3. Compounds identified in methanol-extracted *Humboldtia vahliana*

Sl. No.	Name of the compound	RT	Area percentage	Molecular formula	Molecular weight	Structure
1	9-Octadecenoic acid, (2-phenyl-1,3-dioxolan 4-yl) methyl ester, trans-	3.0417	3.171	C28H44O4	444.324	
2	Decane	4.6215	3.858	C10H22	142.172	
3	Undecane	5.2423	3.328	C11H24	156.188	
4	4-Isopropylbenzenethiol, S -methyl-	5.4461	0.8028	C10H14S	166.082	
5	Acetic acid, phenylmethyl ester	5.7055	16.19	C9H10O2	150.068	
6	Naphthalene	5.9741	1.406	C10H8	128.063	
7	Benzene, 1,3-bis(1,1-dimethylethyl)-	6.3817	0.6247	C14H22	190.172	
8	d-Glycero-d-ido-heptose	7.9336	7.127	C7H14O7	210.074	

9	2,4-Di-tert-butylphenol	8.9760	0.8932	C14H22O	206.167	
10	3-O-Methyl-d-glucose	10.8845	3.599	C7H14O6	194.079	
11	1',1'-Dicarboethoxy-1.beta.,2.beta.-dihydro-3'H-cycloprop[1,2]cholesta-1,4,6-trien-3-one	13.3954	0.1792	C34H50O5	538.366	
12	Hexadecanoic acid, methyl ester	15.6235	9.197	C17H34O2	270.256	
13	Benzenepropanoic acid, 3,5-bis(1,1- dimethylethyl)-4-hydroxy-, methyl ester	16.0312	5.478	C18H28O3	292.204	
14	1,4-Dibutyl benzene-1,4-dicarboxylate	16.3648	1.238	C16H22O4	278.152	
15	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	18.5837	1.378	C19H34O2	294.256	
16	9-Octadecenoic acid, methyl ester, (E)-	18.6810	4.954	C19H36O2	296.272	

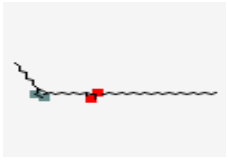
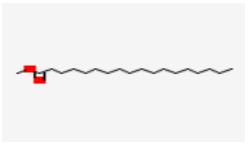
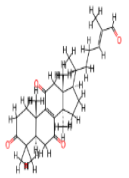
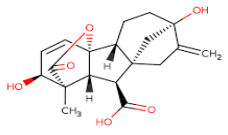
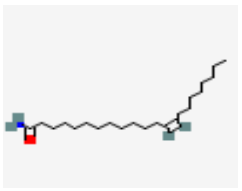
17	Oleic acid, eicosyl ester	18.7829	1.229	C38H74O2	562.569	
18	Methyl stearate	19.1117	4.762	C19H38O2	298.287	
19	(24E)-3,7,11-Trioxolanosta-8,24-dien-26-oic acid, TMS	19.2878	0.34	C33H50O5Si	554.343	
20	Lycopene	19.4963	0.1175	C40H56	536.438	
21	Rhodopin	20.1541	0.1404	C40H58O	554.449	
22	Isogibberellin A3, 3TMS derivative	28.3765	0.3598	C28H46O6Si 3	562.26	
23	13-Docosenamide, (Z)-	29.2893	11.64	C22H43NO	337.334	

Table 4. The pocket binding site of PCSK9

No.	Binding site	Amino acids
1	Strong	VAL-79-A,VAL-149-A,TYR-78-A,CYS-67-A,PHE-115-A,LEU-112-A,ILE-143-A,VAL-114-A,SER-148-A,LEU-74-A,ASP-146-A,LEU-119-A,LEU-118-A,PHE-122-A,VAL-81-A,SER-147-A,LEU-123-A,GLY-121-A,GLU-145-A,LEU-82-A,HIS-113-A,GLY-117-A,VAL-80-A,PRO-120-A,GLU-144-A,HIS-116-A,TYR-142-A,THR-77-A
2	Medium	GLU-132-A,SER-127-A,LEU-130-A,ALA-62-A,ARG-66-A,HIS-65-A,THR-63-A,GLY-76-A,LEU-133-A,TYR-78-A,ARG-73-A,THR-77-A,GLY-128-A,GLU-145-A,LEU-135-A,LEU-131-A,MET-126-A,ASP-129-A,PHE-64-A,PRO-75-A
3	Low	LYS-83-A,PRO-120-A,LEU-119-A,GLU-85-A,PHE-115-A,GLN-90-A,LEU-118-A,ASP-141-A,LEU-88-A,LEU-82-A,VAL-81-A,SER-91-A,GLU-84-A,SER-89-A,HIS-116-A,GLY-117-A,GLY-121-A,TYR-142-A,THR-86-A,HIS-87-A
4	Low	GLU-132-A,ALA-62-A,HIS-139-A,LYS-83-A,THR-63-A,LEU-133-A,LEU-137-A,ASP-141-A,THR-61-A,VAL-140-A,LEU-135-A,LYS-136-A,LEU-131-A,PHE-64-A,PRO-138-A,ALA-134-A

Table 5. Energy binding and phytochemical inhibition constants of *Humboldtia vahliana* methanol bark extracts with the binding sites of PCSK9 from docking analysis and quantification of each compound through GC-MS/MS analysis

No.	Components	GC-MS/MS % peak area	Binding energy (kcal/Mol)	Inhibition constant (Ki)
1	Small-molecule PCSK9 inhibitor (Positive control)		-4.9	77.424 uM
2	Phenyl methyl ester/Acetic acid	16.19	-6.0	40 uM
3	d-Glycero-d-ido-heptose	7.13	-5.5	94 uM
4	Hexadecanoic acid	9.20	-5.1	180 uM
5	13-docosenamide	11.64	-4.9	260 uM
6	Methyl stearate	4.6	-4.4	590 uM
7	9, Octadecenoic acid	3.17	-4.0	1170 uM
8	Decane	3.86	-4.3	71 uM
9	Undecane	3.33	-4.3	710 uM
10	Naphthalene	1.41	-4.6	427 uM
11	3-0-Methyl-d-glucose	3.60	-4.2	840 uM
12	Benzene propanoic acid, 3-5-bis 4-hydroxymethyl ester	5.48	-3.4	3200 uM
13	1,4-Dibutyl benzene-1,4-dicarboxylate	1.24	-4.5	510 uM
14	9,12-Octa decadienoic acid	1.38	-4.8	305 uM
15	Oleic acid, eicosyl ester	1.23	-3.4	3200 um

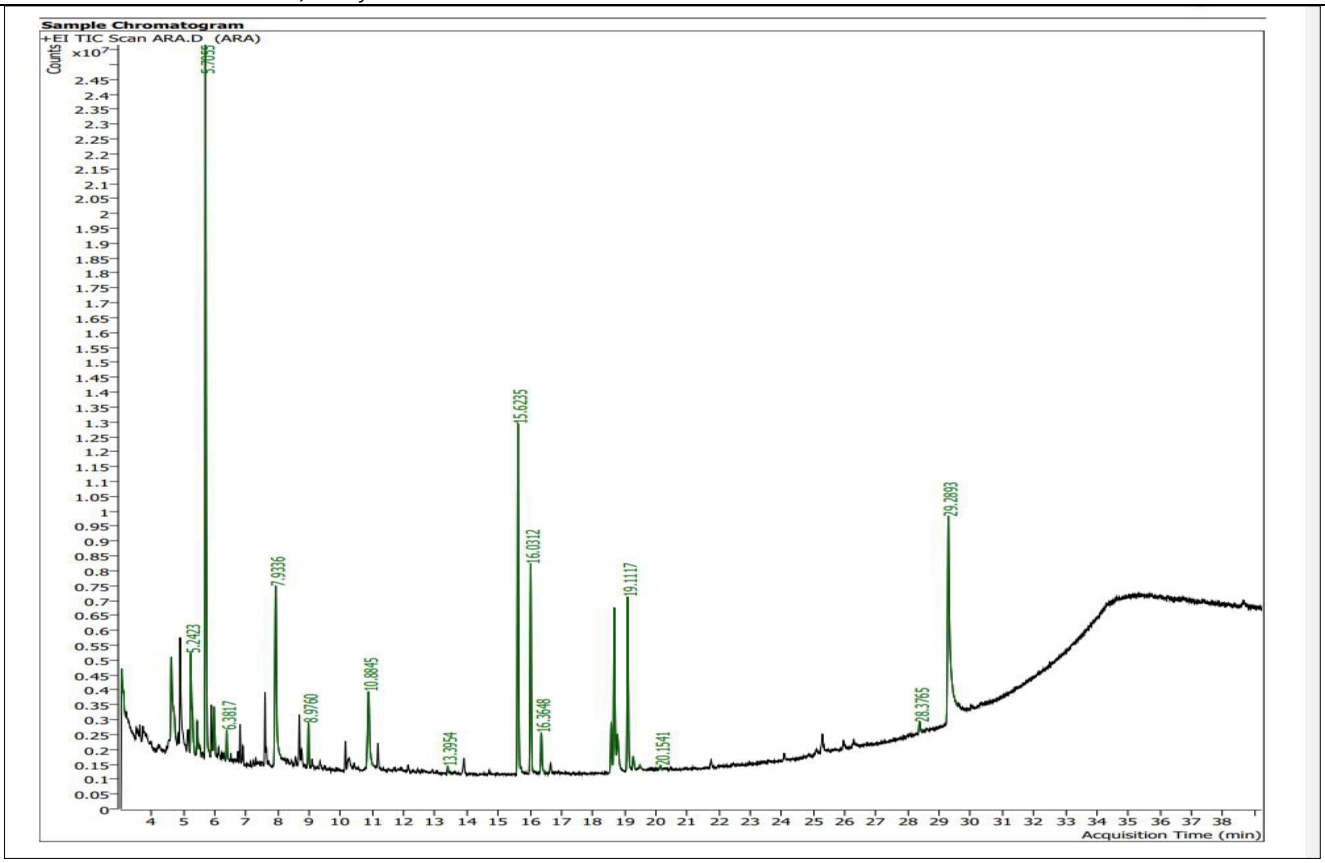


Fig. 2. GC-MS chromatogram of methanolic bark extracts of *Humboldtia vahliana.*

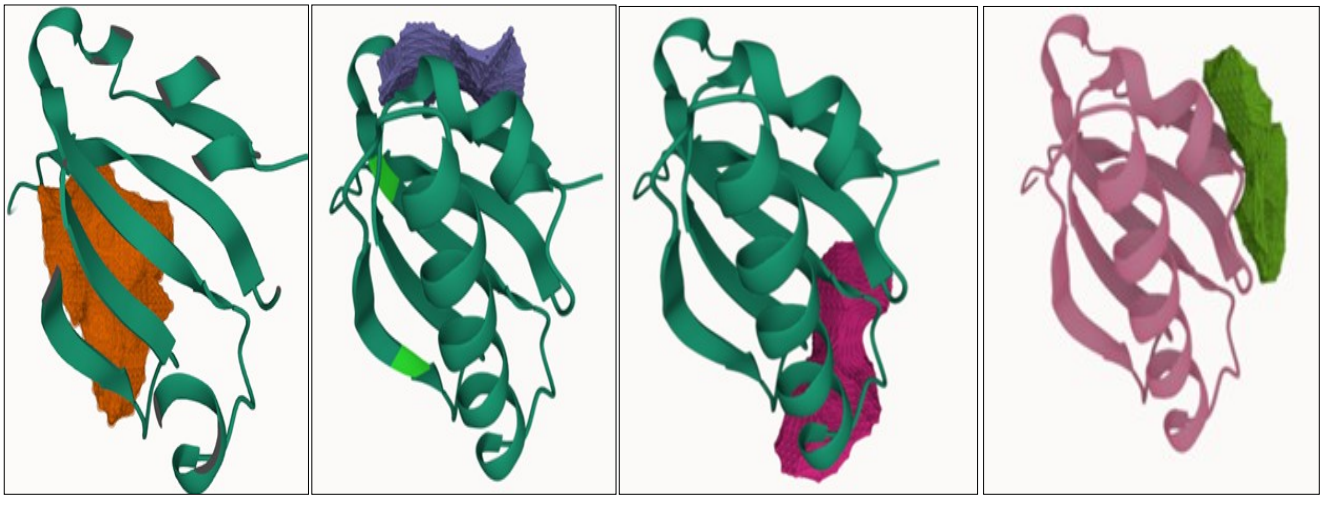


Fig. 3. The pocket binding sites of the PCSK9 protein at (A) high, (B) medium (C) and (D) low binding affinity was analysed with CavityPlus.

3200 µM) and Oleic acid, eicosyl ester (-3.4 kcal/mol, 3200 µM) displayed weak interactions, suggesting minimal inhibitory potential (17).

Integration of docking and GC-MS/MS quantification

When correlating quantitative abundance (GC-MS/MS percentage peak area) with binding potential (ΔG and Ki). Phenyl methyl ester/ acetic acid emerged as the lead compound due to its high abundance (16.19 %) and strongest binding energy (-6.0 kcal/mol). Other abundant compounds like 13-docosenamide (11.64 %) and Hexadecanoic acid (9.20 %) exhibited moderate binding but are present in significant quantities, potentially contributing synergistically. Low-abundance compounds with weak binding (e.g. Oleic acid, eicosyl ester) likely play a negligible role in overall inhibition (18).

2D interaction of phenyl methyl ester shows Pi-Sigma interaction between the aromatic ring of the phenyl methyl ester and proline 436, which stabilises the binding affinity. Hydrogen bond interaction between the ligand and Isoleucine:417, Arginine:357, Asparatine:360 of the receptor strengthen the interaction. Hydrogen bonds are strong contributors to binding specificity and affinity. Both hydrogen and pi interactions are essential for anchoring the ligand in the active site of the receptor. Hydrophobic interactions between the ligand and residues Leu:440, Leu: 435, Val:450, Val:359 of PCSK9 enhance ligand stability inside the binding pocket. Presence of this hydrophobic residue nearby suggests the binding site is nonpolar, favourable for phenyl derivatives. This interaction pattern supports the strong binding affinity of phenyl methyl ester (-6.0 kcal/mol) with the receptor PCSK9 (19).

In the case of D-Glycero-d-ido-heptose multiple conventional hydrogen bonds are present between hydroxyl groups of the ligand and residues ARG:357, TRP:461, VAL:450. These interactions indicate strong polar contacts, crucial for stabilising sugar molecules in protein binding pockets. Unfavourable donor-donor/acceptor-acceptor interactions are observed with the ligand and residues like VAL:450 and ASP :360, suggests some steric/ electrostatic repulsion in the binding site, which might reduce binding affinity slightly. Hydrophobic contacts, LEU:435, LEU:440, provide additional stabilisation through weak non-polar interactions. Thus, Phenyl methyl ester having Pi and hydrophobic interactions make it better fit in nonpolar pocket. D-Glycero-d-ido-heptose with Polar H-bond network and repulsions due to dense hydroxyl groups make it less optimal binding.

Hexadecanoic acid (palmitic acid) is a common fatty acid. Its hydrophobic chain fits into PCSK9's lipophilic pocket. Although Pi-alkyl interactions between the hydrophobic alkyl chains of the ligand with aromatic residues, contributing to binding affinity. The observed interactions like van der Waals and hydrogen bonds suggest stability but not high binding specificity, make it as a supportive modulator rather than a potent inhibitor alone (20). In the case of 13-docosenamide, multiple residues form van der Waals contacts, suggesting hydrophobic stabilisation between the long aliphatic chain of the ligand and the PCSK9 pocket. Conventional hydrogen bond is seen, contributing to moderate specificity (20). Some steric clashes or unfavourable interactions exist with aromatic residues which may slightly reduce binding affinity. Hydrophobic alkyl-pi stacking interactions occur with aromatic residues, aiding in hydrophobic fit. Thus, contributes less to PCSK9 inhibition potential due to steric clashes and less optimal binding. 13-Docosenamide having binding energy (-4.9 kcal/mol) compared to Hexadecanoic acid (-5.1 kcal/mol) and phenyl methyl ester (-6.0 kcal/mol).

Being an unsaturated fatty acid, 9,12–octadecadienoic acid (-4.8 kcal/mol) aligns into PCSK9's hydrophobic cavity but shows suboptimal orientation due to steric interference. Alone, it is unlikely to contribute significantly to PCSK9 inhibition but could enhance activity in a synergistic mixture with other fatty acids.

Observed 2D interaction of naphthalene with PHE and LEU residues form π-sigma interactions which stabilise aromatic–hydrophobic contacts. π-alkyl interactions between the ligand with LEU 268 and ALA 300, contributing additional hydrophobic stabilisation. Detected unfavourable bump with GLU269 and LEU 265, indicating steric clashes or unfavourable positioning that may slightly reduce binding affinity (21). Thus, it shows a moderate binding affinity with the protein pocket with -4.6 kcal/mol binding energy.

Methyl Stearate fits into a hydrophobic pocket of the protein, mainly stabilised by hydrophobic interactions and van der Waals. The absence of strong hydrogen bonds or ionic interactions reduces its binding affinity compared to other ligands like Phenyl methyl ester or Hexadecanoic acid (22). Its moderate energy (-4.4 kcal/mol) suggests it might have supportive or weak inhibitory activity rather than being a potent binder.

Decane interacts mainly through hydrophobic contacts via alkyl and Pi-alkyl Interactions with residues such as LEU- 118, VAL-81 and ALA-296 between the receptor and ligand, stabilises the interaction of the molecules within the hydrophobic binding pockets. Steric clashes reduce binding efficiency compared to other molecules and also absence of polar or charged interactions leads to weaker binding affinity (23). The longer chain length of Undecane increases hydrophobic contact area via alkyl and pi-alkyl Interactions between the ligand and residues like LEU -118, VAL- 81, ALA -296, PHE -115, TYR -142. Several steric clashes with GLU -296, GLU -269, indicating the chain may extend beyond the optimal binding pocket region. Similar binding energy (-4.3 Kcal/mol) to decane, but with slightly more hydrophobic interactions due to chain length. Thus, 2D interaction suggests decane and undecane show weak binding and are less promising as inhibitors individually but may contribute to synergistic hydrophobic stabilisation in a mixture.

2D interaction of 9-Octadecenoic acid shows hydrophobic interactions through alkyl and Pi-alkyl with residues like LEU, VAL, ALA, stabilises the interaction. No strong hydrogen bonds or electrostatic interactions. 9-octadecenoic acid interacts weakly with the receptor protein PCSK9 having a binding energy (-4.0 Kcal/mol), similar to decane and undecane and is unlikely to have significant inhibitory effect individually.

3-0-Methyl-d-glucose with (-4.2 kcal/mol) binding energy, slightly stronger than 9-Octadecenoic acid (-4.0) but still weak compared to phenyl methyl ester (-6.0). It forms hydrogen bonds between the glucose hydroxyl groups of the ligand with polar residues, ARG, HIS, GLU of the receptor, stabilises the interaction. Van der Waals interactions, though weak between the hydrophobic residues like ALA, LEU, VAL. Also stabilises the interaction. Presence of polar hydroxyls allows better hydrogen bonding in 3-0-methyl-d-glucose than pure hydrocarbons like decane and undecane. This suggest that 3-0-methyl-d-glucose act

as a secondary stabiliser rather than primary inhibitor.

1,4-Dibutyl benzene-1,4-dicarboxylate having binding energy, -4.5 kcal/mol, slightly better than Methyl stearate (-4.4) and 3-O-Methyl-D-glucose (-4.2), but weaker than hexadecanoic acid (-5.1) and phenyl methyl ester, suggest weak-to-moderate binding range. Hydrophobic interactions via Alkyl and Pi-alkyl contacts from the butyl chains with hydrophobic residues LEU, VAL, ALA and Pi-Pi or Pi-cation interactions between the benzene ring of the ligand with aromatic residues PHE and TYR stabilises the interaction. It shows better hydrophobic stabilisation than straight -chain hydrocarbons due to aromatic core + ester groups.

Benzene propanoic acid forms hydrogen bonds between the ligand and the residues ARG-60 and GLU-145. 2-dimensional interaction suggests that it also forms hydrophobic interactions via alkyl and Pi-alkyl with PRO-75, LEU-128, LEU-131, LEU-135 and PHE-134, stabilises the ligand in the active pocket of the receptor. This suggests that the ligand having -3.4 Kcal/mol with moderate binding energy, binds in the receptor pocket, but need more optimisation for stronger interaction.

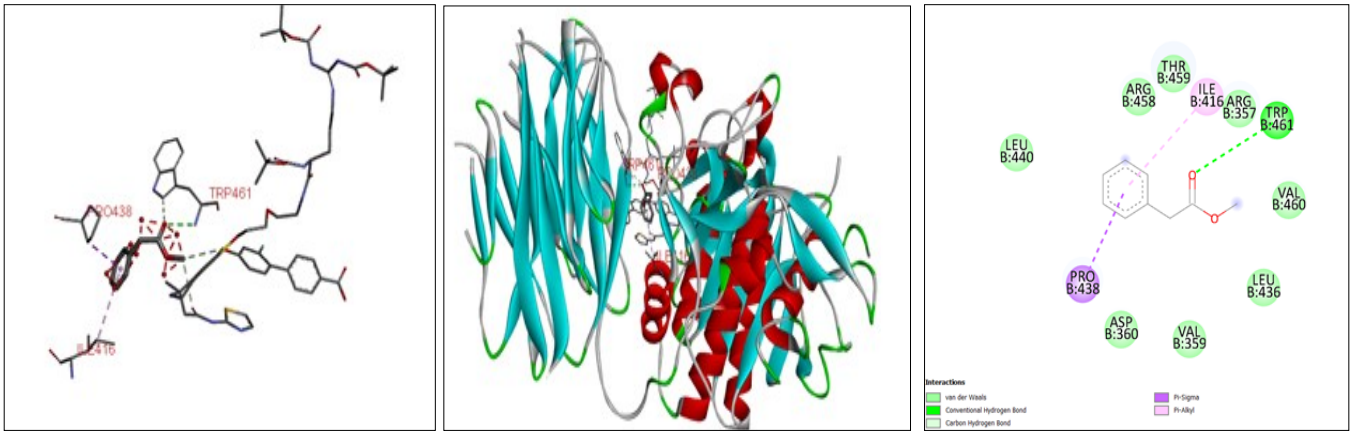
Binding strength of Oleic acid (-3.4 Kcal/mol) is moderate. Due to hydrophobic interaction via alkyl and Pi-alkyl between the ligand and the residues like ASN-61, ARG-60 and ASN-132, make Oleic acid fits into the hydrophobic groove of the protein, PCSK9. Long aliphatic chain of the Oleic acid interacts predominantly through Van der Waals and hydrophobic interactions, which is typical for fatty acids.

Cross docking analysis of the positive control, small-molecule PCSK9 inhibitor with PCSK9

Small-molecule PCSK9 inhibitor having binding energy (-4.9 Kcal/mol) similar with 13-docosenamide reflects some favourable polar interactions, via hydrogen bonds with the residues GLU- 481and ARG- 476. Unfavourable multiple interactions weakened the total binding energy. As a control it performs well, but not optimally in this small molecule interaction. Phenyl methyl ester with stronger binding energy by 1.1 Kcal/mol, implying better fit in the receptor cavity than small-molecule PCSK9 inhibitor, make it a potential lead compound for PCSK9 inhibition (24, 25).

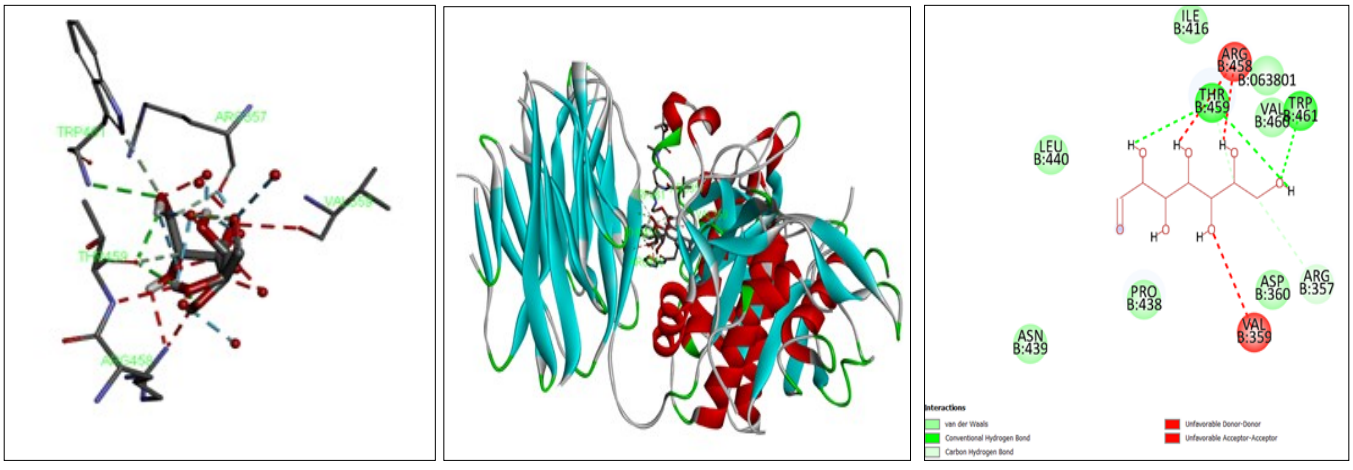
Among the compounds tested, phenyl methyl ester showed the most favourable binding energy (-6.0 kcal/mol), primarily due to aromatic stacking and multiple hydrophobic interactions that enhance stability within the PCSK9 pocket. D-Glycero-d-ido-heptose demonstrated moderate binding (-5.5 kcal/mol) through polar hydrogen bonding, indicating potential for interacting with PCSK9's hydrophilic regions. Hexadecanoic acid exhibited moderate hydrophobic binding (-5.1 kcal/mol), stabilised mainly by van der Waals interactions. Meanwhile, 13-Docosenamide had the lowest affinity (-4.9 kcal/mol) due to steric hindrance (unfavourable bumps) and limited hydrogen bonding, suggesting a less optimal fit in the binding site (Fig. 4–18).

These findings suggest that phenyl methyl ester is the most promising compound for PCSK9 inhibition among the studied



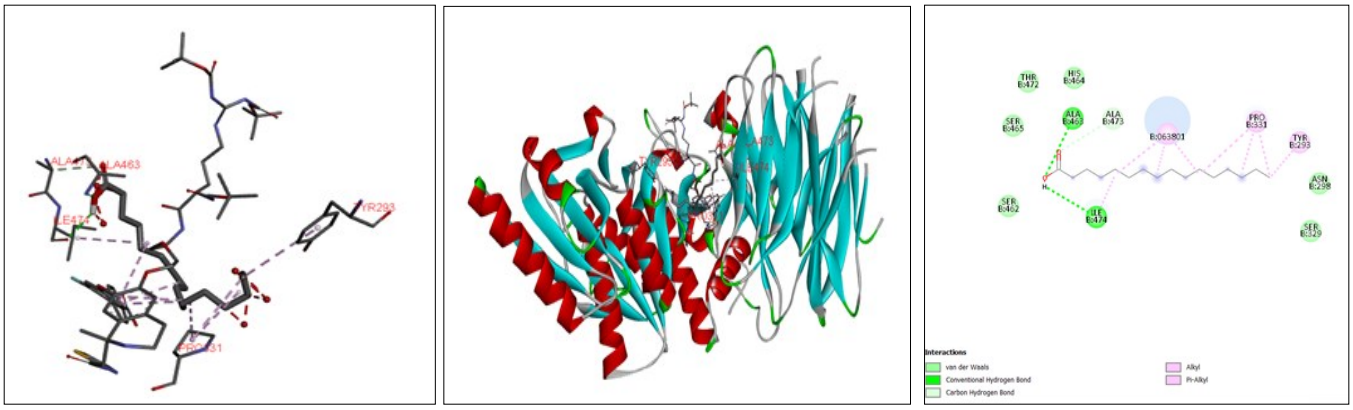
A B C

Fig. 4 A–C. Ligand binding site, 2D & 3D molecular docking visualisation of phenyl methyl ester.



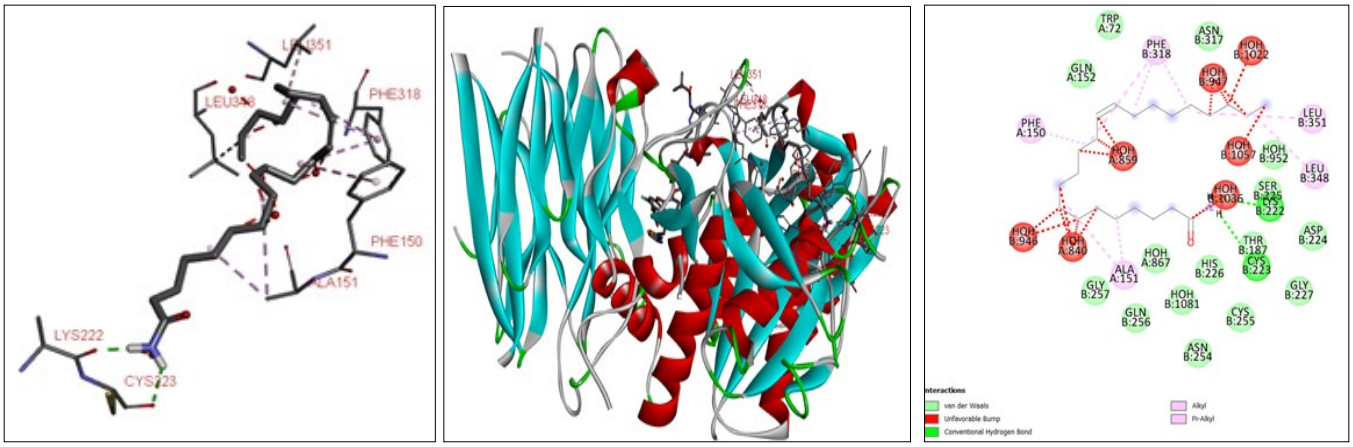
A B C

Fig. 5 A–C. Ligand binding site, 2D & 3D molecular docking visualisation of D-Glycero-d-ido-heptose.



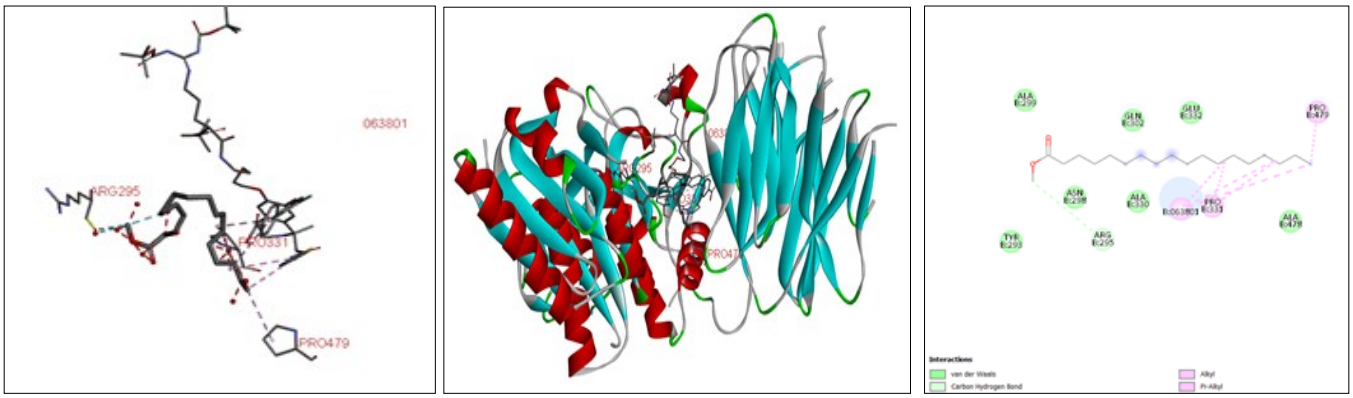
A B C

Fig. 6 A–C. Ligand binding site, 2D & 3D molecular docking visualisation of hexadecanoic acid.



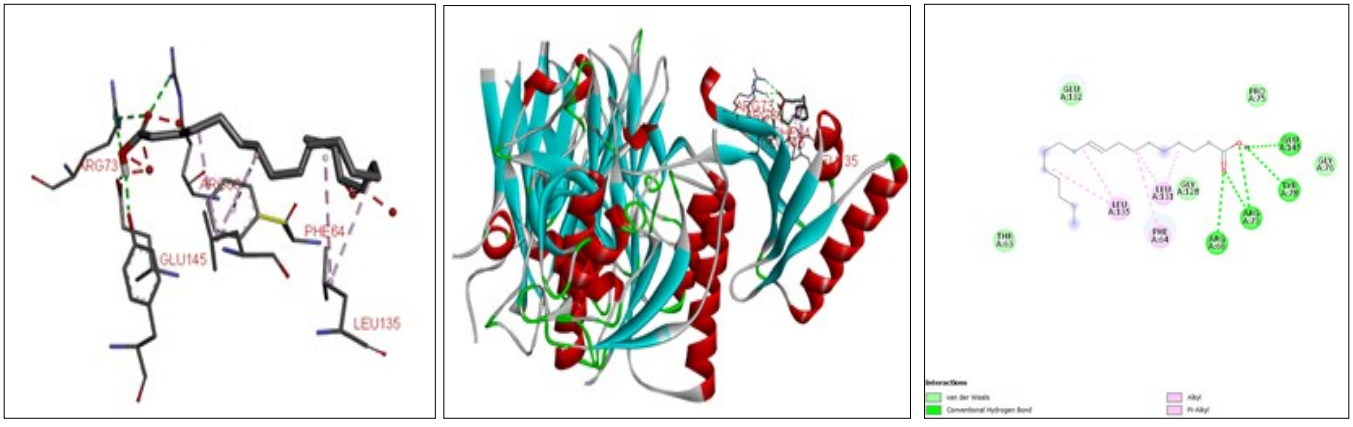
A B C

Fig. 7 A–C. Ligand binding site, 2D & 3D molecular docking visualisation of 13-docosenamide.



A B C

Fig. 8 A–C. Ligand binding site, 2D & 3D molecular docking visualisation of methyl stearate.



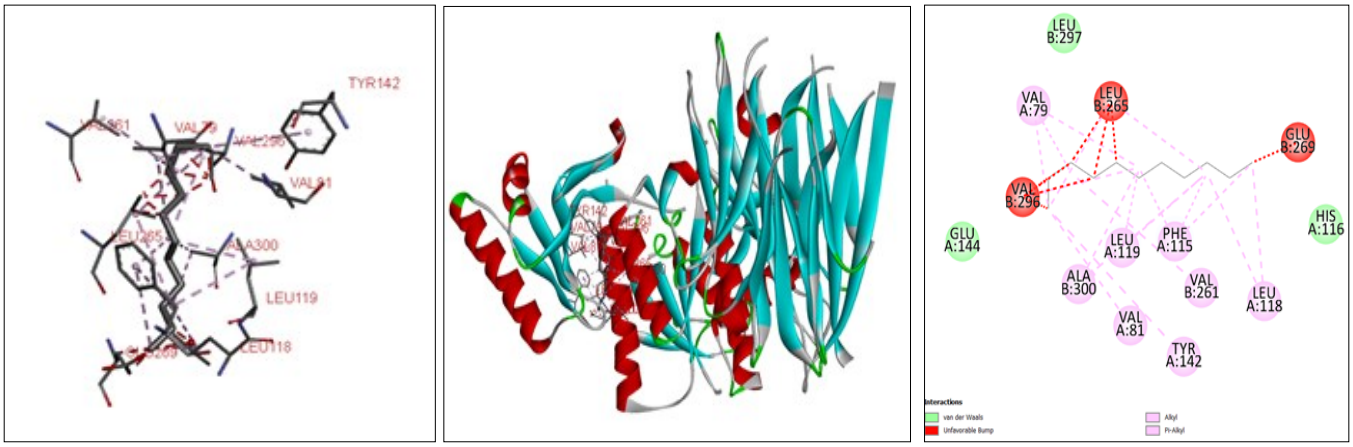
A B C

Fig. 9 A–C. Ligand binding site, 2D & 3D Molecular docking visualisation of 9-octadecenoic acid.



A B C

Fig. 10 A–C. Ligand binding site, 2D & 3D molecular docking visualisation of decane.



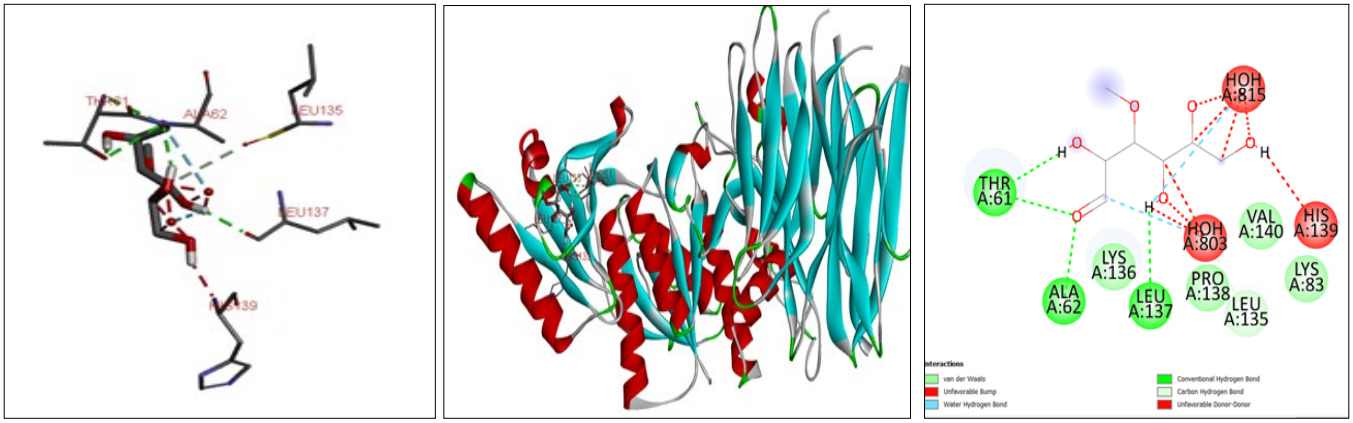
A B C

Fig. 11 A–C. Ligand binding site, 2D & 3D molecular docking visualisation of undecane.



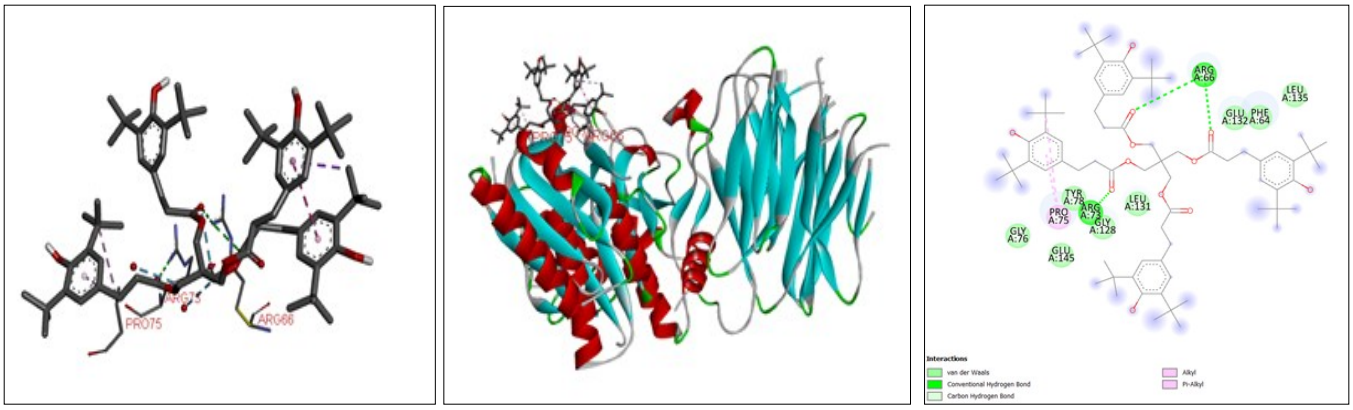
A B C

Fig. 12 A–C. Ligand binding site, 2D & 3D molecular docking visualisation of naphthalene.



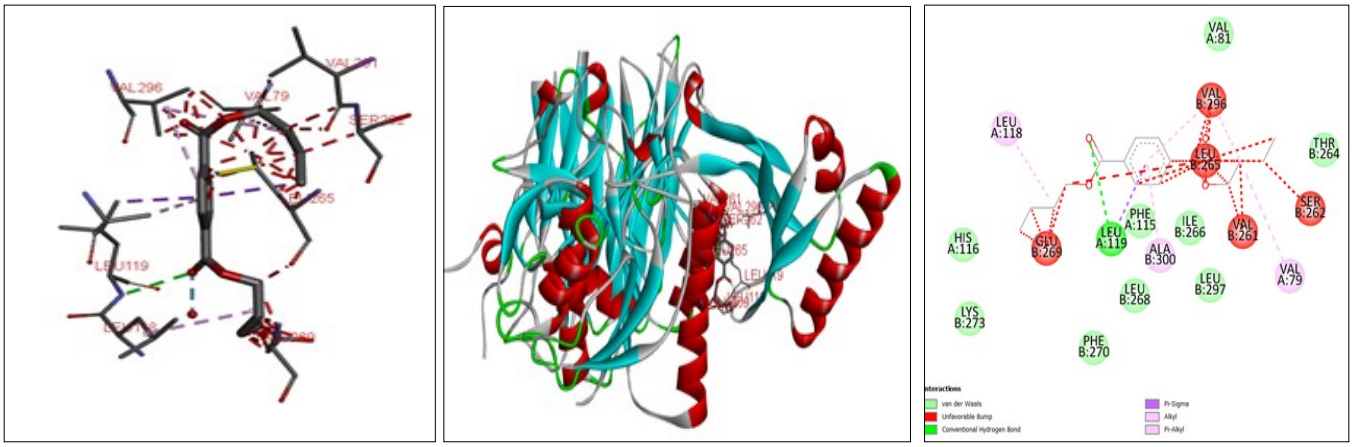
A B C

Fig. 13 A–C. Ligand binding site, 2D & 3D molecular docking visualisation of 3-0-methyl-d-glucose.



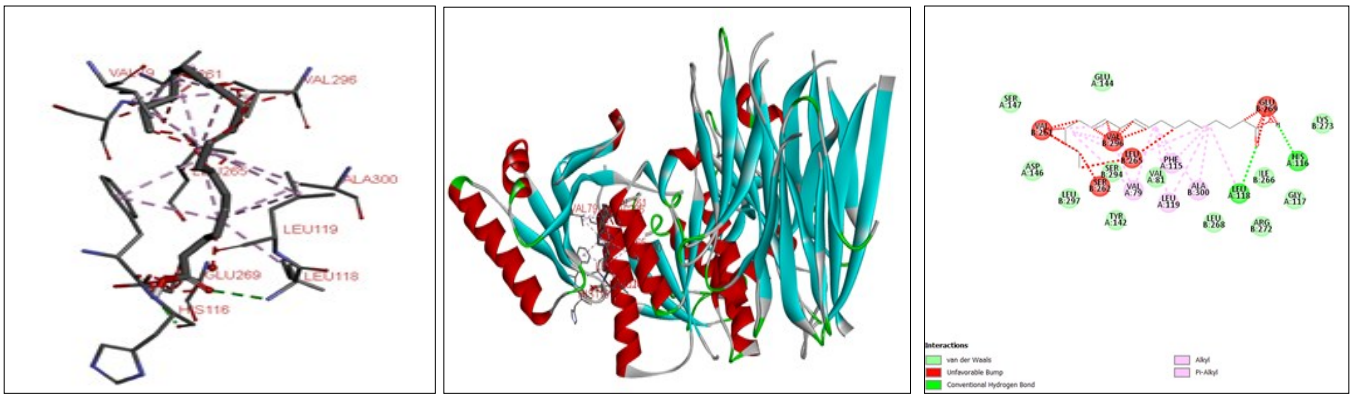
A B C

Fig. 14 A–C. Ligand binding site, 2D & 3D molecular docking visualisation of benzene propanoic acid.



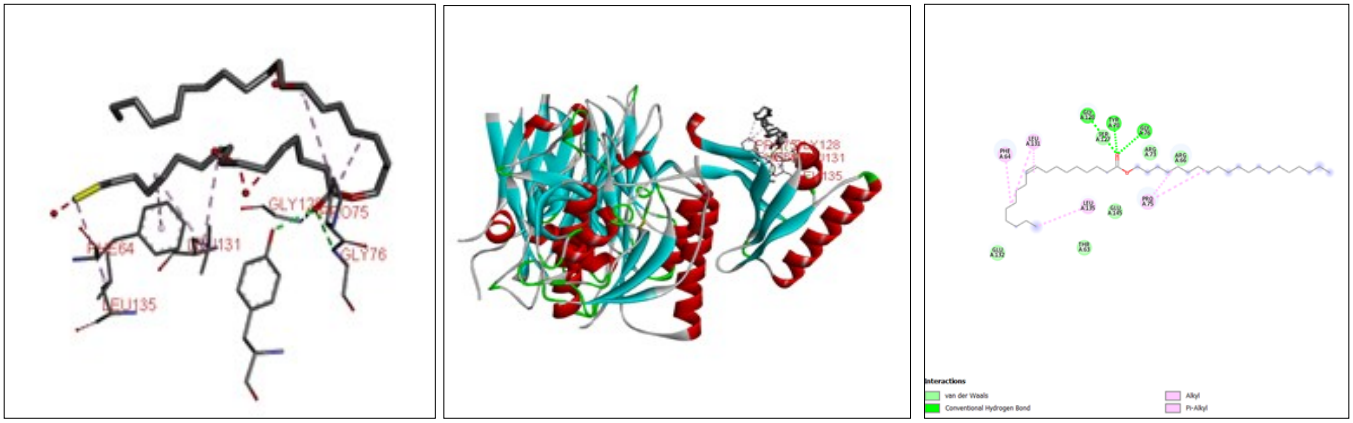
A B C

Fig. 15 A–C. Ligand binding site, 2D & 3D molecular docking visualisation of 1,4-Dibutyl benzene-1,4-dicarboxylate.



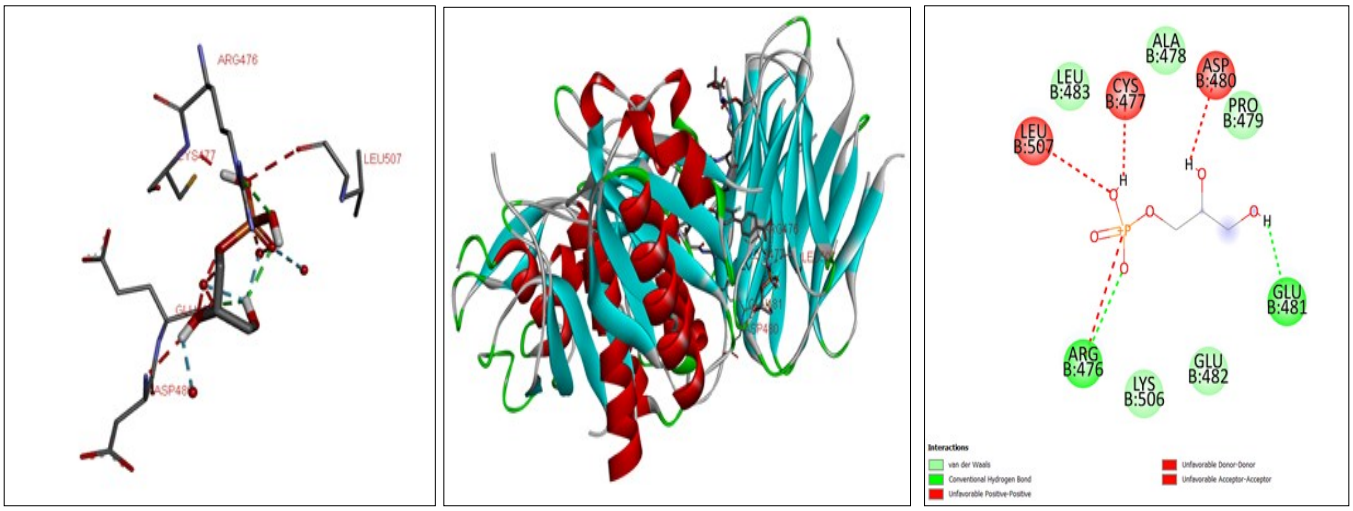
A B C

Fig. 16 A–C. Ligand binding site, 2D & 3D molecular docking visualisation of 9,12 –octa decadienoic acid.



A B C

Fig. 17 A–C. Ligand binding site, 2D & 3D molecular docking visualisation of oleic acid, eicosyl ester.



A B C

Fig. 18 A–C. Ligand binding site, 2D & 3D molecular docking visualisation of the positive control small-molecule PCSK9 inhibitor.

molecules, while others may play supportive roles or contribute synergistically when present in natural extracts.

The docking results highlight that phenyl methyl ester/ acetic acid and d-Glycero-d-ido-heptose are promising natural inhibitors of PCSK9, potentially lowering cholesterol by modulating LDL receptor recycling. The combined presence of multiple moderate-binding compounds suggests a polypharmacological effect of *H. vahliana* extract. Further, *in vivo* and *in vitro* studies are needed to carry out these compounds close to clinical trials.

ADMET analysis

ADMET analysis of phenyl methyl ester, suggested that it has good membrane permeability due to its low topological polar surface area (TPSA: 28 Å^2) which is below 140 Å^{2}threshold. It has 2 rotatable bonds, which indicate low molecular flexibility, which make it favourable for absorption. Water soluble property of the compound makes it favourable for oral uptake with a consistent bio availability score -0.55. D-glycero-D-ido-heptose is intrinsically highly polar and due to the higher number of hydrogen-bond donors (6HBD), it violates the Lipinski's rule of 5. Its high TPSA (140-170 Å^2) and multiple H-bonding functionalities predict poor passive intestinal permeability and thus poor oral bioavailability without chemical modifications.

Conclusion

Humboldtia vahliana Wight is a promising natural source of PCSK9 inhibitors with potential hypocholesterolemic effects. Bioactive constituents identified by GC-MS/MS profiling of the methanolic bark extract noticed the presence of compounds like phenyl methyl ester and D-glycero-D-ido-heptose, that demonstrated favourable binding affinities toward PCSK9 in molecular docking studies. The superior binding energy of phenyl methyl ester compared with small-molecule PCSK9 inhibitor underscores its potential as a lead molecule for further development. This study presents a preliminary *in silico* evaluation of selected natural and small-molecule compounds against the target protein, providing initial insight into their possible binding modes and interaction patterns. While the docking analysis suggests feasible and reproducible interactions within the active site, the predicted binding affinities are modest and should be interpreted cautiously. The results serve as a basis for future experimental studies, including biochemical assays and cellular models, to validate biological activity and assess the potential of these compounds as lead structures for further optimisation. The potential polypharmacological or synergistic effects suggested by the docking analysis should be interpreted with caution. While the observed interactions indicate that phenyl methyl ester may engage multiple binding sites or targets *in silico*, these findings are predictive in nature and do not constitute experimental evidence of synergy. Further *in vitro* and *in vivo* studies are required to validate whether such multi-target interactions translate into biologically meaningful synergistic effects Docking scores primarily provide qualitative insight into binding propensity rather than definitive affinity measurements. Weak to moderate interactions may still be biologically relevant for compounds that act through transient or modulatory mechanisms rather than strong competitive inhibition. Therefore, phenyl methyl ester should be considered a preliminary lead or scaffold for further optimisation rather than a high-affinity inhibitor. Phenyl methyl ester satisfies all 4 criteria of Lipinski's rule of 5 with no violation. Its low molecular weight, moderate lipophilicity, limited hydrogen bond capacity and low polar surface area indicate favourable oral drug likeness and absorption potential. Thus, Phenyl methyl ester is suitable candidate for further pharmacokinetics and biological evaluation. These findings provide a scientific basis for advancing *H. vahliana* derived compounds into preclinical studies aimed at developing novel, plant-based cholesterol-lowering agents.

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Authors' contributions

MM, VV and AK performed the study. AK wrote the manuscript. VV did the bioinformatics studies, MM conceived the study and participated in its design and coordination. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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