Research Article

Novel Drug Development for Anti-Cholesterol Activity by *in silico* Analysis: Identification Cholesterol Inhibiting Compounds

Ananya M Kura[1], Karukuvelraja R[2], Thulasi G Pillai[3,*]

[1]Department of Information Technology, K. J. Somaiya Institute of Technology, Mumbai, Maharashtra, INDIA.
[2]Genolites Research and Development Laboratory, Coimbatore, Tamil Nadu, INDIA.
[3]Basic Science and Humanities Department, K. J. Somaiya Institute of Technology, Mumbai, Maharashtra, INDIA.

Submission Date: 14-02-2024; Revision Date: 15-03-2024; Accepted Date: 19-04-2024.

Correspondence:

Thulasi G Pillai
Basic Science and Humanities Department, K. J. Somaiya Institute of Technology, Mumbai, Maharashtra, INDIA.

Email: thulasi@somaiya.edu, akura403@gmail.com

ABSTRACT

Aim: Cholesterol is required for cellular metabolism and steroid hormone production. However, the excessive level leads to the condition known as hypercholesterolemia, which causes cardiovascular illnesses. A number of drugs can lower blood cholesterol levels, but these medications have a variety of negative effects. The microalgae possess wide range of bioactive compounds, which would be used to treat the abnormal cholesterol levels. This study aims to determine the anti-cholesterol activity of bioactive compounds of the selected microalgal species (*Chlorella, Anabaena*, *Oscillatoria*, and *Lyngbya*) via in silico study. **Materials and Methods:** Druglikeness analysis were carried out for the 226 compounds, based upon the results 81 compounds were selected for molecular docking. **Results:** The ADMET analysis and PASS prediction was performed for the 5 best docked compounds such as Debromoaplysiatoxin, Spiroidesin, Oscillatoxin A, Ergosta-8,(9)14-dien-3beta-ol, and Cyclindrospermopsin. **Conclusion:** Hence, these compounds can be used to develop the novel cholesterol lowering drugs.

Keywords: Cholesterol, Molecular docking, *Chlorella, Anabaena*, *Oscillatoria, Lyngbya*.

INTRODUCTION

Cardiovascular Disease (CVD) is considered to be one of the main cause of death worldwide. Atherosclerosis arises in the medium to large arteries at branching points where blood flow is disrupted. It is a chronic inflammatory disease that does not resolve. Atherosclerosis starts with disruption to the vascular Endothelial Cells (ECs), which usually maintains the blood flow and safeguard the coronary arteries from inflammation and blood clots.[1] High LDL (low-density lipoprotein) levels have been linked to the formation of plaques at the coronary artery followed by CVDs, according to clinical, genetic, and epidemiological research.[2] Hypercholesterolemia is a key risk factor for cardiovascular disease that enhances the occurrence of atherosclerotic diseases. An increase in plasma concentration is the main characteristic that links cholesterol to atherosclerosis. Apart from elevated levels of plasma cholesterol, additional risk factors like diabetes, hypertension, and smoking can also exacerbate endothelial permeability, inflammation, oxidation, and coagulation, which can lead to the development of atherosclerosis.[3]

Cholesterol is essential for cellular metabolism and the synthesis of steroid hormones in the plasma as well as tissues of the body. Excessive levels of cholesterol in the blood can lead to coronary artery disease, elevated blood pressure, and peripheral strokes.[4] Hypercholesterolemia is an ailment in which the levels of cholesterol in the blood exceed the normal range. It results from an elevated level of LDL cholesterol and a decrease in HDL cholesterol.[5] Cholesterol is a vital biological amphipathic compound with numerous

SCAN QR CODE TO VIEW ONLINE

www.ajbls.com

DOI: 10.5530/ajbls.2024.13.7

roles in the body. It is an essential component of all cell membranes, regulating their fluidity and in specialised tissues, cholesterol is a precursor of steroids, bile acids, Vitamin D, and hormones.[6] The human body needs a specific amount of cholesterol to preserve the structural integrity as well as the fluidity of the membranes. Cholesterol also serves as a precursor for numerous biological processes.[7,8]

Since the prevalence of hypercholesterolemia rises with age, accounting for about 7% of patients over the age of 20 and 44% of patients over 60, it is not unanticipated that the illness is thought to be the cause of 2.6 million deaths annually.[9] Global Burden of Disease Research stated that India has an estimated age-standardized mortality rate from cardiovascular disease of 272 per 100,000, greater than the overall death rate from the same condition worldwide of 235 per 100,000 individuals.[10] Cardiovascular diseases are also one of the leading cause for death as like cancer,[11] MDR infections,[12-14] and respiratory diseases.[15]

Cardiovascular events are primarily associated with excessive amounts of Low-Density Lipoprotein (LDL) and extremely high amounts of Triglycerides (TG). However, these conditions are also known to the risk factors for cardiovascular diseases. Agents that can raise HDL cholesterol, reduce TG, LDL/VLDL or overall cholesterol levels are helpful in reducing cardiovascular diseases.[16] Blood cholesterol levels can be lowered with a variety of medications. A common class of medications used to decrease cholesterol are statins, such as atorvastatin, lovastatin, and simvastatin. Statins function by blocking the enzyme HMG-CoA reductase in the liver, which is involved in cholesterol synthesis.[17] However, such medications are expensive, and unfavourable side effects have been recorded, including muscle soreness, liver inflammation, type II diabetes, and neurological adverse effects. Hence, the allopathic hypolipidemic medications have advanced significantly and are now widely available. However, they invariably include adverse effects and contraindications.[18] This has logically encouraged patients to look for safer alternatives.[19]

Many investigations have been carried out in the last few years to find anti-hyperlipidemic medicines in both natural and synthetic sources.[20] Now-a-days, the microalgae are gaining more attention among the researchers due to its various biological activity and biotechnological benefits. Likewise, the numerous researches have documented that the water-soluble components of microalgae or extracted algal polysaccharides exhibited hypocholesterolemic benefits in experimental animals. The diets supplied with either the total algal biomass or its lipid extract resulted in significant reduction in cholesterol levels in the liver and plasma.[21] Microalgae and Cyanobacteria exhibit enormous biodiversity and are a literally unexplored resource. Microalgae and Cyanobacteria have been recognized as a promising and highly profitable sources of value-added products in the food and aquaculture sectors.[22]

Chlorella is a unicellular green alga rich in nutrients such as minerals, vitamins, carotenoids, chlorophyll, and long-chain polyunsaturated fatty acids. The *Chlorella* is helpful in lowering the elevated cholesterol levels of hypercholesterolemic individuals. The *Chlorella* has been found in animal studies to prevent hypercholesterolemia by inhibiting the intestinal absorption of elevated cholesterol from the diet and increasing faecal steroid excretion.[23,24]

Cyanobacteria also known as blue-green algae, are Gram-negative photoautotrophic bacteria with a blue-green pigment.[25] *Anabaena*, *Lyngbya*, *Oscillatoria*, Phormidium, *Nostoc*, *Microcystis* and *Spirulina* are among the most promising species for the production of a broad varieties of bioactive chemicals such as fatty acids, polysaccharides, carotenoids, and lipopeptides.[26] Oral administration of *Oscillatoria* sp. resulted in a notable reduction in blood cholesterol, TG, LDL,VLDL levels along with the rise in HDL levels in hyperlipidemic rats that was closer to that of standard medication.[27]

In this study, we have investigated the *in silico* anti-cholesterol activity of the bioactive compounds of selected microalgal species such as *Chlorella*, *Oscillatoria*, *Anabaena*, and *Lyngbya*

MATERIALS AND METHODS

Retrieval and preparation of ligands

About 226 compounds produced by microalgal species were identified. The 3D structure of each ligands were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/). In order to create 3D atomic coordinates for the molecules, the ligands are further prepared by identifying the torsion root, adjusting the torsion angles, allocating charges, optimization using the Universal Force Field (UFF), and ultimately converting them to pdbqt format.

Screening of the ligands for drug likeness

SwissADME, an online server, is used to assess the compounds' druglikeness (http://swissadme.ch/index.php). The druglikeness of a test compound is a required characteristic for validating it as a possible ligand against the targets.[28] Using Lipinski's Rule of Five, 226

bioactive compounds were screened, and those that demonstrated drug-likeness were employed in docking studies.

Target proteins preparation

The Human HMG-COA reductase (1DQ8) was taken as the target in this study. The crystallographic 3D structure of the protein was retrieved from the Protein data bank (http://www.rcsb.org/). The protein was viewed in Biovia studio, and the water molecules, ligands, and co-crystal ligand complexes that were attached to the target protein were eliminated. Chain D was likewise deleted from the target protein. Additionally, the protein was prepared by adding charges, minimizing energy, and converting it to pdbqt format using Auto Dock Tools.

Determination of functional sites of targets

An accurate evaluation of the active site is essential for docking analysis in order to set the grid box. The online CASTp service (Computated Atlas for Surface Topography) was utilized to identify the amino acid residues linked to the active pocket site development of the target compounds. The binding site is the biggest pocket size of the protein.[29,30]

Molecular docking and protein-ligand interaction analysis

81 compounds were chosen based on their druglikeness, and the PyRx tool by Autodock wizard was used as the docking engine for the molecular docking study. The protein was anticipated to be hard during the docking phase, while the ligands were thought to be flexible. The binding amino acid analysis from CASTp served as the basis for the grid parameter setup file. The ligand with the highest binding affinity was identified as the one with the highest binding energy, or most negative. Using Biovia Drug Discovery Studio, 2020, a visual analysis of the docking site and protein-ligand interactions was carried out.[31]

ADMET analysis of the selected ligands

ADMET analysis uses web-based algorithms to evaluate the substances' levels of toxicity, distribution, metabolism, absorption, and excretion. Numerous offline software programmes and internet databases are available to assist in forecasting the behaviour of potential medication candidates. For ADMET predictions in this work, we employed admet SAR9.[32] Potential phytochemicals exhibiting higher binding energies were assessed for their ability to penetrate the brain barrier *in vivo*, absorb nutrients through the human gut, permeability of caco-2 cells *in vitro*, CYP4502C9 substrate, and various toxicity parameters such as rat carcinogenicity and AMES test mutagenicity.

Prediction of Activity Spectra for Substances (PASS)

The prediction of Activity Spectra for Substances (PASS) algorithm was utilised to evaluate the anti-cholesterol action of the identified compounds.[33] An extensive variety of physiological outcomes are predicted for a multitude of medications using the PASS algorithm.

RESULTS

Screening of the ligands for drug likeness

The various bioactive compounds present in the selected microalgal species (*Chlorella*, *Anabaena*, *Lyngbya*, and *Oscillatoria*) were screened for druglikeness. 226 bioactive compounds were evaluated for druglikeness depends on the Lipinski Rule of five (RO5). The molecular weight, MlogP, hydrogen bond donor, and hydrogen bond acceptor were examined. Based upon the result of druglikeness, 81 compounds were satisfied the Lipinski Rule of five (RO5). The compounds with druglikeness property were given in the Table 1.

Table 1: Compounds showing drug likeness property

			Druglikeness analysis				
Sl. No	Compound	Source organism	Molecular weight (g/mol)	H-bond donor	H-bond acceptor	MLogP	Rule of five
1	12,13-trans-Epoxy-9-oxo-10E,15Z-octadecadienoic acid	*Chlorella pyrenoidosa*	308.41 g/mol	1	4	2.26	Yes; 0 violation
2	13-Hydroperoxy-octadecadienoic acid	*Chlorella pyrenoidosa*	312.44 g/mol	2	4	3.55	Yes; 0 violation
3	13-Hydroxyocta-decadienoic acid	*Chlorella pyrenoidosa*	296.44 g/mol	2	3	3.59	Yes; 0 violation
4	Ergosta-8,(9)14-dien-3beta-ol	*Chlorella ellipsoidea*	398.66 g/mol	1	1	6.43	Yes; 1 violation: LOGP>4.15

5	Jasmonic acid	*Chlorella sp.*	210.27 g/mol	1	3	1.68	Yes; 0 violation
6	Linolenic acid	*Chlorella vulgaris*	278.43 g/mol	1	2	4.38	Yes; 1 violation: LOGP>4.15
7	Methyl jasmonate	*Chlorella sp.*	224.30 g/mol	0	3	1.95	Yes; 0 violation
8	Poriferasterol	*Chlorella spp.*	412.69 g/mol	1	1	6.62	Yes; 1 violation: MLOGP>4.15
9	Anatoxin alpha	*Oscillatoria sp. PCC9240*	165.23 g/mol	1	2	1.27	Yes; 0 violation
10	Lipopurealin A	*Oscillatoria agardhii*	728.56 g/mol	5	6	3.09	Yes; 1 violation: MW>500
11	Homoanatoxin-a	*Oscillatoria sp. PCC6407*	179.26 g/mol	1	2	1.56	Yes; 0 violation
12	Glucose	*Anabaena sp.*	180.16 g/mol	5	6	-2.75	Yes; 0 violation
13	Linolenic acid	*Anabaena flos-aquae*	278.43 g/mol	1	2	4.38	Yes; 1 violation: LOGP>4.15
14	Norharman	*Anabaena cylindrica Lemmermann*	168.19 g/mol	1	1	1.62	Yes; 0 violation
15	Circinamide	*Anabaena circinalis*	386.49 g/mol	4	7	-0.85	Yes; 0 violation
16	Arabinose	*Anabaena sp.*	150.13 g/mol	4	5	-2.48	Yes; 0 violation
17	Spiroidesin	*Anabaena spiroides*	601.73 g/mol	5	6	2.89	Yes; 1 violation: MW>500
18	Cyclindrospermopsin	*Anabaena bergii*	416.43 g/mol	4	8	-0.3	Yes; 1 violation: NorO>10
19	Guanidoacetic acid	*Anabaena bergii*	117.11 g/mol	3	3	-1.42	Yes; 0 violation
20	9S,12R,13S-Trihydroxy-10E,15Z-octadecadienoic acid	*Lyngbya majuscula*	328.44 g/mol	4	5	1.91	Yes; 0 violation
21	13S-Hydroperoxy-9Z,11E,15Z-octadecatrienoic acid	*Lyngbya majuscula*	310.43 g/mol	2	4		; violation
22	(+)-Curacin A	*Lyngbya majuscula*	373.60 g/mol	0	2	4.21	Yes; 1 violation: MLOGP>4.15
23	(E)-7-Methoxytetradec-4-enoic acid	*Lyngbya majuscula*	256.38 g/mol	1	3	2.96	Yes; 0 violation
24	Agelastatin C	*Lyngbya wollei*	357.16 g/mol	4	4	-0.38	Yes; 0 violation
25	Agelastatin D	*Lyngbya wollei*	327.13 g/mol	4	3	-0.27	Yes; 0 violation
26	Apramide A	*Lyngbya majuscula*	337.46 g/mol	1	3	1.11	Yes; 0 violation
27	Apramide B	*Lyngbya majuscula*	323.43 g/mol	1	3	0.87	Yes; 0 violation
28	Apramide D	*Lyngbya majuscula*	322.42 g/mol	3	1	0.87	Yes; 0 violation
29	Apramide E	*Lyngbya majuscula*	323.43 g/mol	3	1	0.87	Yes; 0 violation
30	Barbamide	*Lyngbya majuscula*	461.83 g/mol	0	3	2.68	Yes; 0 violation
31	Curacin D	*Lyngbya majuscula*	359.57 g/mol	0	2	3.99	Yes; 0 violation
32	Dechloro-barbamide	*Lyngbya majuscula*	427.39 g/mol	0	3	2.47	Yes; 0 violation
33	Dysidenamide	*Lyngbya majuscula*	506.08 g/mol	2	3	2.19	Yes; 1 violation: MW>500
34	Louludinium	*Lyngbya gracilis*	272.45 g/mol	0	0	4.34	Yes; 1 violation: LOGP>4.15
35	Madangolide	*Lyngbya bouillonii*	377.56 g/mol	3	1	3.78	Yes; 0 violation
36	Pseudodysidenin	*Lyngbya majuscula*	546.17 g/mol	1	3	2.5	Yes; 1 violation: MW>500
37	Yanucamide A	*Lyngbya majuscula*	587.75 g/mol	2	7	2.09	Yes; 1 violation: MW>500
38	Yanucamide B	*Lyngbya majuscula*	601.77 g/mol	2	7	2.27	Yes; 1 violation: MW>500
39	Ypaoamide	*Lyngbya majuscula*	456.57 g/mol	2	5	2.31	Yes; 0 violation
40	Besarhanamide A	*Lyngbya majuscula*	381.59 g/mol	2	3	3.15	Yes; 0 violation
41	Besarhanamide B	*Lyngbya majuscula*	369.54 g/mol	1	4	3	Yes; 0 violation
42	Nordysidenin	*Lyngbya majuscula*	532.14 g/mol	2	3	2.29	Yes; 1 violation: MW>500
43	Antillatoxin	*Lyngbya majuscula*	503.67 g/mol	2	5	2.14	Yes; 1 violation: MW>500
44	Dolabellin	Lyngbya majuscula	611.56 g/mol	2	10	1.05	Yes; 1 violation: MW>500

45	Lyngbyabellin C	*Lyngbya* sp.	609.54 g/mol	2	10	1.05	Yes; 1 violation: MW>500
46	Lyngbyabellin G	*Lyngbya majuscula*	595.51 g/mol	2	10	0.85	Yes; 1 violation: MW>500
47	Malyngamide T	*Lyngbya majuscula*	468.03 g/mol	1	5	2.78	Yes; 0 violation
48	Malyngamide U	*Lyngbya majuscula*	409.56 g/mol	2	5	1.32	Yes; 0 violation
49	Malyngamide V	*Lyngbya majuscula*	409.56 g/mol	2	5	1.32	Yes; 0 violation
50	Malyngamide W	*Lyngbya majuscula*	409.56 g/mol	2	5	1.32	Yes; 0 violation
51	Palau'imide	*Lyngbya* sp.	428.56 g/mol	1	4	2.31	Yes; 0 violation
52	Semiplenamide A	*Lyngbya semiplena*	365.59 g/mol	2	2	4.3	Yes; 1 violation: MLOGP>4.15
53	Semiplenamide B	*Lyngbya semiplena*	407.63 g/mol	1	3	4.59	Yes; 1 violation: MLOGP>4.15
54	Semiplenamide C	*Lyngbya semiplena*	325.53 g/mol	2	2	3.74	Yes; 0 violation
55	Semiplenamide D	*Lyngbya semiplena*	423.67 g/mol	1	3	4.88	Yes; 1 violation: MLOGP>4.15
56	Semiplenamide E	*Lyngbya semiplena*	395.62 g/mol	1	3	4.47	Yes; 1 violation: MLOGP>4.15
57	Semiplenamide F	*Lyngbya semiplena*	369.58 g/mol	2	3	3.05	Yes; 0 violation
58	Taveuniamide A	*Lyngbya majuscula* x *Schizothrix* sp.	202.76 g/mol	0	0	4.76	Yes; 1 violation: MLOGP>4.15
59	Taveuniamide B	*Lyngbya majuscula* x *Schizothrix* sp.	202.76 g/mol	0	0	4.76	Yes; 1 violation: MLOGP>4.15
60	Taveuniamide C	Lyngbya majuscula x Schizothrix sp.	166.30 g/mol	0	0	5.25	Yes; 1 violation: MLOGP>4.15
61	Taveuniamide D	*Lyngbya majuscula* x *Schizothrix* sp.	44.10 g/mol	0	0	2.28	"
62	Taveuniamide E	*Lyngbya majuscula* x *Schizothrix* sp.	42.08 g/mol	0	0	2.13	Yes; 0 violation
63	Taveuniamide F	*Lyngbya majuscula* x *Schizothrix* sp.	42.08 g/mol	0	0	2.13	Yes; 0 violation
64	Taveuniamide K	*Lyngbya majuscula* x *Schizothrix* sp.	42.08 g/mol	0	0	2.13	Yes; 0 violation
65	Carmabin A	*Lyngbya majuscula*	155.24 g/mol	1	1	1.87	Yes; 0 violation
66	Dragonamide A	*Lyngbya majuscula*	452.57 g/mol	4	5	-0.1	Yes; 0 violation
67	Dragonamide B	*Lyngbya majuscula*	452.57 g/mol	4	5	-0.1	Yes; 0 violation
68	Deacetyl-hectochlorin	*Lyngbya majuscula*	623.57 g/mol	2	10	1.25	"
69	Hermitamide A	*Lyngbya majuscula*	359.55 g/mol	1	2	4.12	Yes; 0 violation
70	Hermitamide B	*Lyngbya majuscula*	398.58 g/mol	2	2	3.41	Yes; 0 violation
71	Malyngamide C	*Lyngbya majuscula*	456.02 g/mol	2	5	1.74	Yes; 0 violation
72	Malyngamide J	*Lyngbya majuscula*	607.78 g/mol	2	9	0.48	Yes; 1 violation:MW>500
73	Malyngamide K	*Lyngbya majuscula*	424.02 g/mol	1	3	3.3	Yes; 0 violation
74	Lyngbyaloside	*Lyngbya* sp.	661.62 g/mol	2	10	1.29	Yes; 1 violation: MW>500
75	Lyngbyaloside B	*Lyngbya* sp.	649.61 g/mol	3	10	1.19	Yes; 1 violation: MW>500
76	Debromoaplysiatoxin	*Lyngbya majuscula*	592.72 g/mol	3	10	1.78	Yes; 1 violation: MW>500
77	Isomalyngamide A	*Lyngbya majuscula*	553.13 g/mol	0	6	2.12	Yes; 1 violation: MW>500
78	Lyngbyatoxin A acetate	*Lyngbya majuscula*	479.65 g/mol	2	3	3.49	Yes; 0 violation
79	Oscillatoxin A	*Lyngbya majuscula*	578.69 g/mol	3	10	1.6	Yes; 1 violation: MW>500
80	Grenadamide B	*Lyngbya majuscula*	369.97 g/mol	1	2	4	Yes; 0 violation
81	Grenadamide C	*Lyngbya majuscula*	404.41 g/mol	1	2	4.21	Yes; 1 violation: LOGP>4.15

Determination of functional sites of targets

CastP was employed to analyze the functional binding sites of the target protein Human HMA-COA reductase. The amino acid molecules present in the active pocket of the protein can be found using the CASTp. The position of the amino acid present in the active site of target was provided in the Table 2. The Figure 1 demonstrated the CastP results of the chain A, B, and C of the target protein. The grid boxes were created to cover the binding site of the active protein.

Table 2: Amino acid residues in the binding sites.

Sl. No.	Target	Chain	Aminoacid residues in the binding sites
1.	HMG-COA reductase	A	471- VAL, 472-ASN, 475- HIS, 476 - ILE, 479 - TYR, 807 –GLY, 808-GLY, 809-THR, 841-GLN
		B	536- ILE, 540-VAL, 542- GLY, 552-GLN, 556-ALA, 557-THR, 559-GLU, 862- LEU, 863-VAL, 865-SER,
		C	536- ILE, 540-VAL, 542- GLY, 552-GLN, 556-ALA, 557-THR, 559-GLU, 862- LEU, 863-VAL, 865-SER,

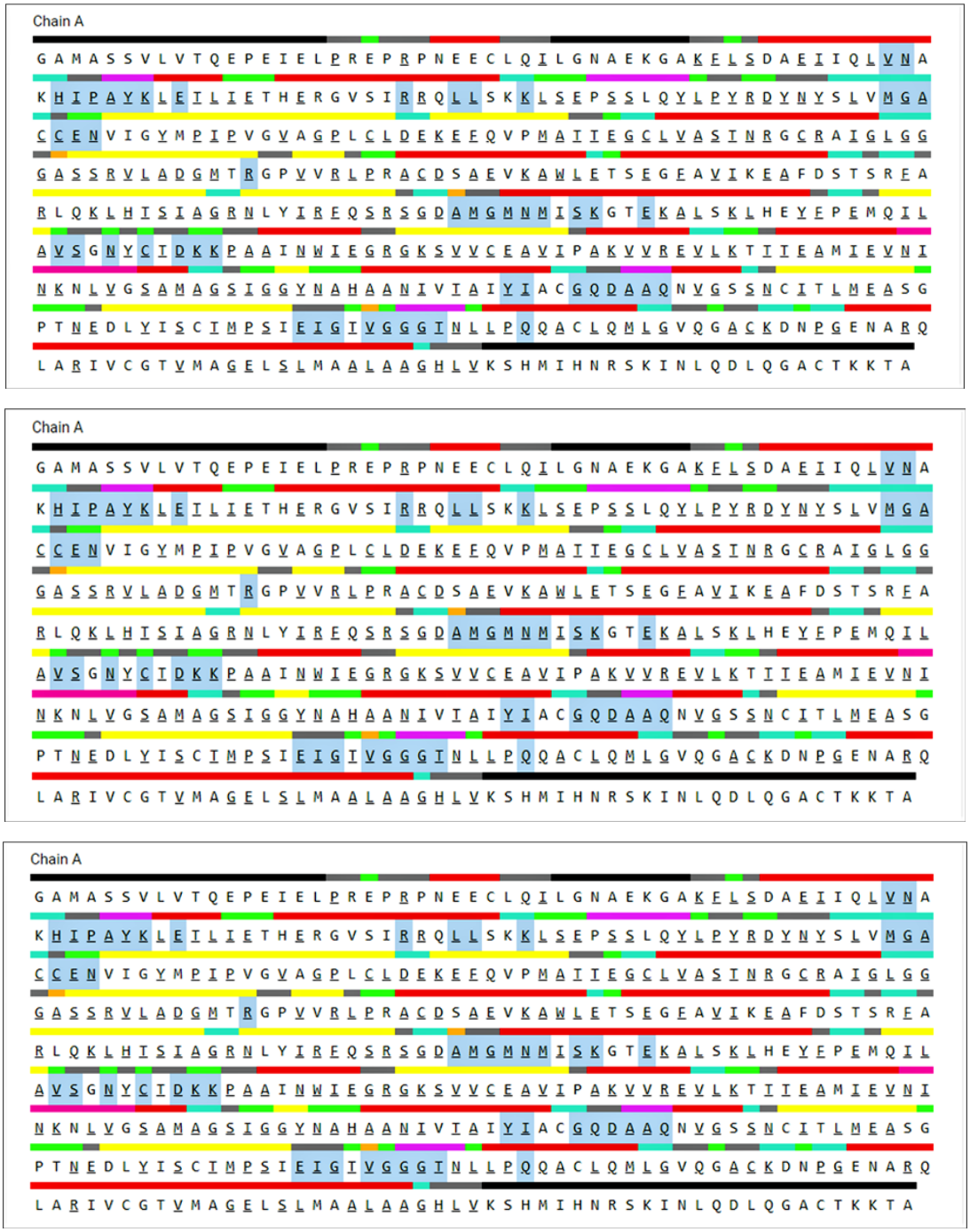

Figure 1: Binding site of the target protein. Binding sites were highlighted in blue.

Molecular docking and protein-ligand interaction analysis

PyRx was employed for docking all the 81 compounds against the target HMG-COA reductase (A, B, and C chain). The binding affinity of the all the 81 compounds were examined (Table 3) and the compounds showing binding energy lower than -7 K/mol were chosen. The aminoacid residues in the protein-ligand interaction of selected five compounds were provided in the Table 4.

Table 3: Binding affinity of the bioactive compounds

Sl. No.	Compound	Source organism	Binding affinity
1.	12,13-trans-Epoxy-9-oxo-10E,15Z-octade-cadienoic acid	Chlorella pyrenoidosa	-5.9
2.	13-Hydrope-roxyoctade-cadienoic acid	Chlorella pyrenoidosa	-5.7
3.	13-Hydrox-yoctade-cadienoic acid	Chlorella pyrenoidosa	-6.3
4.	Ergosta-8,(9) 14-dien-3beta-ol	Chlorella ellipsoidea	-9.0
5.	Jasmonic acid	Chlorella sp.	-6.3
6	Linolenic acid	Chlorella vulgaris	-4.5
7	Methyl jasmonate	Chlorella sp.	-5.8
8	Poriferasterol	Chlorella spp.	-5.2
9	Anatoxin alpha	Oscillatoria sp. PCC9240	-5.5
10	Lipopurealin A	Oscillatoria agardhii	-6.0
11	Homoanatoxin-a	Oscillatoria sp. PCC6407	-5.9
12	Glucose	Anabaena sp.	-6.0
13	Linolenic acid	Anabaena flos-aquae	-4.5
14	Norharman	Anabaena cylindrica Lemmermann	-6.9
15	Circinamide	Anabaena circinalis	-5.7
16	Arabinose	Anabaena sp.	-6.2
17	Spiroidesin	Anabaena spiroides	-9.2
18	Cyclindrospermopsin	Anabaena bergii	-8.5
19	Guanidoacetic acid	Anabaena bergii	-5.2
20	9S,12R,13S-Trihydroxy-10E,15Z-octadecadienoic acid	Lyngbya majuscula	-5.7
21	13S-Hydroperoxy-9Z,11E,15Z-octadecatrienoic acid	Lyngbya majuscula	-5.1
22	(+)-Curacin A	Lyngbya majuscula	-6.1
23	(E)-7-Methoxytetradec-4-enoic acid	Lyngbya majuscula	-5.1
24	Agelastatin C	Lyngbya wollei	-6.8
25	Agelastatin D	Lyngbya wollei	-8.1
26	Apramide A	Lyngbya majuscula	-5.7
27	Apramide B	Lyngbya majuscula	-6.9
28	Apramide D	Lyngbya majuscula	-6.6
29	Apramide E	Lyngbya majuscula	-5.8
30	Barbamide	Lyngbya majuscula	-6.8
31	Curacin D	Lyngbya majuscula	-6.1
32	Dechlorobarbamide	Lyngbya majuscula	-6.9
33	Dysidenamide	Lyngbya majuscula	-6.1
34	Louludinium	Lyngbya gracilis	-5.3
35	Madangolide	Lyngbya bouillonii	-7.7
36	Pseudodysidenin	Lyngbya majuscula	-6.6
37	Yanucamide A	Lyngbya majuscula	-8.1
38	Yanucamide B	Lyngbya majuscula	-7.1
39	Ypaoamide	Lyngbya majuscula	-8.3
40	Besarhanamide A	Lyngbya majuscula	-5.9
41	Besarhanamide B	Lyngbya majuscula	-6.1
42	Nordysidenin	Lyngbya majuscula	-6.7
43	Antillatoxin	Lyngbya majuscula	-8.4
44	Dolabellin	Lyngbya majuscula	-6.4
45	Lyngbyabellin C	Lyngbya sp.	-7.1
46	Lyngbyabellin G	Lyngbya majuscula	-7.6
47	Malyngamide T	Lyngbya majuscula	-5.3
48	Malyngamide U	Lyngbya majuscula	-6.8
49	Malyngamide V	Lyngbya majuscula	-6.0

50	Malyngamide W	Lyngbya majuscula	-6.5
51	Palau'imide	Lyngbya sp.	-7.8
52	Semiplenamide A	Lyngbya semiplena	-6.2
53	Semiplenamide B	Lyngbya semiplena	-5.3
54	Semiplenamide C	Lyngbya semiplena	-5.4
55	Semiplenamide D	Lyngbya semiplena	-5.4
56	Semiplenamide E	Lyngbya semiplena	-6.8
57	Semiplenamide F	Lyngbya semiplena	-6.1
58	Taveuniamide A	Lyngbya majuscula x Schizothrix sp.	-6.1
59	Taveuniamide B	Lyngbya majuscula x Schizothrix sp.	-5.9
60	Taveuniamide C	Lyngbya majuscula x Schizothrix sp.	-6.6
61	Taveuniamide D	Lyngbya majuscula x Schizothrix sp.	-6.1
62	Taveuniamide E	Lyngbya majuscula x Schizothrix sp.	-6.8
63	Taveuniamide F	Lyngbya majuscula x Schizothrix sp.	-5.8
64	Taveuniamide K	Lyngbya majuscula x Schizothrix sp.	-5.6
65	Carmabin A	Lyngbya majuscula	-3.7
66	Dragonamide A	Lyngbya majuscula	-5.4
67	Dragonamide B	Lyngbya majuscula	-5.9
68	Deacetylhectochlorin	Lyngbya majuscula	-7.7
69	Hermitamide A	Lyngbya majuscula	-6.1
70	Hermitamide B	Lyngbya majuscula	-6.5
71	Malyngamide C	Lyngbya majuscula	-6.8
72	Malyngamide J	Lyngbya majuscula	-7.3
73	Malyngamide K	Lyngbya majuscula	-6.6
74	Lyngbyaloside	Lyngbya sp.	-8.0
75	Lyngbyaloside B	Lyngbya sp.	-7.6
76	Debromoaplysiatoxin	Lyngbya majuscula	-9.3
77	Isomalyngamide A	Lyngbya majuscula	-5.6
78	Lyngbyatoxin A acetate	Lyngbya majuscula	-7.6
79	Oscillatoxin A	Lyngbya majuscula	-9.2
80	Grenadamide B	Lyngbya majuscula	-6.1
81	Grenadamide C	Lyngbya majuscula	-6

Table 4: Protein-ligand interaction analysis.

Sl. No.	Compounds	AMES Test	Carcinogenicity
1.	Debromo-aplysiatoxin (-9.3)	-	-
2.	Spiroidesin (-9.2)	3	A: GLU-559, A:HIS-752, B: GLY-765
3.	Oscillatoxin A (-9.2)	2	A: GLN-632, A: GLN-648
4.	Ergosta-8,(9) 14-dien-3beta-ol (-9.0)	1	C: ASN-788
5.	Cyclindro-spermopsin (-8.5)	3	A:GLY-560, B:THR-809, B:GLY-806

Protein-ligand interaction

The Biovia Accellrys Discovery Studio Visualizer software was utilised to investigate binding interactions between amino acid residues and the best-docked bioactive compounds on their active sites. Protein-ligand interactions are significantly influenced by the type of bond, quantity of hydrogen bonds, and hydrophobic interactions in addition to binding affinity (Figures 2 to 6).

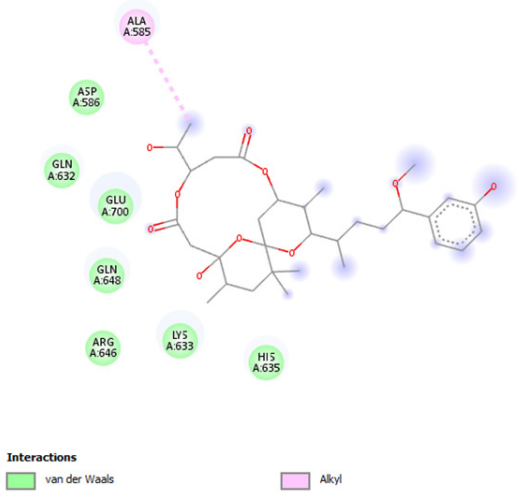

Figure 2: Interaction of Debromoaplysiatoxin on HMG COA reductase

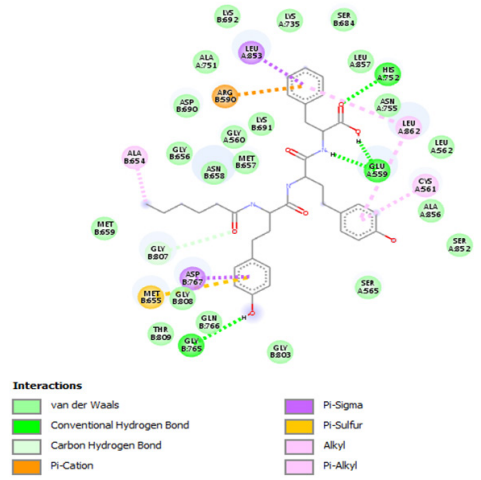

Figure 3: Interaction of Spiroidesin on HMG COA reductase

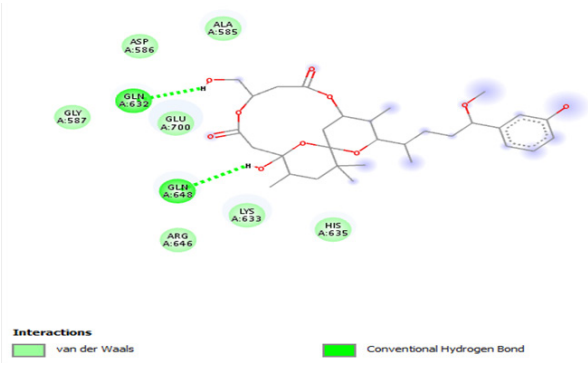

Figure 4: Interaction of Oscillatoxin A on HMG-COA reductase

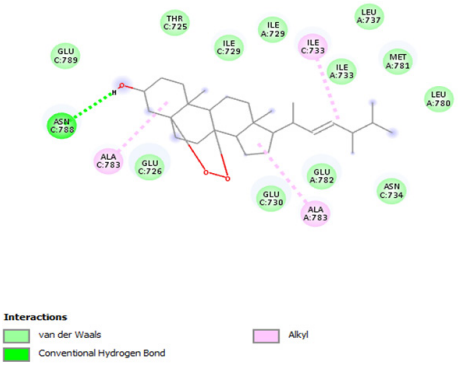

Figure 5: Interaction of Ergosta-8,(9)14-dien-3beta-ol on HMG-COA reductase

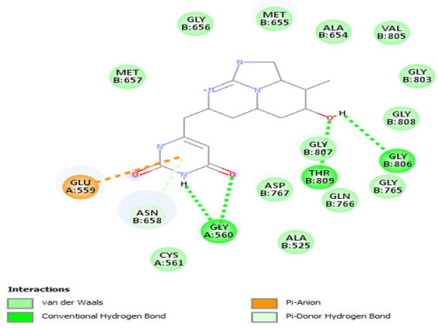

Figure 6: Interaction of Cyclindrospermopsin on HMG-COA reductase. ADMET analysis

ADMET analysis

The admetSAR tool was used to analyze the distribution, absorption, metabolism, excretion, and toxicity of the best-docked five bioactive compounds such as Debromoaplysiatoxin, Spiroidesin, Oscillatoxin A, Ergosta-8,(9)14-dien-3beta-ol, and Cyclindrospermopsin (Table 5). *In vitro* caco-2 cell permeability, human intestinal absorption, *in vivo* blood-brain barrier penetration, mitochondrial distribution, and non-substrate for CYP4502C9 were all investigated. Table 6 illustrated the ADMET features of the compounds. Debromoaplysiatoxin, Spiroidesin, Oscillatoxin A, and Ergosta-8, (9)14-dien-3beta-ol were observed to be AMES non-toxic and non-carcinogenic. The compound Cyclindrospermopsin was observed to be AMES non-toxic but it is carcinogenic.

PASS predictions

The anti-cholesterol effect of the selected bioactive compound was predicted by evaluating the PASS prediction. It is desirable for the Probable activity (Pa) to exceed the Probable inactivity (Pi). Every compound displayed a Pa value greater than the Pi. This shows that the compounds that have been found have anti-cholesterol properties (Table 7).

Table 5: ADMET properties of the selected compounds

Sl. No.	Compounds	*In vivo* blood-brain barrier penetration	Human intestinal	In vitro Caco-2 cell permeability (nm/sec)	Distribution	CYP4502C9
1	Debromoaplysiatoxin	0.5557	0.6975	0.7290	0.7727	0.8264 (NS)
2	Spiroidesin	0.6710	0.8378	0.7667	0.8249	0.7535 (NS)
3	Oscillatoxin A	0.5133	0.7992	0.7382	0.8061	0.8308 (NS)
4	Ergosta-8,(9)14-dien-3beta-ol	0.9825	1.0000	0.8378	0.4911	0.7988 (NS)
5.	Cyclindrospermopsin	0.7590	0.8810	0.6407	0.4570	0.6863 (NS)

Table 6: Toxicity analysis.

Sl. No.	Compounds	AMES Test	Carcinogenicity
1.	Debromo-aplysiatoxin	Non-toxic	No
2.	Spiroidesin	Non-toxic	No
3.	Oscillatoxin A	Non-toxic	No
4.	Ergosta-8, (9)14-dien-3beta-ol	Non-toxic	No
5.	Cyclindro-spermopsin	Non-toxic	Carcinogen

Table 7: PASS predictions.

Sl. No.	Compounds	Cholesterol antagonist		Antioxidant		Atherosclerosis treatment		Cholesterol synthesis inhibitor	
		Pa	Pi	Pa	Pi	Pa	Pi	Pa	Pi
1	Spiroidesin	0.444	0.052	0.296	0.031	0.327	0.063	0.352	0046
2	Ergosta-8(9) 14-dien-3beta-ol	0.915	0.003	0.143	0.111	0.435	0.029	0.663	0.001
3	Oscillatoxin A	-	-	0.148	0.105	-	-	-	-
4	Cyclindro-spermopsin		-	-	-	-	-	-	-
5	Debromoaplysiatoxin	-	-	-	-	-	-	-	-

DISCUSSION

Cardiovascular disease continues to be the leading cause of death worldwide. The burden of cardiovascular disease is not exclusively determined by the number of fatalities caused by it. The epidemiology of cardiovascular disease takes into account the morbidity and related disability, as there are significant fluctuations in both incidence and prevalence.[34] Excessive cholesterol levels in the blood can lead to cardiovascular disease, hypertension, and peripheral strokes. Cholesterol is essential for the generation of steroid hormones and cellular metabolism.[4]

After being substantially oxidised, the low-density lipoprotein aggressively accumulates lipids on the blood vessels of the heart wall and other areas of the body. This promotes the development of inflammation and plaque.[35] Several sources of information remain to show the strong link between antioxidants, inflammation, and cardiovascular diseases. A key factor in the emergence of cardiovascular diseases is inflammation.[36,37] The drug Statins reduce the cholesterol levels by inhibiting the HMG-COA reductase enzyme and have anti-inflammatory effects.[38] Therefore, in order to stop or postpone the onset of hypercholesterolemia before it turns into a chronic ailments, it is essential to reduce blood LDL cholesterol and increase HDL levels.[39] Particularly, a lot of medications were found to have harmful side effects on a variety of body organs. Recent research has indicated that nutrition has a major role in decreasing cholesterol.[40]

Microalgae have garnered widespread attention in the scientific community as a potential source of biological and therapeutic chemicals for use in pharmaceutical applications. This alga has been shown to have a range of bioactive chemicals with hypolipidemic qualities that have been shown to have extremely positive health effects.[41] It requires a comprehensive approach to tackle the challenging issue of identifying bioactive compounds. In this process, the ongoing improvement of analytical and molecular techniques is crucial and a requirement for using high-throughput methodologies to target innovative products.[42]

HMG-CoA reductase (3-hydroxy-3-methylglutaryl reductase) is the enzyme responsible for the production of cholesterol, and LDLR (low-density lipoprotein receptor) is in charge of removing low-density lipoprotein cholesterol from the plasma.[43] Lipid-lowering medications, such as statin, which act on 3-hydroxy-3-methyl-glutaryl-CoA reductase, the first and major enzyme of the cholesterol biosynthesis pathway, are one of the approach for regulating the amount of low density lipoprotein in the bloodstream. One of the primary precursors in the hepatic cholesterol synthesis pathway, mevalonate, is produced at a slower rate when 3-hydroxy-3-methyl-glutarylCoA reductase is inhibited.[8]

The most prevalent approach for comprehending drug-receptor interactions is *in silico* molecular docking studies. These techniques have demonstrated a high degree of support for the generation of new or more effective drug receptor inhibitors based on synthetic or natural substances.[44] This computer-based method aids in the identification of small compounds by orienting and rating them in the active binding region of a protein. Lipinski's rule of five is used by the World Drug Index to screen drugs for suitability for human usage.[45] We simulated the potential binding of the ligands to the active site of HMG-CoA reductase protein using active site prediction methodology. These ligands are associated with primary active site of the HMG-CoA reductase protein capable of inhibiting the activity of the enzyme. It has been established that Debromoaplysiatoxin,

Spiroidesin, Oscillatoxin A, Ergosta-8, (9)14-dien-3beta-ol, and Cyclindrospermopsin satisfy the criteria and are anticipated to be effective in lowering the cholesterol levels.

Cyanobacteria have been found to produce a variety of bioactive substances including toxins. Oscillatoxin is an analogue of aplysiatoxin in which the molecule does not have a six-membered ether ring.[46] Likewise, the bioactive compound Ergosta-8,(9)14-dien-3beta-ol of *Chlorella* sp. was investigated for its anti-bacterial action for the multi-drug resistant infections.[47] The spiroidesin is a unique linear lipopeptide comprising D amino acids, was extracted from cyanobacterium *Anabaena* spiroides. At lower dosages, the peptide demonstrated growth suppression on the poisonous cyanobacterium M. aeruginosa (NIES-88), and it further inhibited chymotrypsin.[48]

Metabolites of microalgae are a potential source of a variety of biological activity. Microalgae metabolites may be the most effective approach when searching for novel medications, given their high reactivity and bioavailability. Numerous metabolites have been found in microalgae, therefore *in silico* investigations make it possible to quickly and efficiently screen for the best molecules. It is possible to identify an efficient molecule with improved ADMET characteristics and target specificity. For this reason, the most effective method for finding new medications is *in silico* screening.[49] ADMET Predictor is an advanced approach that allows researchers to rapidly anticipate a wide range of ADMET attributes based on chemical structure. Its prognostic models depend on the vector algorithm square measure concept, and include characteristics like as absorption, distribution, metabolism, excretion, and toxicity.[50] ADMET properties of the best docked compounds were examined in the present study.

PASS (prediction of activity spectra for substances) used to evaluates the probability of a drug or a compound being active against a target using physicochemical approaches and multiple algorithms.[51] Probable activity (Pa) as well as the Probable inactivity (Pi) were used to predict the estimated activity of a compound. The molecule deemed feasible for a specific therapeutic action was the one with a Pa value greater than Pi.[52] Hence, in the current study the findings of PASS prediction revealed that the test compounds Spiroidesin, and Ergosta-8,(9)14-dien-3beta-ol confirmed the anti-cholesterol effects.

CONCLUSION

This study demonstrated the anti-cholesterol potential of the bioactive compounds of selected microalgal species. The in silico studies revealed that the bioactive compounds such as Debromoaplysiatoxin, Spiroidesin, Oscillatoxin A, Ergosta-8,(9)14-dien-3beta-ol, and Cyclindrospermopsin exhibited greater binding affinity against the target HMG-COA reducatse. The ADMET analysis of the five compounds showed that the compounds were safe and non-carcinogenic. The PASS prediction approach confirmed the anti-cholesterol action of the compounds such as Spiroidesin, and Ergosta-8,(9)14-dien-3beta-ol. Hence, these compounds can serve as lead compound for the development of novel drugs to lower the cholesterol level and also to treat the cardiovascular diseases.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ACKNOWLEDGEMENT

The authors wish to gratefully acknowledge and thank the following for their generous support of this research:
K. J. Somaiya Institute of Technology, Mumbai, India.
Genolites Research and Development Laboratory, Coimbatore, Tamil Nadu.

ABBREVIATIONS

CVD: Cardiovascular disease; **EC:** Endothelial cells; **PASS:** Prediction of activity spectra for substances; **UFF:** Universal Force Field; **LDL:** Low-density lipoprotein.

SUMMARY

Cardiovascular Disease (CVD) is widely regarded as one of the leading causes of death worldwide. One major risk factor for cardiovascular disease that raises the likelihood of atherosclerotic illnesses is hypercholesterolemia. Numerous studies have shown that the water-soluble parts of microalgae or isolated algal polysaccharides benefitted experimental mice with low cholesterol. We evaluated the anti-cholesterol activity of bioactive compounds from Chlorella, Oscillatoria, Anabaena, and Lyngbya via *in silico* studies. Based on *in silico* investigations, it was found that bioactive compounds with higher binding affinities against the target HMG-COA reducatse included Debromoaplysiatoxin, Spiroidesin, Oscillatoxin A, Ergosta-8, and (9)14-dien-3beta-ol. Therefore, these compounds may be used as a model compound in the creation of new medications intended to treat cardiovascular conditions by reducing cholesterol levels.

REFERENCES

1. Roth GA, Mensah GA, Johnson CO, Addolorato G, Ammirati E, Baddour LM, *et al*. Global burden of cardiovascular diseases and risk factors, 1990-2019: update from the GBD 2019 study. J Am Coll Cardiol. 2020;76(25):2982-3021. doi: 10.1016/j.jacc.2020.11.010, PMID 33309175.

2. Hetherington I, Totary-Jain H. Anti-atherosclerotic therapies: milestones, challenges, and emerging innovations. Mol Ther. 2022;30(10):3106-17. doi: 10.1016/j.ymthe.2022.08.024, PMID 36065464.
3. Félix-Redondo FJ, Grau M, Fernández-Bergés D. Cholesterol and cardiovascular disease in the elderly. Facts and gaps. Aging Dis. 2013;4(3):154-69. PMID 23730531.
4. Sivasubramanian P, Gayathri R, Priya VV, Selvaraj J, Kavitha S. Evaluation of anticholesterol and antioxidant potentiality of aqueous extracts of *Citrus aurantifolia*, *Zingiber officinale* and its formulation: A comparative *in vitro* study. J Pharm Res Int. 2021;33(60B):1873-82. doi: 10.9734/jpri/2021/v33i60B34820.
5. Simorangkir M, Silaban S, Roza D. Anticholesterol activity of ethanol extract of Ranti Hitam (*Solanum blumei Nees ex Blume*) Leaves: *in vivo* and *in silico* study. Pharmacia. 2022;69(2):485-92. doi: 10.3897/pharmacia.69.e84913.
6. van der Wulp MY, Verkade HJ, Groen AK. Regulation of cholesterol homeostasis. Mol Cell Endocrinol. 2013;368(1-2):1-16. doi: 10.1016/j.mce.2012.06.007, PMID 22721653.
7. Song Y, Kenworthy AK, Sanders CR. Cholesterol as a co-solvent and a ligand for membrane proteins. Protein Sci. 2014;23(1):1-22. doi: 10.1002/pro.2385, PMID 24155031.
8. Rozano L, Abdullah Zawawi MR, Ahmad MA, Jaganath IB. Comparative docking study of "dukung anak" phytonutrient compounds with anti-cholesterol properties. J Teknol. 2015;77(31):37-43. doi: 10.11113/jt.v77.6900.
9. Oktaviani ARD, Aprillia VS, Suhartono E, Komari N. Hesperidin Interaction with HMG-CoA-reductase enzyme in hypercholesterolemia: a study *in silico*. Berkala Kedokteran. 2021;17(2):173-8. doi: 10.20527/jbk.v17i2.11692.
10. Prabhakaran D, Jeemon P, Roy A. Cardiovascular diseases in India: current epidemiology and future directions. Circulation. 2016;133(16):1605-20. doi: 10.1161/CIRCULATIONAHA.114.008729, PMID 27142605.
11. Mattiuzzi C, Lippi G. Current cancer epidemiology. J Epidemiol Glob Health. 2019;9(4):217-22. doi: 10.2991/jegh.k.191008.001, PMID 31854162.
12. Ayman G, Shamna KP, Muhammad MP, Karkuvelraja R. Antibacterial potentials of *Tamarindus indica* L. leaf extracts against multidrug resistant Pathogens. Annals of Phytomedicine. Annals Phytomed. 2022;11(1):1-12. doi: 10.21276/ap.2022.11.1.0.
13. Selvaraj SP, Chen JY. Conjugation of antimicrobial peptides to enhance therapeutic efficacy. Eur J Med Chem. 2023 J;259:115680. doi: 10.1016/j.ejmech.2023.115680, PMID 37515922.
14. Soriano JB, Kendrick PJ, Paulson KR, Gupta V, Abrams EM, Adedoyin RA. Prevalence and attributable health burden of chronic respiratory diseases, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Respir Med. 2020;8(6):585-96. doi: 10.1016/S2213-2600(20)30105-3, PMID 32526187.
15. Narayan AR, Anilkumar S, Prasad S S. Antibacterial Properties of Antimicrobial peptides (AMP) from Skin Extracts of *Catla catla* against Wound Pathogens. J Univ Shanghai Sci Technol. 2021;23(5):384-91. doi: 10.51201/JUSST/21/05154.
16. Deng R, Chow TJ. Hypolipidemic, antioxidant, and anti-inflammatory activities of microalgae Spirulina. Cardiovasc Ther. 2010;28(4):e33-45. doi: 10.1111/j.1755-5922.2010.00200.x, PMID 20633020.
17. Ward NC, Watts GF, Eckel RH. Statin toxicity. Circ Res. 2019;124(2):328-50. doi: 10.1161/CIRCRESAHA.118.312782, PMID 30653440.
18. Sarin S, Kaman L, Dahiya D, Behera A, Medhi B, Chawla Y. Effects of preoperative statin on liver reperfusion injury in major hepatic resection: a pilot study. Update Surg. 2016;68(2):191-7. doi: 10.1007/s13304-016-0370-3, PMID 27164985.
19. Michael DR, Moss JW, Calvente DL, Garaiova I, Plummer SF, Ramji DP. *Lactobacillus plantarum* CUL66 can impact cholesterol homeostasis in Caco-2 enterocytes. Benef Microbes. 2016;7(3):443-51. doi: 10.3920/BM2015.0146, PMID 26839071.
20. Ibrahim MR, Hamouda RA, Tayel AA, Al-Saman MA. Anti-cholesterol and antioxidant activities of independent and combined microalgae aqueous extracts *in vitro*. Waste Biomass Valorization. 2021;12(9):4845-57. doi: 10.1007/s12649-021-01349-6.
21. Zhao C, Wu Y, Yang C, Liu B, Huang Y. Hypotensive, hypoglycaemic and hypolipidaemic effects of bioactive compounds from microalgae and marine micro organisms. Int J Food Sci Technol. 2015;50(8):1705-17. doi: 10.1111/ijfs.12860.
22. Soundararajan S, KarkuvelRaja R, Sanjay Prasad S. Vishnu Chitthan S, Thajuddin N. Microalgae and cyanobacterial Diversity Analysis from Bharathidasan University campus, Tamil Nadu, India. Int J Life Sci Pharm Res. 2022;12(5):165-77. doi: 10.22376/ijpbs/lpr.2022.12.5.L165-177.
23. Ryu NH, Lim Y, Park JE, Kim J, Kim JY, Kwon SW, *et al*. Impact of daily Chlorella consumption on serum lipid and carotenoid profiles in mildly hypercholesterolemic adults: a double-blinded, randomized, placebo-controlled study. Nutr J. 2014;13(1):57. doi: 10.1186/1475-2891-13-57, PMID 24920270.
24. Kim S, Kim J, Lim Y, Kim YJ, Kim JY, Kwon O. A dietary cholesterol challenge study to assess *Chlorella* supplementation in maintaining healthy lipid levels in adults: a double-blinded, randomized, placebo-controlled study. Nutr J. 2015;15(1):1-7. doi: 10.1186/s12937-016-0174-9.
25. Hamouda RA, Al-Saman MA, El-Sabbagh SM, Abo El-Seoud GW, Hendawy AN. Approach to improve the productivity of bioactive compounds of the cyanobacterium *Anabaena oryzae* using factorial design. Egypt J Basic Appl Sci. 2017;4(3):190-5. doi: 10.1016/j.ejbas.2017.06.003.
26. Gupta V, Ratha SK, Sood A, Chaudhary V, Prasanna R. New insights into the biodiversity and applications of cyanobacteria (blue-green algae)—prospects and challenges. Algal Res. 2013;2(2):79-97. doi: 10.1016/j.algal.2013.01.006.
27. Silambarasan G, Ramanathan T. Antihyperlipidemic Activity of Marine Cyanobacteria *Oscillatoria salina* in High cholesterol Diet Induced hyperlipidemic Wistar albino Rats. Inventi Rapid Pharm Biotechnol Microbiol. 2012;3:1-3.
28. Daina A, Michielin O, Zoete V. SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules. Sci Rep. 2017;7(1):42717. doi: 10.1038/srep42717, PMID 28256516.
29. prasad S. S, Shanthi S. Identification of Angiotensin Converting Enzyme (ACE) Inhibiting phytochemical compounds from *Aegle marmelos, Euphorbia hirta, Senna auriculata, Ocimum tenuiflorum* and *Hibiscus Rosa sinensis* by In Silic. Int J Pharm Biol Sci. 2020;11:79-85. doi: 10.22376/ijpbs.2020.11.3.b.
30. Tian W, Chen C, Lei X, Zhao J, Liang J. CASTp 3.0: computed atlas of surface topography of proteins. Nucleic Acids Res. 2018;46(W1):W363-7. doi: 10.1093/nar/gky473, PMID 29860391.
31. Shamna KP, Shanmugam R, Poyil MM, Bari MDN, Prasad SS, Alfaki MA. SARS-CoV-2 protease inhibiting phytocompounds from *Datura metel* L.: an *in silico* investigation for potential drugs unaffected by viral mutations against COVID-19. Annals Phytomed Int J. 2021;COVID-19(Special Issue 2):S77-85. doi: 10.54085/ap.covid19.2021.10.2.9.
32. Cheng F, Li W, Zhou Y, Shen J, Wu Z, Liu G, *et al*. admetSAR: a comprehensive source and free tool for assessment of chemical ADMET properties. J Chem Inf Model. 2012;52(11):3099-105. doi: 10.1021/ci300367a, PMID 23092397.
33. Hasan MN, Bhuiya NM, Hossain MK. *In silico* molecular docking, PASS prediction, and ADME/T analysis for finding novel COX-2 inhibitor from *Heliotropium indicum*. J Complement Med Res. 2019;10(3):142-54. doi: 10.5455/jcmr.20190525051057.
34. Townsend N, Kazakiewicz D, Lucy Wright F, Timmis A, Huculeci R, Torbica A, *et al*. Epidemiology of cardiovascular disease in Europe. Nat Rev Cardiol. 2022;19(2):133-43. doi: 10.1038/s41569-021-00607-3, PMID 34497402.
35. Jagriti S, Satyam K, Vinay D, Srinath P, Ved Kumar M. Role and importance of antioxidant in medical science-A review. Int J Pharma Bio Sci. 2020;11(3):b129-134. doi: 10.22376/ijpbs.2020.11.2.b129-134.
36. Mangge H, Becker K, Fuchs D, Gostner JM. Antioxidants, inflammation and cardiovascular disease. World J Cardiol. 2014;6(6):462-77. doi: 10.4330/wjc.v6.i6.462, PMID 24976919.
37. Manimegalai M, Prasad SS, Subha TS, Suguna L. Anti-inflammatory activity of ethanolic extract of whole plant of *Merremia tridentata* on murine models. Int J Pharm Sci Res. 2021;12:2870-5. doi: 10.13040/IJPSR.0975-8232.12(5).1000-06.
38. Golia E, Limongelli G, Natale F, Fimiani F, Maddaloni V, Pariggiano I, *et al*. Inflammation and cardiovascular disease: from pathogenesis to therapeutic target. Curr Atheroscler Rep. 2014;16(9):435. doi: 10.1007/s11883-014-0435-z, PMID 25037581.
39. Harini P, Gayathri R, Vishnu Priya R. Antioxidant and antihypercholesterolemic potential of crude aqueous leaf extract of *Annona muricata:* an *in vitro* study. J Res Med Dent Sci. 2021;9(2):354-7.

40. Lim MY, You HJ, Yoon HS, Kwon B, Lee JY, Lee S, *et al*. The effect of heritability and host genetics on the gut microbiota and metabolic syndrome. Gut. 2017;66(6):1031-8. doi: 10.1136/gutjnl-2015-311326, PMID 27053630.
41. Nacer W, Baba Ahmed FZ, Merzouk H, Benyagoub O, Bouanane S. Antihyperlipidemic and antioxidant effects of the microalgae *Nannochloropsis gaditana* in streptozotocin-induced diabetic rats. Rev Agrobiol. 2019;9(2):1474-83.
42. Cutignano A, Nuzzo G, Ianora A, Luongo E, Romano G, Gallo C, *et al*. Development and application of a novel SPE-method for bioassay-guided fractionation of marine extracts. Mar Drugs. 2015;13(9):5736-49. doi: 10.3390/md13095736.
43. Chen J, Jiang Y, Ma KY, Chen F, Chen ZY. Microalga decreases plasma cholesterol by down-regulation of intestinal NPC1L1, hepatic LDL receptor, and HMG-CoA reductase. J Agric Food Chem. 2011;59(12):6790-7. doi: 10.1021/jf200757h, PMID 21561085.
44. Meng XY, Zhang HX, Mezei M, Cui M. Molecular docking: A powerful approach for structure-based drug discovery. Curr Comput Aided Drug Des. 2011;7(2):146-57. doi: 10.2174/157340911795677602, PMID 21534921.
45. Lipinski CA. Lead- and drug-like compounds: the rule-of-five revolution. Drug Discov Today Technol. 2004;1(4):337-41. doi: 10.1016/j.ddtec.2004.11.007, PMID 24981612.
46. Nagai H, Sato S, Iida K, Hayashi K, Kawaguchi M, Uchida H, *et al*. Oscillatoxin I: A new aplysiatoxin derivative, from a marine cyanobacterium. Toxins. 2019;11(6):366. doi: 10.3390/toxins11060366, PMID 31234410.
47. Mohideen AP. Identification of potential metabolites in *Chlorella* species and *Oscillatoria* species for treatment of Multidrug Resistant (MDR) infections: an *in silico* analysis. J Res Med Dent Sci. 2021:86-94.
48. Kaya K, Mahakhant A, Keovara L, Sano T, Kubo T, Takagi H. Spiroidesin, a novel lipopeptide from the cyanobacterium *Anabaena* spiroides that inhibits cell growth of the cyanobacterium Microcystis aeruginosa. J Nat Prod. 2002;65(6):920-1. doi: 10.1021/np010660x, PMID 12088439.
49. Bintener T, Pacheco MP, Sauter T. Towards the routine use of *in silico* screenings for drug discovery using metabolic modelling. Biochem Soc Trans. 2020;48(3):955-69. doi: 10.1042/BST20190867, PMID 32369553.
50. Biswal R A, Aishwarya A, Sharma A, Pazhamalai V. 2D QSAR, Admet prediction and multiple receptor molecular docking strategy in bioactive compounds of *Gracilaria corticata* against *Plasmodium falciparum* (contractile Protein). Inform Med Unlocked. 2019;17:100258. doi: 10.1016/j.imu.2019.100258.
51. Azhaguraj R, Lenin EA, Viswanathan C, Sangeetha B, Selvanayagam M. Predication of biological activity of algal antitumor drugs using pass. Pharmacologyonline. 2010;3:22.e34.
52. Filimonov DA, Lagunin AA, Gloriozova TA, Rudik AV, Druzhilovskii DS, Pogodin PV, *et al*. Prediction of the biological activity spectra of organic compounds using the PASS online web resource. Chem Heterocycl Compd. 2014;50(3):444-57. doi: 10.1007/s10593-014-1496-1.

Cite this article: Kura AM, Karukuvelraja R, Pillai TG. Novel Drug Development for Anti-Cholesterol Activity by *in silico* Analysis: Identification Cholesterol Inhibiting Compounds. Asian J Biol Life Sci. 2024;13(1):48-60.