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Molecular docking studies of active metabolites from *Capsicum frutescens* fruit against hyperlipidemia

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High cholesterol levels are frequently thought to be connected with atherosclerosis. However, over the last 20 years, several studies have demonstrated that the pathophysiology of numerous diseases is significantly influenced by the buildup of excess cholesterol in different tissues and organs. *Capsicum frutescens'* fruit extract shows Hypolipidemic activity in cholesterol-induced rats. To determine the Hypolipidemic characteristic of the active metabolites found in a fruit of *Capsicum frutescens*, an *in silico* docking is performed against six target proteins involved in the synthesis of cholesterol: acetoacetyl thiolase (PDB ID: 2 F2S), HMG-CoA synthase (PDB ID: 2P8U), squalene synthase (PDB ID: 3 V66, Oxido squalene cyclase (PDB ID: 1W6J), Niemann Pick C1 Like 1 (PDB ID: 6V3F), and 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoAR) (PDB ID: 3CDB).The molecular docking of the protein target is done using the PyRx software v8.0. The docking studies show that six target proteins strongly interact with a significant molecule, Ergosterol, with the highest binding affinity. Since Ergosterol can be used as a Hypolipidemic potential agent for further uses.

Keywords: *Capsicum frutescens*, Cholesterol, Ergosterol, Hypolipidemic, *In silico* docking

One of the most prevalent pathological disorders in humans is Hyperlipidemia, which is caused by a disturbance of lipid metabolism in the body and elevates the content of lipids in the serum over normal levels. Genetics, nutrition, lifestyle, medication, and other variables have all contributed to the substantial increase in the incidence of hyperlipidaemia, which is now the most prevalent pathological condition[1]. Triglycerides (TG), very low-density lipoprotein cholesterol (VLDL-C), and circulating low-density lipoprotein cholesterol (LDL-C) are all elevated in hyperlipidemia[2], which causes serious illnesses, including heart disease, stroke, and even atherosclerosis-related mortality. The best course of action is to inhibit the liver's synthesis of cholesterol[3,4]. Acetyl-CoA initiates the isoprenoid pathway, a key route in cholesterol biosynthesis, regulated by various enzymes[5].Six target proteins were selected for molecular docking: HMG-CoA reductase (HMG-CoAR, PDB ID: 3CDB), acetoacetyl thiolase (PDB ID: 2F2S), squalene synthase (PDB ID: 3V66), Oxidosqualene cyclase (PDB ID: 1W6J), Niemann-Pick C1 Like 1 (NPC1L1, PDB ID: 6V3F), and HMG-CoA synthase (PDB ID: 2P8U). Acetoacetyl thiolase catalyzes the conversion of acetyl-CoA to acetoacetyl-CoA *via* the mevalonate pathway and is vital for fatty acid breakdown[6]. HMG-CoA synthase, located in both mitochondria and cytosol, regulates ketogenesis and contributes to HMG-CoA formation[7]. Squalene synthase, essential in sterol biosynthesis, catalyzes the production of squalene from farnesyl diphosphate (FPP), making it a key therapeutic target[8]. Oxidosqualene cyclase converts 23 Oxidosqualene to lanosterol, marking the first steroidal step in cholesterol synthesis[9].NPC1L1 facilitates cholesterol absorption in the liver and intestines through lattice-protein-dependent endocytosis[10]. HMG-CoAR, a critical rate-limiting enzyme in this pathway, is widely targeted for cholesterol-lowering therapies[11]. *Capsicum frutescens L.,* belonging to the family *Solanaceae,* is an extremely valuable medicinal herb distributed throughout India. In traditional medicine, due to the presence of phytochemicals, it has been used for the treatment of cough, toothache, sore throat, parasitic infections, rheumatism, wound healing, etc[12].This plant sterol plays a major role in the absorption of cholesterol, stanols, and some important inhibitors like ezetimibe, also being found out along with that as

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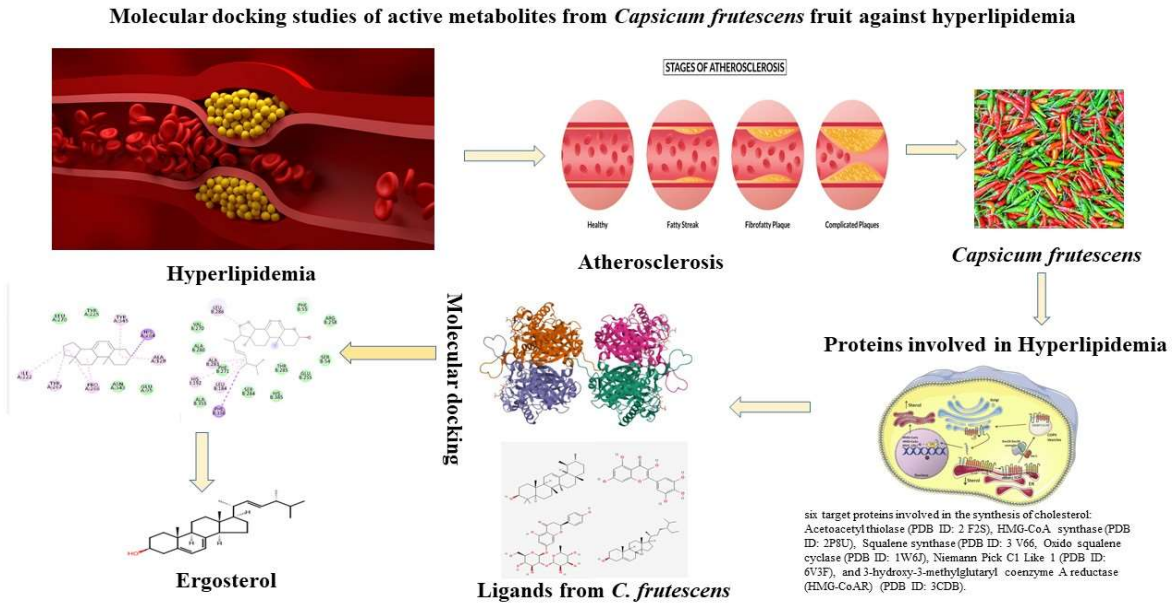

Graphical abstract

Hypocholesterolemia agents[13]. The purpose of this study was to investigate the active metabolites that inhibit cholesterol synthesis through molecular docking analysis. This study will further help to find out the effective inhibitor for all the targeted proteins which can be utilized as an Anti-Hypolipidemic agent for future applications.

Materials and Methods

Ligand preparation

The list of Phytocompounds were taken from various literatures and IMMPAT database (https://cb.imsc.res.in/imppat/). The chemical structures of capsicum's constituents were gathered from published works. The PubChem Compound Database (http://www.ncbi.nlm.nih.gov/pccompound) provided the ligands' chemical structures. The ligand molecules' 3D structure was verified and downloaded in SDF format. To guarantee ideal conformation and remove any steric conflicts, energy reduction was carried out, preparing the ligands to make effective interactions in the following docking studies[14].

Protein preparation

The Protein Data Bank (PDB) at http://www.pdb.org provided the crystallographic three-dimensional structures of a selection of chosen target proteins (PDB ID: 2F2S, PDB ID: 2P8U, PDB ID: 6V3F, PDB ID: 3CDB, PDB ID: 3V66, and PDB ID: 1W6J). To enable precise docking studies, polar hydrogen atoms were added to the protein structures and water molecules were eliminated before docking (Table 1). The receptor was given Kollman charges, and the non-polar hydrogens were combined with carbons. To make sure the protein structures were in their most stable conformations for docking analysis, energy minimization was also carried out[15].

Table 1 — Name of the targeted protein selected for Molecular docking with their PDB ID

S. No	Name of the Protein	PDB ID
1.	HMG-CoA reductase	3CDB
2.	Acetoacetyl thiolase	2F2S
3.	Squalene synthase	3V66
4.	Oxidosqualene cyclase	1W6J
5.	Niemann-Pick C1 Like 1	6V3F
6.	HMG-CoA synthase	2P8U

Molecular docking

PyRx was used to perform molecular docking, which uses Auto Dock Vina for virtual screening. Grid boxes were created around binding sites after target proteins and ready-made ligands were input into the program. Parameters were changed during the docking procedure to achieve the best outcomes. Binding affinities and interaction modes were analyzed to evaluate each ligand's potential as an inhibitor, offering insightful information about their potential therapeutic applications[16].

Results and Discussion

In rats with hyperlipidemia, the crude extract of *Capsicum frustescens* significantly reduced the lipid profile. Rats with hyperlipidemia treated with *C. frutescens* showed persistent reductions in CAI, AI, and CI indices, indicating the extract's potential for cardio protection. On the basis of those findings, performed docking research utilizing *C. frutescens* Phytocompounds with various target proteins that are involved in the transportation and production of cholesterol. The results showed good binding poses and the lowest energy values. The Phytocompounds' optimal binding nodes and amino acid interactions at six target protein active sites are shown, and the (Table 2) lists the relevant energy values.

The docking results revealed that several compounds exhibited high binding affinities with individual target proteins. In the case of sterols such as Beta-Sitosterol, Campesterol, Ergosterol, and Stigmasterol, among which Ergosterol shows

Table 2 — The docking score between the Phytocompounds against the selected six protein targets.

List of the Compounds with Respective PubChem ID		Molecular docking Scores (kcal/mol)					
Phytocompound	**Compound ID**	**2F2S**	**2P8U**	**3V66**	**1W6J**	**6V3F**	**3CDB**
Alpha Amyrin	73170	-8.6	-8.9	-10	-9.3	-9.2	-10.4
alpha Humulene	5281520	-5.7	-6.1	-7.2	-6.3	-6.9	-5.8
Ascorbic acid	54670067	-5.8	-6.1	-5.1	-5.5	-5.4	-5.9
b -bisabolene	10104370	-5.9	-6	-7.7	8.7	-6.5	-6.5
beta caryophyllene	20831623	-5.7	-6.5	-7.8	-6.2	-6.9	-7
Beta Sitosterol	222284	-7.4	-7.1	-9.6	-9.8	-9.5	-8.4
Beta-lonone	5282108	-5.7	-5.5	-6.5	-5.8	-6	-6.6
Caffeic Acid	689043	-6.5	-7.5	-5.9	-7.1	-6.4	-6.8
Campesterol	173183	-7.7	-8.9	-9.8	-7.2	-9.2	-8.8
Capsaicin	1548943	-5.9	-6.5	-7	-9.3	-5.2	-6.7
Catechin	9064	-7	-8.9	-8	-7.6	-7.4	-8
Chrysoeriol	5280666	-7.7	-8.8	-8.5	-10.2	-7.7	-8.5
d -elemene	156582221	-5.6	-5.5	-6.5	-8.7	-7	-6.1
Dihydrocapsaicin	107982	-5.6	-6.7	-6.9	-5.2	-5.4	-6.3
Ergosterol	444679	-8.6	-9.7	-10.1	-9.6	-9.1	-9.5
Ethyl linolenate	5367460	-6.3	-6.2	-6	-4.7	-5.8	-5.4
Ethyl palmitoleate	6436624	-5.1	-6	-5.9	-5.2	-4.4	-5.3
Farnesyl	444108	-5.3	-7.1	-7.3	-6.2	-6.3	-6.1
Ferulic Acid	445858	-5.6	-7.5	-6	-6.9	-5.7	-6.3
g -himachalene	577062	-6.2	-8.7	-7.1	-8.8	-7.7	-6.7
Gemma Cadinene	5281520	-5.9	-6.1	-7.2	-6.2	-7.1	-6.5
Geranyl	637566	-4.3	-6.2	-6	-5.5	-5.3	-5.3
Heptadecane	12398	-3.8	-5.9	-6	-4.4	-4.8	-4.4
Hexadecanal	984	-5.5	-5.3	-5.8	-4.8	-4.3	-4.7
Hydroxycinnamic Acid	637542	-6.7	-7.2	-5.9	-6.9	-5.5	-6.1
Kaempferol	5280863	-7	-9	-8	-7.2	-7.6	-8.3
Linoleic acid	5280450	-5.2	-5.4	-6.7	-5.5	-5.7	-5.5
Lutein	5281243	-8.1	-8.1	-10	-8.1	-8.4	-8.1
Methyl linoleate	5284421	-4.7	-4.9	-6.4	-5.2	-4.8	-5.6
Methyl linolenate	5319706	-5.1	-4.4	-6.1	-4.9	-5.1	-5.5
Myricetin	5281672	-7.1	-8.1	-8.2	-9.3	-7.7	-8.2
Myristic acid	11005	-4.8	-5.9	-5.7	-5.2	-4.9	-4.6
Naringenin	439246	-7.6	-8.5	-8	-7.4	-8.3	-8
Naringin	442428	-8.3	-9.1	-9.1	-8.7	-9.3	-9
Neophytadiene	10446	-4.9	-5.5	-6.5	-5.3	-5.3	-5.2
Nerolidol	5284507	-6.6	-7.1	-6.7	-6.4	-5.7	-5.8
Palmitic acid	985	-4.7	-5.4	-5.7	-4.3	-4.7	-4.6
Pentadecanal	17697	-4.9	-4.3	-5.8	-5.2	-5.1	-4.6
Phytol	5280435	-5.3	-5.9	-6.9	-5.5	-5.6	-5.3
Quercetin	5280343	-7.3	-9.6	-8.2	-9.8	-7.7	-8.4
Retinol	445354	-6.2	-6.6	-8.4	-6.8	-7.3	-7.4
Rosmarinic Acid	5281792	-7.6	-9.1	-7.7	-7.1	-7.7	-7.2
Stigmasterol	5280794	-8.1	-9.5	-10	-7.8	-9.3	-8.9
Tetradecanal	31291	-4.5	-4.4	-5.6	-4.7	-4.5	-4
Vanillic Acid	8468	-5.8	-6.8	-5.8	-6.9	-5.5	-5.8
Zeaxanthin	5280899	-7.9	-8.6	-9.8	-7.5	-8.8	-8.5

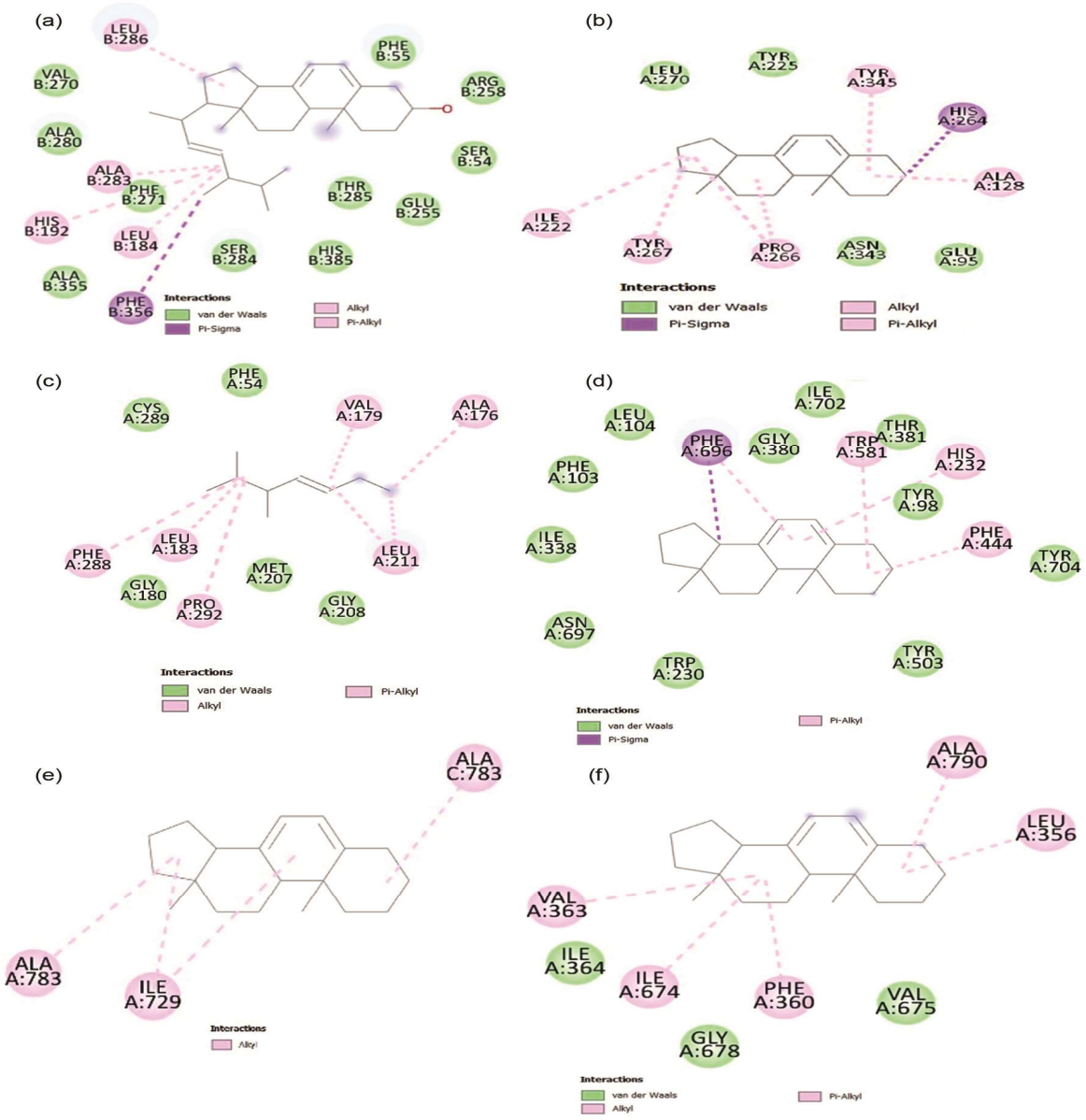

Fig. 1 — 2D Interaction of Ergosterol with Six targeted protein receptors. (a) Ergosterol with Acetoacetyl thiolase - 2F2S; (b) Ergosterol with HMG-CoA synthase - 2P8U; (c) Ergosterol with Squalene synthase - 3V66; (d) Ergosterol with Oxidosqualene cyclase – 1W6J; (e) Ergosterol with HMG-CoA reductase - 3CDB; and (f) Ergosterol with Niemann-Pick C1 Like 1 – 6V3F

consistent and strong interactions, were demonstrated across all six receptors. The 2D interaction between Ergosterol with six targeted proteins is given in the (Fig. 1).

This suggests its broad-spectrum potential in modulating cholesterol-related pathways. Given its superior and comprehensive binding profile, Ergosterol has been identified as a key candidate for further analysis.

The chemical structure of Ergosterol were given in the (Fig. 2).When the Ergosterol (ligand) interacted

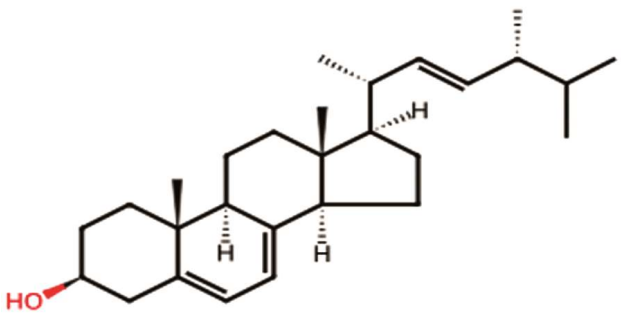

Fig. 2 — Chemical structure of Ergosterol

with Acetoacyl-CoA thiolase (2F2S), the amino acids ALA (alanine), HIS (histidine), LEU (leucine), and PHE (phenylalanine) attracted the ligand's C-3OH. Specifically, the docked binding energy is -8.6kcal/mol. The condensing enzyme acetoacetyl-CoA thiolase, also known as thiolase II, catalyzes the conversion of two acetyl-CoA molecules into acetoacetyl-CoA. Most of these thiolases are involved in anabolic activities as the first step in the synthesis of isoprenoids via the mevalonate (MVA) pathway in eukaryotes and polyhydroxybutyrate (PHB) via the thiolase II pathway in bacteria, even though thiolase II catalyzes reversible events. The inhibition of this enzyme resulted in rats with lower levels of triglycerides and plasma cholesterol. Acetoacetyl-CoA formation may be inhibited by the ergosterol that binds with Acetoacetyl-CoA thiolase in docking studies. The production of HMG-CoA by HMG-CoA synthase (2P8U) initiates the isoprenoid pathway. Cholesterol is the main byproduct of this route. In order to tackle dangerous Gram-positive bacteria, including Enterococcus, Staphylococcus, and Streptococcus species that depend on the mevalonate pathway for survival, this enzyme may be utilized as a therapeutic target to regulate serum cholesterol levels and develop novel antibiotics. In our investigation, three amino acids, LLE (isoleucine), TYR (tyrosine), and PRO (proline) formed hydrogen bonds with ergosterol and the active site residues of HMG-CoA synthase (2P8U), a C-3OH ligand. Furthermore, histidine (HIS) was joined to form a pi-sigma link. Its corresponding binding energy is -9.7 kcal/mol. Ergosterol may reduce cholesterol by inhibiting the HMG-CoA synthase enzyme by binding to its amino acids. A protein-ligand binding interaction with PHE (phenylalanine) and PRO (proline) was found by squalene synthase (3V66) docking analysis, with a binding energy value of -10.1 kcal/mol. Because it plays a critical regulatory role in directing the flow of the metabolite FPP (Farnesyl pyrophosphate) to either the sterol or the non-sterol branch of the pathway, squalene synthase is a desirable target for antilipogenic and antiatherogenic medications[17]. According to a previous research report, the squalene synthase inhibitor RPR107393 prevented the production of new cholesterol and reduced the plasma total cholesterol (TC) levels in rats by at least 35%[18]. The ligand ergosterol's binding energy was -9.6 kcal/mol, and it binds to the amino acids histidine (HIS) and tryptophan (TRP). Ergosterol and oxidosqualene cyclase (1W6J) have binding interactions. Hypercholesterolemia is one of the primary risk factors for the development of atherosclerotic vascular diseases. Reducing the rate of cholesterol production is an effective therapy method for lowering elevated plasma cholesterol levels[19]. Oxidosqualene cyclase, one of the enzymes in the cholesterol synthesis pathway, may be a useful target from the perspective of drug therapy. In our current investigation, ergosterol docked with the oxidosqualene cyclase protein (3V66), suggesting a positive protein–ligand interaction. This implies that ergosterol might be an effective inhibitor of oxidosqualene cyclase, which could affect the production of lanosterol and prevent the generation of cholesterol[20].Interfering with NPC1L1 may prevent cellular cholesterol uptake because it is a crucial transporter for this process. Since the N-terminal domain of NPC1L1 can specifically bind cholesterol, molecular docking was done at the N-terminus to determine whether ergosterol binds to NPC1L1. The findings indicated that the amino acid residues VAL (valine) and ALA (alanine) were implicated in the interaction with PTL, and that ergosterol had a binding affinity of -9.1kcal/mol for the N-terminal domain of the cholesterol-binding site of NPC1L1.A crucial enzyme in the metabolism of cholesterol, human cholesterol oxidase, has a crystal structure referred to as the 3CDB receptor. Because it catalyzes the transformation of cholesterol to cholestenone, this enzyme is crucial for regulating lipid homeostasis. Ergosterol interacts with ALA (alanine) and LLE (isoleucine) and has a strong binding affinity of -9.5 kcal/mol for this receptor. This suggests possible inhibitory or modulatory effects by demonstrating a persistent contact between the receptor and Ergosterol.

Conclusion

The molecular docking investigation was performed between active metabolites from *Capsicum frutescens* with six distinct target proteins associated with cholesterol synthesis. The Docking results revealed that Alpha-amyrin, Ergosterol, Lutein, Naringin, and Stigmasterol exhibit great inhibitory action among all six Protein receptors. Despite this, Ergosterol was well-docked with all the chosen proteins compared to the other sterols, providing additional information about potential inhibitory effects. In summary, ergosterol's *in silico* docking analysis with six target proteins

produced superior docking results. This demonstrates the ergosterol's Hypolipidemic properties; therefore, it may be taken into consideration for the creation of Hypolipidemic medications.

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Conflicts of interest

All authors declare no conflicts of interest.

References

1 He N & Ye H, Exercise and hyperlipidemia. In: Xiao J (Ed.), *Physical Exercise for Human Health*. Springer Nature Singapore, (2020) 79.
2 Su X, Peng H, Chen X, Wu X & Wang B, Hyperlipidemia and hypothyroidism. *Clin Chim Acta*, 527 (2022) 61.
3 Krečman V, Škottová N, Walterová D, Ulrichová J & Šimének V, Silymarin inhibits the development of diet-induced hypercholesterolemia in rats. *Planta Med*, 64 (1998) 138.
4 Liscum L, Cholesterol biosynthesis. *Biochem Lipids, Lipoproteins Membr*, (2008) 399.
5 Cerqueira NMFSA, Oliveira EF, Gesto DS, Santos-Martins D, Moreira C, Moorthy HN, et al., Cholesterol biosynthesis: A mechanistic overview. *Biochemistry*, 55 (2016) 5483.
6 Nes WD, Biosynthesis of cholesterol and other sterols. *Chem Rev*, 111 (2011) 6423.
7 Hegardt FG, Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase: A control enzyme in ketogenesis. *Biochem J*, 338 (1999) 569.
8 Radisky ES & Poulter CD, Squalene synthase: Steady-state, pre-steady-state, and isotope-trapping studies. *Biochemistry*, 39 (2000) 1748.
9 Risley JM, Cholesterol biosynthesis: Lanosterol to cholesterol. *J Chem Educ*, 79 (2002) 377.
10 Ge L, Wang J, Qi W, Miao HH, Cao J, Qu YX, et al., The cholesterol absorption inhibitor ezetimibe acts by blocking the sterol-induced internalization of NPC1L1. *Cell Metab*, 7 (2008) 508.
11 Mahdavi A, Bagherniya M, Fakheran O, Reiner Ž, Xu S & Sahebkar A, Medicinal plants and bioactive natural compounds as inhibitors of HMG-CoA reductase: A literature review. *BioFactors*, 46 (2020) 906.
12 Muthuswamy R, S A& NisonQ M, Review on *Capsicum frutescens*, a tribal herbal food used as medicine. *Res J Pharmacogn Phytochem*, 13 (2021) 191
13 Nguyen TT, The cholesterol-lowering action of stanol esters. *J Nutr*, 129 (1999) 2109.
14 Rizkita AD, Ella T, Gulo J, Wahyuni NS, Eka R & Selay P, Interaction of BCN OH (bicyclononyn derivate) and pertuzumab in the development of linker ADC (antibody drug conjugation) through molecular docking. *Indones J Chem Sci*, 13 (2024).
15 Bhowmik R, Roy S, Sengupta S & Sharma S, Biocomputational and pharmacological analysis of phytochemicals from *Zingiber officinale* (ginger), *Allium sativum* (garlic), and *Murraya koenigii* (curry leaf) in contrast to type 2-diabetes. *Int J Appl Pharm*, 13 (2021) 280.
16 Dallakyan S & Olson AJ, Small-molecule library screening by docking with PyRx. *Methods Mol Biol*, 1263 (2015) 243.
17 Tansey TR & Shechter I, Squalene synthase: Structure and regulation. *Prog Nucleic Acid Res Mol Biol*, 65 (2000) 157.
18 Amin D, Rutledge RZ, Needle SN, Galczenski HF, Neuenschwander K, Scotese AC, et al., RPR 107393, a potent squalene synthase inhibitor and orally effective cholesterol-lowering agent: Comparison with inhibitors of HMG-CoA reductase. *J Pharmacol Exp Ther*, 281 (1997) 746.
19 Butterfield DA, Castegna A, Pocernich CB, Drake J, Scapagnini G & Calabrese V, Nutritional approaches to combat oxidative stress in Alzheimer's disease. *J Nutr Biochem*, 13 (2002) 444.
20 Mark M, Müller P, Maier R & Eisele B, Effects of a novel 2, 3-oxidosqualene cyclase inhibitor on the regulation of cholesterol biosynthesis in HepG2 cells. *J Lipid Res*, 37 (1996) 148.