INNOVARE JOURNAL OF MEDICAL SCIENCES

Vol 11, Issue 3, 2023

ISSN - 2321-4406

Research Article

MOLECULAR DOCKING STUDY TO TREAT FAMILIAL HYPERCHOLESTEROLEMIA USING NATURAL PHYTOCOMPOUNDS FROM INDIAN *PANICUM MILIACEUM*

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Received: 30 March 2023, Revised and Accepted: 26 April 2023

ABSTRACT

Objectives: Lipoprotein disorders like Familial Hypercholesterolemia are crucial from the clinical point of view. In atherogenesis and the associated risk of atherosclerotic cardiovascular disease, lipoproteins are crucial. Therefore, to treat the disease, naturally active phytocompounds from an Indian millet *Panicum miliaceum* were used for molecular docking study and drug-likeness prediction along with absorption, distribution, metabolism, excretion (ADME) analysis.

Methods: Proprotein convertase subtilin/kexin type 9 (PCSK9) decreases low-density lipoprotein (LDL) cholesterol levels *in vivo* by forming a complex with an LDL Receptor disruptor, according to a 3D structure retrieved from protein data bank (PDB). Plant phytocompound names and their structures were obtained from Indian medicinal plant, phytochemistry and therapeutics database and PDB, respectively. Docking was performed using two different docking software – PyRx.

Results: Molecular docking study and drug-likeness prediction were carried out with the help of various computer-aided drug-designing tools and techniques. Five phytocompounds from *P. miliaceum* show prominent binding affinity toward PCSK9-disruptor complex, namely Diosgenin, Yamogenin, Miliacin, Germanicol, and beta-Amyrin are observed to possess drug-like properties that were confirmed through ADMET and drug likeliness studies.

Conclusion: According to the present research, it has been concluded that Diosgenin, Yamogenin, Miliacin, Germanicol, and beta-Amyrin show specific interactions with the PCSK9-disruptor complex.

Keywords: Hypercholesterolemia, Proprotein convertase subtilisin/kexin type 9, *Panicum miliaceum,* Indian medicinal plant, Phytochemistry and therapeutics, PyRx, Protein data bank.

 DOI: http://dx.doi.org/10.22159/ijms.2023v11i3.47980. Journal homepage: https://innovareacademics.in/journals/index.php/ijms

INTRODUCTION

Hypercholesterolemia can be described as a disorder in which low-density lipoprotein (LDL) or bad cholesterol levels are high. These increased LDL levels lead to a medical condition called Atherosclerosis, where fat is collected in the arteries. Lipoprotein abnormalities are clinically important due to their role in atherogenesis and the risk they pose for atherosclerotic cardiovascular disease (ASCVD). In patients with established ASCVD who are middle-aged or later, lowering cholesterol consistently reduces cardiovascular mortality and cardiovascular events [1].

Lipoproteins are biomolecules made up of lipids and proteins. Since lipids like cholesterol and triglycerides are not soluble in water, they must be carried in the bloodstream along with proteins called lipoproteins [2]. To prevent toxicity, large amounts of fatty acids from meals must be delivered as triglycerides. These lipoproteins are essential for the small intestine to absorb and transport dietary lipids, for lipids to travel from the liver to peripheral tissues, and for lipids to travel to the liver and intestine from peripheral tissues (reverse cholesterol transport). Transporting harmful external hydrophobic and amphipathic substances, including bacterial endotoxin, away from invasion and infection sites is a secondary role. Very LDL and intermediate-density lipoproteins (IDL) particles give rise to LDLs, a class of lipoproteins, which are then further concentrated in cholesterol. Circulation of most of the cholesterol is carried by LDL. Small, dense LDL particles remain in the bloodstream for a longer period because they have a lower affinity for the LDL receptor (LDLR). They also more readily penetrate the artery wall and bind to intra-arterial proteoglycans with greater affinity, which traps them in the arterial wall [2].

Hypercholesterolemia has both acquired and inherited causes. Familial hypercholesterolemia, which is caused by mutations in the LDLR gene, is the most prevalent genetic disease. Mutations in the gene that encodes for LDLR that result in loss-of-function are the root cause of familial hypercholesterolemia, which is at least 85% the result of this LDLR deficiency [3,4]. The liver's decreased LDLR function results in a slow removal of LDL from the bloodstream. The primary cause of the high LDL cholesterol (LDL-C) levels in familial hypercholesterolemia is a delayed elimination of LDL from circulation. The creation of LDL from IDL is also increased since the elimination of IDL is also put off. Two mutated LDLR alleles (familial hypercholesterolemia homozygotes or compound heterozygotes) are associated with much higher LDL-C levels than one mutation allele alone (familial hypercholesterolemia heterozygotes). Men and women around the age of 45 and 55 years, respectively; Early ASCVD in the family history (younger than 55 years in a male and younger than 65 years in a female); Hypertension\Diabetes; Smoking; Low high-density lipoprotein (HDL) cholesterol levels (males: Fewer than 40 mg/dL, females: <55 mg/dL) are some of the major risk elements in high LDL-C levels [4]. According to estimates, elevated cholesterol contributes to 29.7 million DALYS, or 2% of all DALYS, and 2.6 million fatalities (4.5% of all deaths). Elevated total cholesterol is a major cause of disease burden in both industrialised and emerging countries as a risk factor for ischemic heart disease and stroke. In 2008, 39% of individuals worldwide (37% of men and 40% of women) had elevated total cholesterol [4].

LDL-C levels in plasma have a positive relationship with the risk of CVD. Through encouraging LDLR degradation, proprotein convertase subtilin/kexin type 9 (PCSK9) plays a crucial part in controlling plasma cholesterol homeostasis. While loss-of-function mutations are linked to lower plasma levels of LDL-C, gain-of-function mutations in PCSK9 result in autosomal dominant hypercholesterolemia [5].

Panicum miliaceum, also known as Proso-millet, is highly nutritious and has proven and important medicinal values. The protein from millet has a positive effect on how cholesterol is metabolized. Studies were done on how dietary proso-millet protein affected the plasma levels of HDL cholesterol in various rats [6]. According to reports, millet protein consumption raises plasma levels of HDL cholesterol, similar to our past studies. Given that HDL has an anti-atherogenic effect, millet protein could be a beneficial new food ingredient because it can control cholesterol metabolism [6]. The avoidance of liver damage is another benefit associated with this protein.

An attempt was made in this research to study the gain of function of the PCSK9 gene using new and naturally occurring active phytocompounds as ligands from an Indian medicinal plant *P. miliaceum,* with the help of molecular docking techniques aiming toward therapeutic application.

METHODS

Protein preparation

The three-dimensional structure of the PCSK9-disruptor complex (Protein data bank [PDB] ID: 7KFA) was retrieved from the research collaboratory for structural bioinformatics-PDB (https://www.rcsb.org/) [7]. It has A and B chains with a total of 442 amino acids. Protein preparation was done before docking according to standard protocols of BIOVIA Discovery Studio Visualizer by Dassault Systems [8]. It is a molecular visualization and simulation tool. Side chains excluding the A chain were removed along with heteroatoms and water molecules to prevent their interaction during the docking process. In case of the presence of ligands, they should also be removed. The addition of polar hydrogen atoms to the protein structure is an important and final step in refining the protein structure.

Ramachandran plot

A peptide's amino acids' torsional angles, phi, and psi are plotted using the Ramachandran algorithm. The MolProbity Server (http://molprobity.biochem.duke.edu/index.php) was used to analyze Ramachandran plots [9] C-Alpha Based Low-resolution Annotation Method (CaBLAM) outliers, favored rotamers, etc., contribute to the knowledge of the protein structure's geometry. To analyze the Ramachandran plot with outliers that are labeled by residue type, residue number, and chain and display all the labels, the PDB file for Protein (7KFA) was uploaded.

Ligand selection

To obtain the potential inhibitors of the PCSK9-disruptor complex, 21 naturally occurring phytocompounds were retrieved from the Indian medicinal plant, phytochemistry and therapeutics (IMPPAT) database, which is a manually curated database that was created by digitizing data from more than 7000 published research articles, more than 100 books on traditional Indian medicine, and other sources [10]. The three-dimensional structure data file format used by PubChem, a compound database, was used to retrieve the structures of phytocompounds. Utilizing the PyRx program, the ligand synthesis process involved ligand optimization, energy minimization, and conversion of the ligand to 3D PDB format [11].

Molecular docking

By simulating the interaction between a small molecule and a protein at the atomic level, the molecular docking method enables us to characterize how small molecules behave at the binding site of target proteins and to better understand fundamental biological processes. The molecular docking employed the PyRx virtual screening tool [12]. All 21 active phytocompounds of *P. miliaceum* were docked with the PCSK9-disruptor complex (PDB ID: 7KFA) using the PyRx program. Prepared receptor files were chosen as the target for the docking investigation. OpenBabel plugin. The ligands were subjected to energy minimization following which there were concerted to.pdbqt format. To check all potential combinations of ligand and protein binding, the grid box (X: 33.2310, Y: 34.7447, Z: 45.1742) was formed by maximization after the protein and ligand molecules were determined. After making the necessary adjustments, docking was initiated through which we were able to retrieve the results as CSV files, and the efficacy of the ligand as PCSK9-disruptor was evaluated based on their binding affinity and based on it, the top 5 ligands with the highest binding affinity were chosen for further investigation once the docking was finished saving the compounds as PDB files [11]. With the aid of BIOVIA Discovery studio visualizer 21.1, an interactive 2D–3D visualization study was carried out [8].

Absorption, distribution, metabolism, excretion (ADME) analysis

In pharmacokinetics and pharmacology, the terms "Absorption, Distribution, Metabolism, and Excretion" or "ADME" refer to how a medication is eliminated from the body. The four criteria all have an effect on drug levels and the kinetics of drug exposure to tissues, which affects the compound's efficacy and pharmacological activity as a medicine. Toxicity, an indispensable parameter when you are developing a drug, is also documented in the ADME study. In this investigation, the top 5 compounds with the highest binding affinity were chosen for the ADMET analysis and drug-likeness test. Using SwissADME (http://www.swissadme.ch/) and Prediction of TOXicity of chemicals (ProTox-II) (https://tox-new.charite.de/protox II/), drug-likeness and ADMET analysis were performed [13,14]. The SWISSADME tool was also used for BOILED-Egg analysis. For ADME analysis, the Lipinski rule of five was taken into consideration. When a molecule meets two or more of the following requirements, Lipinski's rule of five predicts whether a drug-likeness will be successful or not: Molecular mass must not be >500 Dalton, logP value must not exceed 4.15, H-bond donors should not be any more than 5, H-bond acceptors should be <10, and molar refractivity ranging between 40 and 130 is acceptable [11].

RESULTS

Ramachandran plot

We identified 386 favored rotamers and 532 Ramachandran favored as 82.66% and 97.44%, respectively, in the Ramachandran plot when protein geometry was taken into account, indicating high protein quality. There were a few outliers and bad rotamers. It was discovered that the protein's Rama distribution Z-score was -1.05±0.32. The Rama-Z score compares a model's "normality" to a set of high-resolution structures. A Rama-Z score between –2 and 2 indicates a normal protein backbone [15]. There were 7 CaBLAM outliers when low-resolution criteria were taken into account, or 1.3% (Fig. 1). CaBLAM is a system designed to use protein CA geometry to evaluate main chain geometry and identify areas of probable secondary structure. Compound errors or ambiguities in a model may make the results of highly-sensitive measures of protein conformation, such as Ramachandran analysis, difficult or impossible to interpret. Hence, the criteria were set to consider low-resolution CaBLAM outliers.

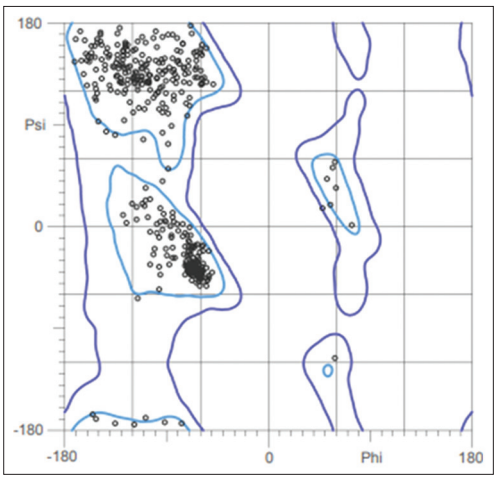

Fig. 1: Ramachandran plot analysis of Proprotein convertase subtilin/kexin type 9-disruptor complex

Molecular docking
Molecular docking studies provide insight into five active phytocompounds from *P. miliaceum* that were able to significantly bind to the PCSK9-disruptor complex. Table 1 shows the top 5 phytocompounds with the highest binding affinities.

Based on the findings from molecular docking studies performed with PyRx, we discovered that 8 out of the 21 compounds from *P. miliaceum* exhibit considerable binding affinity (higher than 8Kcal/moL) with the PCSK9-disruptor complex. We chose the top 5 compounds (Table 1) for Drug-likeness Prediction and ADME analysis based on docking data.

Molecular visualization
Using the BIOVIA Discovery Studio Visualizer, interactions between the receptor and ligand of the top 5 phytocompounds with the highest binding affinity were viewed. Using PyRx, docked ligands were stored in PDB format and opened with pure protein PCSK9. Different interactions between phytocompounds and the PCSK9-disruptor complex were seen in the 2D and 3D planes. Van-der Waal forces, conventional and carbon-hydrogen bonds, π-sulfur interactions, alkyl and π-alkyl interactions, π-π T-shaped interactions, and unfavorable interactions are only a few of the interactions that may be seen in the diagram of 2D interactions.

Diosgenin
Diosgenin is a sapogenin, a naturally occurring substance found in the Dioscorea (wild yam) species that serves as the precursor for the synthetic production of several steroids, including cortisone, pregnenolone, and progesterone. It functions as a metabolite, antineoplastic agent, antiviral agent, and apoptosis inducer [16]. It interacts with the PCSK9-disruptor complex by forming a Conventional Hydrogen bond with Leu 118, a Pi-sigma bond with Phe 115, a Carbon Hydrogen bond with His 116, and Alkyl bonds with Val 79 and Leu 119 (Fig. 2).

Table 1: Top binding affinity results obtained using PyRx

Ligand pubchem ID	Binding affinity (Kcal/mol)	Name of phytocompound
purified_7kfa_99474 _uff_E=741.60	-7.7	Diosgenin
purified_7kfa_441900 _uff_E=753.48	-7.6	Yamogenin
purified_7kfa_73145 _uff_E=694.14	-7.4	beta-Amyrin
purified_7kfa_122857 _uff_E=781.09	-6.9	Germanicol
Purified_7kfa_15560540 _uff_E=821.42	-6.8	Miliacin

Yamogenin
Yamogenin is a natural product, a triterpenoid found in Cordyline australis, Solanum spirale, and other organisms [16]. It forms a Pi-sigma bond with Phe 115, a Carbon Hydrogen bond with His 116, and Alkyl bond with Val 79 and Leu 118 and 119 (Fig. 3).

Germanicol
Germanicol is a pentacyclic triterpenoid, a natural product found in Barringtonia racemosa, Euphorbia nicaeensis, and other organisms [16]. It interacts significantly with the PCSK9-disruptor complex and forms conventional hydrogen bonds with it (Fig. 4).

Beta-amyrin
Beta-amyrin is a pentacyclic triterpenoid that is oleanane, a natural product found in Ficus pertusa, Ficus septica, and other organisms [16]. PCSK9-disruptor complex forms a bond with beta-Amyrin during their interaction (Fig. 5).

Miliacin
It forms a Carbon Hydrogen bond with residue Glu 132 while interacting with the PCSK9-disruptor complex (Fig. 6).

Drug-likeness prediction and ADMET analysis
Lipinski's rule of five, which bases a distinction between compounds that resemble drugs and non-drug-like molecules on five factors, is useful. The best-docked compounds' drug-likeness was predicted using Lipinski's rule of five, and ADME analysis was carried out using the SwissADME web server [13] (Table 2). SwissADME tool was used to do BOILED-egg analysis as well. In addition, the Boiled-egg analysis method was used to predict the passive brain access blood–brain barrier (BBB) and gastrointestinal absorption human intestinal absorption (HIA) of particular phytocompounds with the aid of the SwissADME program (Fig. 7) [19]. In addition, the standard scale was used to examine the water solubility (LogS), HIA, BBB, Permeability Glycoprotein substrate (Pgp-sub), carcinogenic effects, and Lipinski's rule validation of the best-docked compounds. The water solubility (LogS), HIA, BBB, Pgp-sub, carcinogenic effects, and Lipinski's rule validation of the best-docked compounds were also examined within the standard scale.) [11]. In addition, the standard scale was used to examine the water solubility (LogS), HIA, BBB, Pgp-sub, carcinogenic effects, and Lipinski's rule validation of the best-docked compounds. The water solubility (LogS), HIA, BBB, Pgp-sub, carcinogenic effects, and Lipinski's rule validation of the best-docked compounds were also examined within the standard scale. The BOILED-Egg plot was observed to gain an insight into the molecules for BBB, HIA, and PGP, which helped select the molecules that were satisfying those parameters allowing the molecules to be BBB permeant, show high gastrointestinal absorption and Pgp-sub (Fig. 7). Molecules being out of range indicates that the above-mentioned conditions were not satisfied.

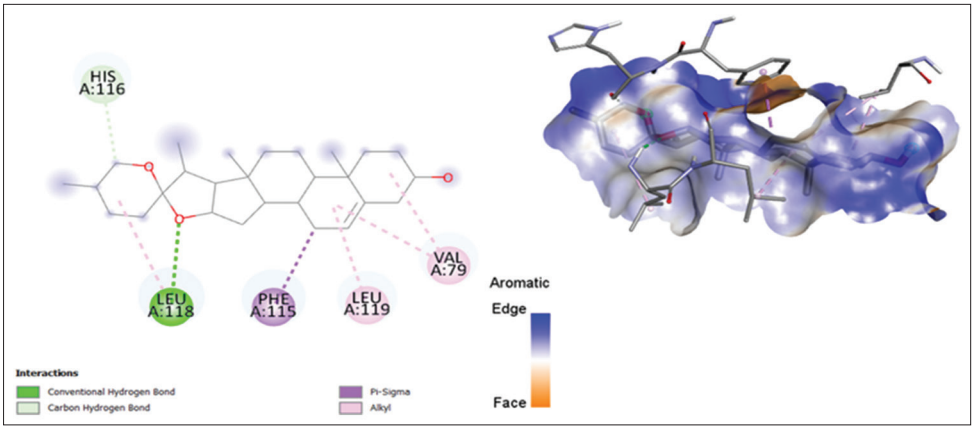

Fig. 2: 2D and 3D diagrams of interactions of diosgenin with proprotein convertase subtilin/kexin type 9-disruptor complex (7KFA)

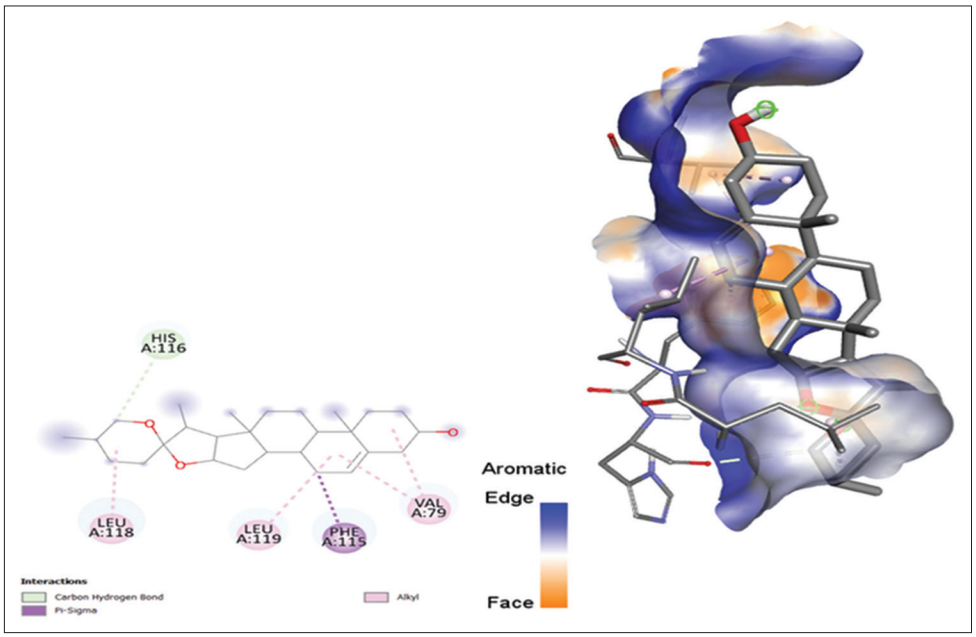

Fig. 3: 2D and 3D diagrams of interactions of yamogenin with proprotein convertase subtilin/kexin type 9-disruptor complex (7KFA)

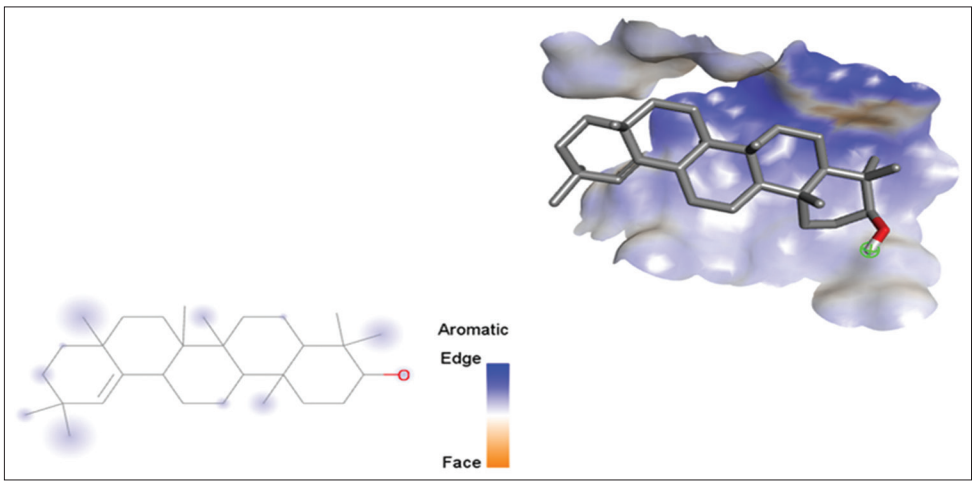

Fig. 4: 2D and 3D diagrams of interactions of germanicol with proprotein convertase subtilin/kexin type 9-disruptor complex (7KFA)

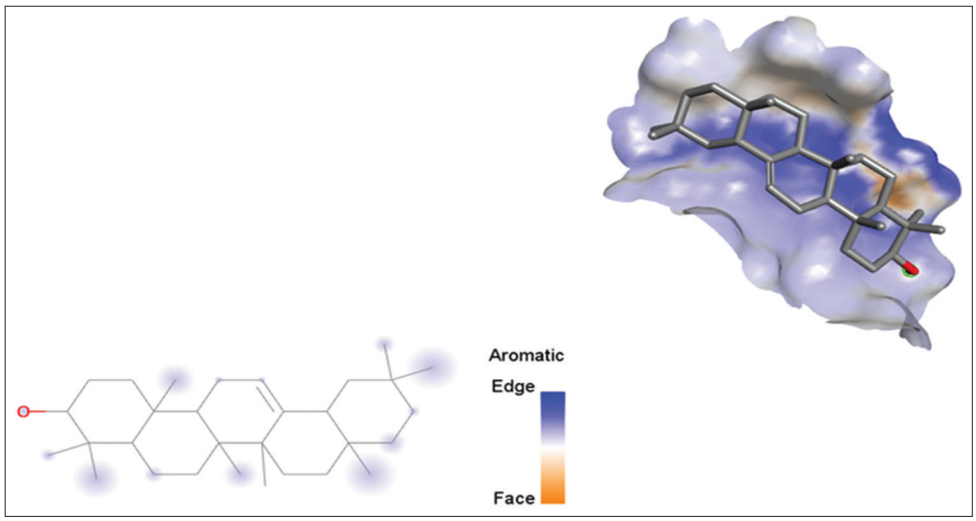

Fig. 5: 2D and 3D diagrams of interactions of beta-amyrin with proprotein convertase subtilin/kexin type 9-disruptor complex (7KFA)

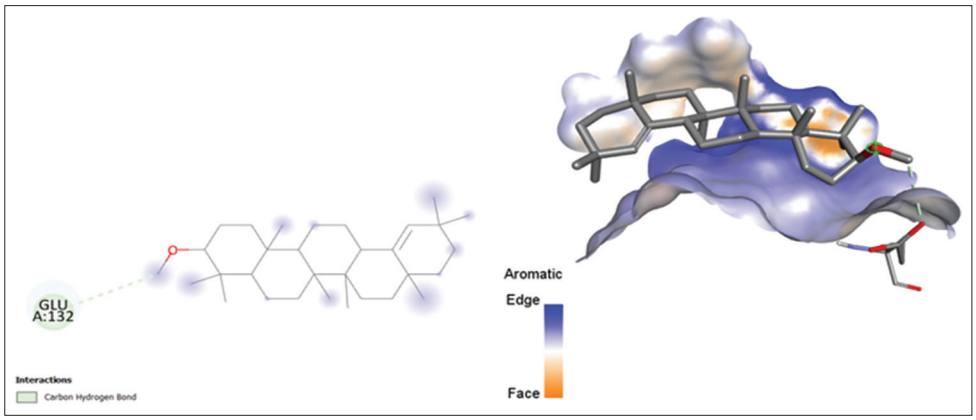

Fig. 6: 2D and 3D diagrams of interactions of miliacin with proprotein convertase subtilin/kexin type 9-disruptor complex (7KFA)

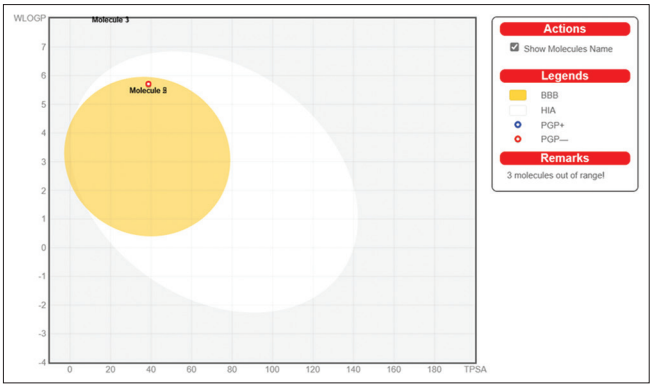

Fig. 7: BOILED-Egg analysis of top 5 docked compounds from IMPPAT server

Table 2: ADME analysis of best-docked compounds from IMPPAT server using swissADME based on Lipinski's rule

Molecule names	Molecular weight	H-bond acceptors	H-bond donors	Molar refractivity	LogP
Miliacin	440.74	1	0	139.61	7.12
beta-Amyrin	426.72	1	1	134.88	6.92
Diosgenin	414.62	3	1	121.59	4.94
Germanicol	426.72	1	1	134.88	6.92
Yamogenin	414.62	3	1	121.59	4.94

ADME: Absorption, distribution, metabolism, excretion, IMPPAT: Indian medicinal plant, phytochemistry and therapeutics

DISCUSSION

Familial hypercholesterolemia is a lipid disorder where LDL levels in the body are high. Many medically prescribed lipids-lowering drugs such as Probucol, Neomycin, and D-thyroxine reduce LDL concentrations by 10% to 15% in single drug use [17]. Among the two causes of this disorder, the genetic cause was studied in this research, which led to the understanding of the mutations in the PCSK9 gene that either caused gain or loss of function in the gene. The main function of this gene is to synthesize the PSCK9 protein responsible for the regulation of plasma cholesterol homeostasis through LDLR degradation [18]. An identified 3D structure of PCSK9 complex with PCSK9i, a 13 mer cyclic peptide LDLR disruptor complex, was seen impairing the normal functioning of PCSK9 protein. LDL is removed at a slower rate from the liver when the LDLR activity is disrupted, leading to elevated levels of LDL-C. This is called the gain-of-function mutation in the PCSK9 gene or the loss-of-function of the LDLR gene, which is the root cause of hypercholesterolemia. This made the PCSK9-disruptor (PDB ID: 7KFA) an ideal target for this research study, and Ramachandran plot analysis measured the purity of the protein molecule (7KFA).

Proso-millets (*P. miliaceum*) are proven for their natural medicinal properties against lipid-related disorders [6]. IMPPAT, an Indian medicinal plant database, was used to retrieve 21 natural phytocompounds from *P. miliaceum* as the plant source for this purpose. With the help of molecular docking tools and techniques, it was possible to identify the most suitable five compounds for drug development. PyRx, a molecular docking tool, was used to dock the phytocompounds from *P. miliaceum* on the target molecule (7KFA). BIOVIA Discovery Studio Visualizer, a molecular visualization tool, was used to further visualize the best-docked compounds' both 3D and 2D structures of the ligand-receptor interactions.

Toxicity prediction of the top 5 best-docked compounds from *P. miliaceum* was performed using the ProTox-II web server (ProTox-II – [charite.de]) [14]. An essential step in developing a drug's design is predicting a compounds toxicities. In addition to being quicker than testing harmful doses on animals, computational toxicity calculations can also help to cut down on the number of animal tests. It was discussed that these docked molecules were safe to be further processed as a drug as the phytocompounds were less toxic. Compounds with LD50 values >5000 mg/kg, falling in toxicity class 6 are considered to be non-toxic [14].

Among the top 5 docked compounds, namely, Diosgenin, Germanicol, beta-Amyrin, Miliacin, and Yamogenin, the two compounds Diosgenin and Yamogenin are the most suitable candidates for drug development considering their binding affinities, ADME properties along with their toxicities and BOILED-egg analysis. These two compounds are BBB permeant, high in GIA, and capable of binding with the PCSK9-disruptor complex. This binding will suppress the PCSK9-disruptor complex activity and hence improve the PCSK9 protein function to degrade LDLR, eventually regulating the LDL levels.

CONCLUSION

The objective of the current study regarding the molecular docking of natural phytocompounds from *P. miliaceum* on the PCSK9 complex with PCSK9i a 13 mer cyclic peptide LDLR disruptor (PDB ID: 7KFA) was achieved. Among the many compounds obtained from our plant source, five compounds, namely, beta-Amyrin, Diosgenin, Germanicol, Miliacin, and Yamogenin were best docked, out of which Diosgenin and Yamogenin emerged to be prominent candidates. Thus, using computational methods and technologies, we were able to stop our protein molecule complex's debilitating activity and attempt to introduce a pharmacological therapy.

ACKNOWLEDGMENT

I hereby acknowledge the Department of Bioinformatics, BioNome, Bengaluru, India, for providing computational facilities and support in

the scientific research services. I thank Mr. Shubham Wanarase for his assistance throughout the project.

AUTHORS' CONTRIBUTION

All the authors have contributed equally to the paper.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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