Original Research

IDENTIFICATION OF HMG CoA REDUCTASE INHIBITOR FROM NUTRACEUTICALS FOR ATHEROSCLEROSIS

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ABSTRACT

Atherosclerosis (also known as Arteriosclerotic Vascular Disease or ASVD) is a condition in which the artery wall thickens due to the deposition of fatty materials such as cholesterol. Atherosclerosis can affect any artery in the body, including arteries in the heart, brain, arms, legs and pelvis. Atherosclerosis develops from low-density lipoprotein molecules (LDL) becoming oxidized by free radicals, particularly oxygen free radicals (ROS). A key target enzyme to inhibit Atherosclerosis is 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. The present study was designed to find the potential inhibitor from the nutraceuticals for Atherosclerosis using *in silico* methods. PDB, Catalytic site atlas and PubChem database were used to get the 3D structure of protein, active site residues and ligand structures, respectively. Glide (a Schrodinger module) was used to dock HMG-CoA reductase with nutraceutical compounds. From the results, Gallotannin, Cynaroside and Hesperidin were found to be better inhibitors for HMG-CoA reductase than the synthetic drug fluvastatin. Hence, this study suggests that Pomegranate, Grapes, Green tea, Artichoke and Citrus limon that contain these compounds which were found to be HMG CoA reductase inhibitors can be used as dietary supplements.

Key words: Atherosclerosis, HMG-CoA reductase, nutraceutical compounds, docking studies.

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INTRODUCTION

In the present scenario, fast foods rich in fatty ingredients which may lead to heart diseases, become inevitable in the diet of the people. Atherosclerosis is a disease of arterial blood vessels where fats, cholesterol, blood cells and fibers form hardened plaques on the arterial wall and these plaques restrict blood flow to tissues such as the heart and brain by narrowing the artery[1]. These plaques can suddenly rupture, resulting in blood clots that completely block blood flow and lead to heart attack or stroke[2]. Atherosclerosis can be caused by high blood pressure, high fat and high cholesterol diets, smoking, diabetes, low High Density Lipoprotein-cholesterol concentrations, hypertension and obesity[3]. Cholesterol is a main causing agent of Atherosclerosis whose level in blood was affected by many factors including the lifestyle choices, the diet, physical activity and weight. Atherosclerosis follows the deposition, retention and oxidative modification of lipoproteins, especially low-density lipoprotein (LDL) in the walls of large arteries[1].

A key enzyme in the sterol and nonsterol isoprenoids biosynthesis pathway is 3-Hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase. This enzyme is highly regulated at the level of synthesis as well as at the level of degradation of the protein[4]. HMG-CoA reductase (HMGR) catalyzes the rate-determining reaction of the conversion of HMG-CoA to mevalonic acid in cholesterol and polyisoprenoid biosynthesis[5]. The well known inhibitors of HMG-CoA reductase are statins with usage in cholesterol-lowering therapy and reduce the incidence of myocardial infarctions and stroke[6]. Statin treatment is not safe when consumed for a long period as it leads to several side effects[7]. In the case of Atherosclerosis there is no permanent cure through treatment with synthetic drugs as it only acts as a reliever. When compared to the synthetic drug, nutraceutical compounds are very cheap and easily available and non toxic. Docking is an efficient *in silico* method playing an ever increasing role in structure-based drug design[8]. The present study was aimed to find potential inhibitor for HMG-CoA reductase from nutraceutical compounds using docking studies.

MATERIALS AND METHODS

Retrieval of protein structure & Active site prediction

The 3D structure of HMG CoA reductase (PDB ID: 1DQ8) was retrieved from the Protein Data Bank and the active site was predicted using Catalytic site atlas, a database documenting enzyme active sites and catalytic residues in enzymes of 3D structure[9].

Retrieval of ligands

The structure of the nutraceutical compounds were retrieved from the PubChem database. All these ligand molecules were retrieved from the PubChem database as .SDF file format and they were converted into PDB file format through ChemSketch and Open babel.

Interaction of HMG CoA reductase with ligands

Docking of flexible ligands and the receptor was carried out using Glide which performs a series of hierarchical searches for locations of possible ligand affinity within the binding site of a receptor. A rough positioning and scoring algorithm was applied during the initial search step, followed by torsional energy optimization on an OPLA-AA non-bonded potential energy grid for the candidate poses[10]. The docking results were evaluated based on the Glide Score and interactions.

RESULTS

Retrieval of protein structure

The 3D structure of HMG CoA reductase was retrieved from the Protein Data Bank and their PDB ID is 1DQ8. The 3D structure of HMG CoA reductase is shown in figure 1.

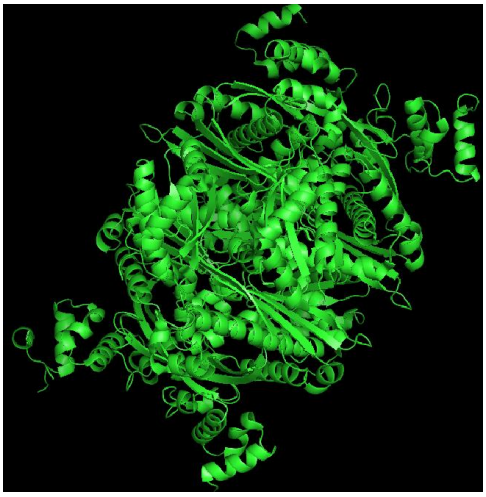


Figure 1: 3D structure of HMG CoA reductase

Active site prediction

Active site residues of HMG CoA reductase were obtained from Catalytic site atlas and the residues are GLU 559, ASP 690, LYS 735, SER 684, ARG 590, ASN 735, GLY 560, ASN 658 and LYS 692.

Retrieval of ligands

The 2D and 3D structure of ligands were obtained from PubChem database and Chemsketch and the structures are shown in table1.

Interaction of HMG CoA reductase with nutraceutical compounds

Docking analysis was carried out for the protein HMG CoA reductase with nutraceutical compounds using Glide (a Schrodinger module). The results were analyzed using the same package and the interactions involved in the active site of the target protein were examined. Lowest glide score represents the good activity. Hence, based on the glide score, the same predictions were performed for all the ligands and the results are shown in table 2.

HMG CoA reductase was docked with the ligands and the results were analyzed. The glide score of docked complex of Gallotannin was found to be –15.81 Kcal/mol. and had hydrogen bond interactions with ARG590, LYS692 and ASP690. The glide score of docked complex of Cynaroside was found to be -12.73 Kcal/mol and it formed hydrogen bonds with residues of ASP690, SER684, LYS735, LYS692, GLY560 and GLU559. The glide score of Hesperidin from docking was found to be -12.71 Kcal/mol. Hesperidin formed hydrogen bonds with GLY560, LYS691, ASP690 and ARG590.

Another four compounds Chlorogenic acid, Orientin, Rutin and Caffeic acid also had very low glide score and good interactions than the synthetic drug, fluvastatin (-5.3 Kcal/mol.). Interaction of HMG-CoA reductase with nutraceutical compounds are shown in figure2.

DISCUSSION

Ligands that bind specifically to certain proteins can lead to enzyme inhibition or modulation of signal transduction and thus can be used as drugs[11]. The drug is most commonly an organic small molecule which activates or inhibits the function of a biomolecule such as a protein which in turn results in a therapeutic benefit to the patient. One goal in drug design is to make drugs which bind to their target with the highest binding affinity[12]. The inhibition of HMG CoA reductase can be considered as a valid drug target as it plays a key role in Atherosclerosis. There is no permanent relief for Atherosclerosis by using synthetic drug fluvastatin and it gives some side effects also. To overcome this problem, this study is mainly focused on the nutraceutical compounds.

Lipinski's rule states that compound classes that are substrates for biological transporters are exceptions to the rule[13]. In another report only 70% of the drugs fit into the Lipinski's rule, and it would be unwise to use these criteria so stringently that the other 30% of profitable drugs on the market were excluded from consideration[14]. Natural compounds are another important exception of ADMET properties or RO5 (Rule Of five) [15]. Hence, the phytocompounds and the nutraceutical compounds need not to be tested for ADME Tox properties. With these parameters the compounds from natural origin which do not need any ADMET properties were taken into consideration for blocking the residues which are responsible for the inhibition of HMG CoA reductase.

Interaction of HMG CoA reductase with synthetic fluvastatin showed the glide score of -5.3 Kcal/mol . When compared, the Glide score of seven nutraceutical compounds were higher than the synthetic drug, fluvastatin. Among the seven compounds, Gallotannin, Cynaroside and Hesperidin were having very good Glide score of -15.81 Kcal/mol, -12. 73 Kcal/mol and -12.71 Kcal/mol, respectively. Based on this, it was found that the Gallotannin, Cynaroside and Hesperidin were very good inhibitors for HMG-CoA reductase. Further, these compounds are present in Pomegranate, Grapes, Green tea, Artichoke and Citrus limon and these plants are edible and having medicinal value.

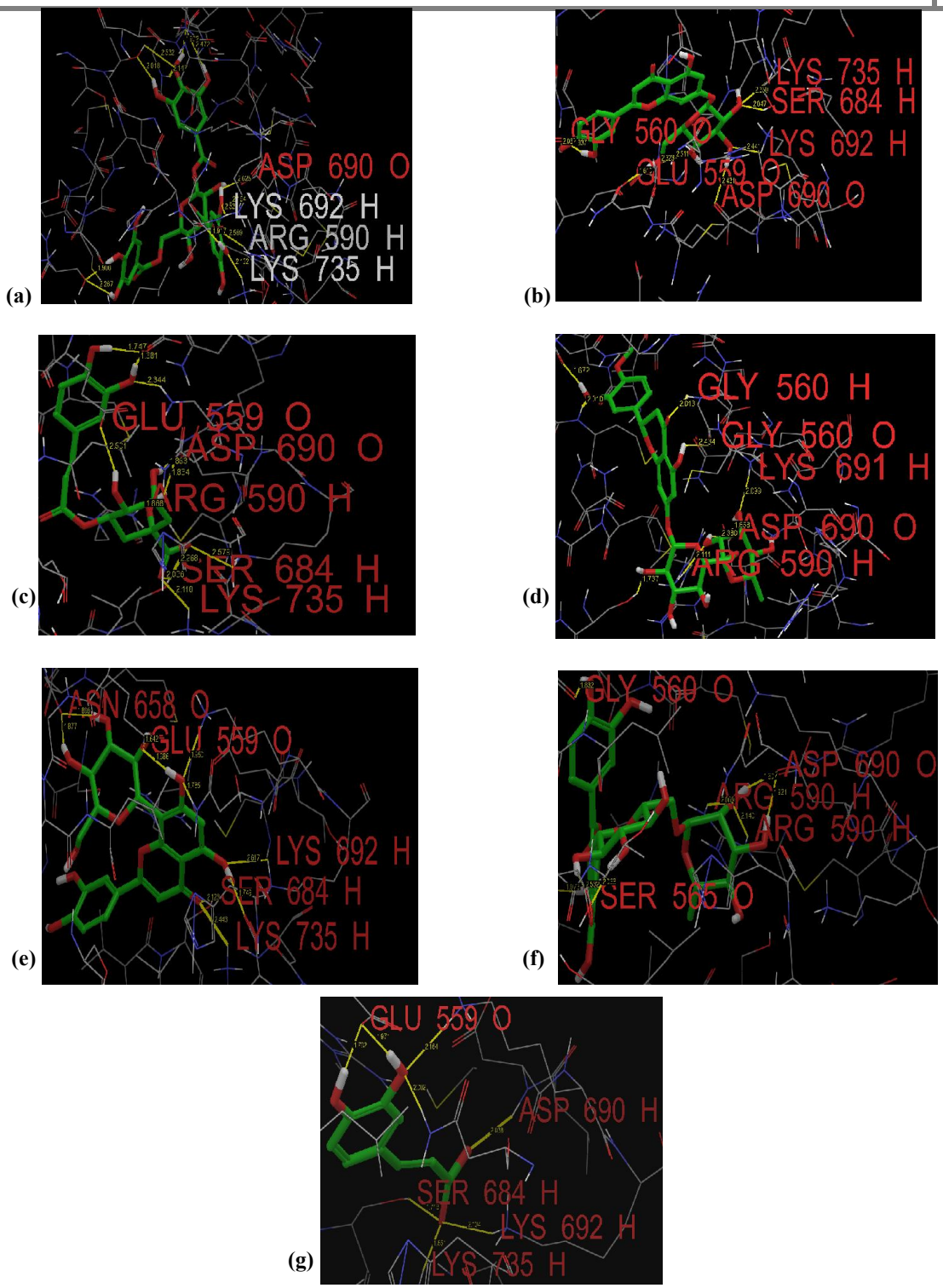
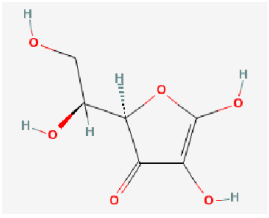
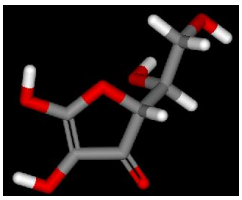
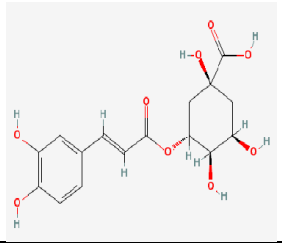
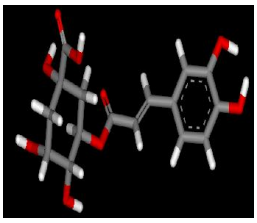
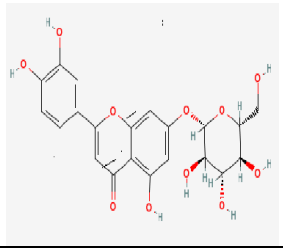
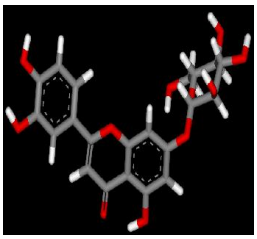
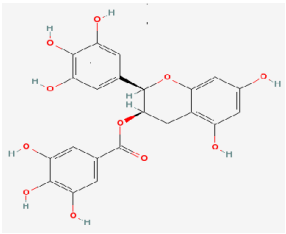
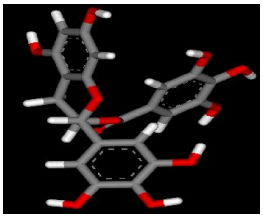
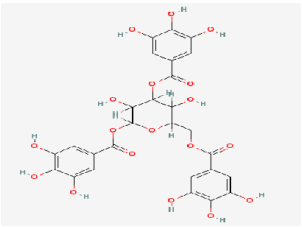
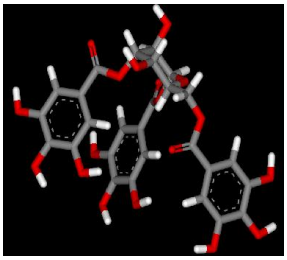
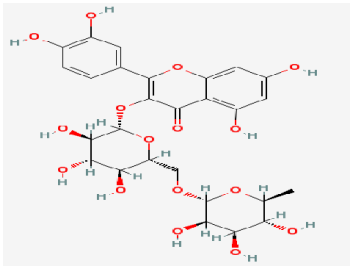
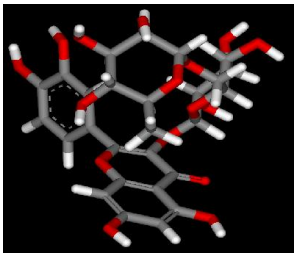
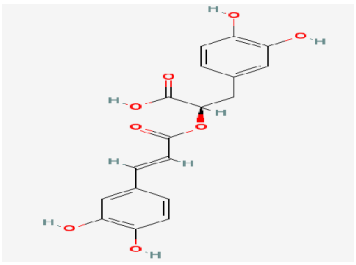
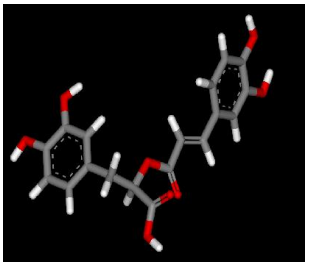
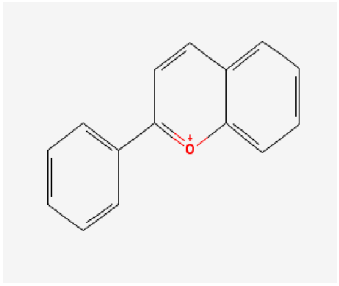
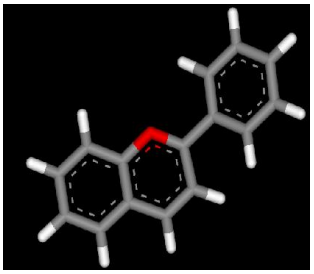
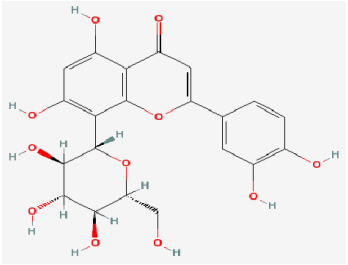
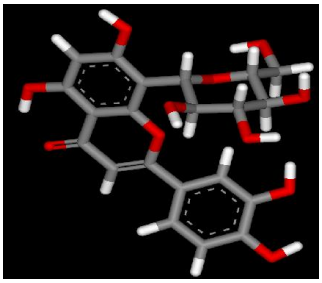


Figure 2: Interaction of HMG CoA reductase with nutraceutical compounds a) Gallotannin, b) Cynaroside, c) Hesperidin, d) Rutin, e) Chlorogenic acid, f) Orientin and g) Caffeic acid. Yellow color lines represent the hydrogen bond interactions present in docked complex.

Table 1: 2D and 3D structures of ligands

Name of the ligands	2D Structure of ligands	3D Structure of ligands
Ascorbic Acid		
Chlorogenic Acid		
Cynaroside		
EGCG		
Gallotannin		

Rutin		
Rosmarinic Acid		
Proanthocyanidin		
Orientin		

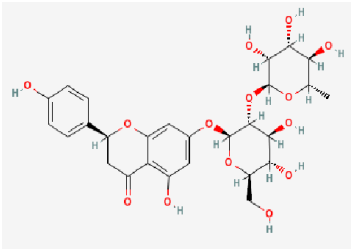
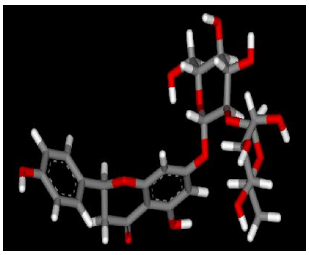
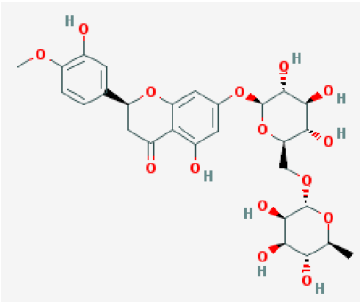
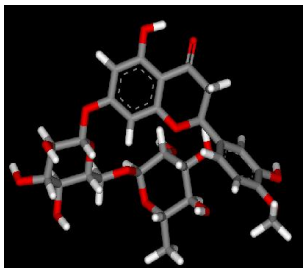
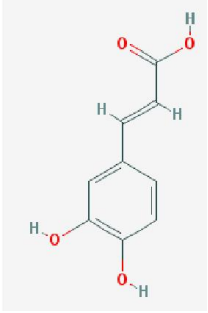
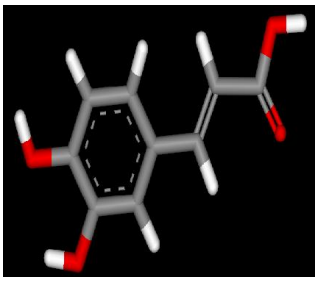
Naringin		
Hesperidin		
Caffeic acid		

Table 2: The results of interaction of HMG-CoA reductase with nutraceutical compounds

S.No.	Name of the nutraceutical compound	Glide score (Kcal/mol)	Number of interactions	Interacting residues and Bond length (Å)
1	Gallotannin	-15.81	13	ARG590 (2.569, 2.152), LYS692 (2.534), ASP690 (2.625)
2	Cynaroside	-12.73	8	ASP690 (2.431), SER684 (2.047), LYS735 (2.350), LYS692 (2.444), GLY560 (1.837), GLU559 (1.610)
3	Hesperidin	-12.71	9	GLY560 (2.013, 2.434), LYS691 (2.099), ASP690 (1.658), ARG590 (2.111)
4	Chlorogenic acid	-11.52	11	GLU559 (2.501), ASP690 (1.863), ARG590 (1.868), SER684 (2.579), LYS735 (2.118)
5	Orientin	-11.48	10	LYS735 (2.443), LYS692 (2.617), SER684 (1.749), ASN658 (1.896), GLU559 (1.950)
6	Rutin	-11.36	8	GLY560 (1.832), ASP690 (1.607), ARG590 (2.069, 2.140), SER565 (2.532)
7	Caffeic acid	-11.20	8	GLU559 (1.671), ASP690 (2.038), SER684 (1.718), LYS692 (2.134), LYS735 (1.551)
8.	Fluvastatin	-5.3	4	LYS 735 (1.730, 2.550), GLU559 (1.772), ASN755 (2.080)

Conclusion

From this study, it was concluded that all the seven nutraceutical compounds showed better result than the synthetic drug and particularly Gallotannin showed the best result when compared to other the compounds. Finally it was concluded that all these compounds are present in edible plants Pomegranate, Grapes, Green tea, Artichoke and Citrus limon and it is better to afford for those nutraceuticals which are easily available and can be had as food supplements to avoid or reduce the risk of Atherosclerosis.

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Author contributions

MJ design the concept and guided the entire work, MSA carried out the work, PR drafting the manuscript and MI helped to carry out the work.

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