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MOLECULAR DOCKING STUDY OF ISOLATED PHYTOCONSTITUENTS FROM *BOUGAINVILLEA GLABRA* AND *MUCUNA PRURIENS*

Rakam Gopi Krishna[1] and Raja S.*[2]

[1]Department of Pharmaceutical Chemistry, Chaitanya College of Pharmacy Education and Research, Kishanpura, Hanamkonda, Warangal, Telangana State, India-506001
[2]GITAM Institute of Pharmacy, GITAM University, Visakhapatnam- Andhra Pradesh.

***Corresponding Author: Dr. Raja S.**
GITAM Institute of Pharmacy, GITAM University, Visakhapatnam- Andhra Pradesh.

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ABSTRACT
Cardiovascular diseases are common and result in much of mortality of people throughout the world. Myocardial infarction is particularly formidable in cardiovascular disease case. Natural products isolated from plants have played an imperative role in discovery of drugs against various diseases of heart. *In silico* docking techniques are being used to investigate the complementarily at the molecular level of a ligand and a protein target. Each of the natural compounds was docked with associated proteins individually, to determine the best binding affinity using Autodock4.2. Four different phytoconstituents *like quercetin 3-β-D-glucoside, hesperetin 7-rutinoside, genistein 4-β-D-glucopyranoside and β-sitosterol β-D-glucoside* were isolated from *Bougainvillea glabra* and *Mucuna pruriens* which were carried out for docking study. The present work was premeditated to assess the phytoconstituents based on their capability to bind with cardiovascular disarray receptors such as PKR1 (5KJY), C protein (1B09) and G Protein (3N7S). In the result, the compound quercetin 3-β-D-glucoside was found to possess highest binding affinity with energy of -8.582 towards the receptor PKR1 (Prokineticin receptor-1). Simultaneously, other ligands such as hesperetin-7-rutinoside (-7.037), genistein 4-β-D-glucopyranoside (-4.386) and β-sitosterol β-D-glucoside (-6.838) were shown low binding affinity towards the active site of the receptors like C protein (1B09), and G Protein (3N7S), respectively. The binding energy values towards Cprotein (1B09) for the ligands quercetin 3-β-D-glucoside, hesperetin-7-rutinoside, genistein 4-β-D-glucopyranoside and β-sitosterol β-D-glucoside were found to be -6.362, -5.071, -4.326 and -4.034 respectively. The binding energy values of all the ligands towards G protein (quercetin 3-β-D-glucoside -5.182, hesperetin-7-rutinoside -4.022, genistein 4-β-D-glucopyranoside -3.486 and β-sitosterol β-D-glucoside -3.123) were found to be comparatively less than other two receptors. Most of the drugs currently used for the treatment of heart related diseases produce side effects, and hence, study focused on plant-based compounds, which exhibit the less poisonous effect. In the current docking study a ligand quercetin 3-β-D-glucoside revealed the best fitness score with PKR1 (5KJY) receptor.

KEYWORDS: Cardiovascular diseases, *in silico* studies, C Protein, Ligand, Docking study.

INTRODUCTION
Molecular docking studies are used to find out the interaction between a ligand/drug and a protein at the atomic level which allows us to characterize the behavior of our compounds in the binding site of targets as well as to explain fundamental biochemical processes.[1] Each of the natural compounds was docked with associated proteins individually, to determine the best binding affinity using Autodock4.2.[2] Numerous software packages have been developed with the implementation of various molecular docking algorithms based on different search methods.[3] Computational (*In silico*) methods have been developed and widely applied to pharmacology hypothesis development and testing. These *insilico* methods include database searching, quantitative structure activity relationships, similarity searching, pharmacophore identification, computational modeling and docking. Such techniques have seen recurrent use in the discovery and optimization of novel molecules with affinity to a target, the clarification of absorption, distribution, metabolism, excretion and toxicity properties as well as physicochemical characterization.[4] Molecular docking methods are used to calculate how a protein interrelates with small vitamin-like molecules. This ability governs a significant part of the protein's dynamics which may enhance/inhibit its biological function.[5] Dual features are of paramount prominence in molecular docking studies: augmenting the candidate ligand for the accurate native conformation in the presence of which it can achieve a best fit orientation to bind with a protein of interest, and the conformational flexibility or elasticity of

ligand and protein.[6] Therefore, the exact estimate of the binding modes among the ligand and protein is of fundamental importance in modern structure-based drug design. Computer-based molecular modeling targets to speediness of drug discoveries by calculating potential efficiency of ligand-protein interactions preceding to laborious experiments and costly preclinical trials.

On the other hand, over the past year there have been some interesting and significant advances in computer based ligand protein docking techniques and related rational drug-design. Docking is estimated by energy values. As a result, the successful use of computational tools to help generate interesting new drugs for targeted receptors.[7] Molecular docking allows providing agonism/antagonism to the target receptor protein selected and the most favorable orientation for the formation of a stable complex and the position of one molecule (ligand) in relation to other molecule (target).[8] Docking allows reducing expenses and time due to carrying out the procedure that is similar to high-performance biological screening. The main objective of the current study was to find out most suitable positions and orientations of ligands in the ligand binding center of the enzyme or receptor and to evaluate the effect of isolated phytoconstituents from *Bougainvillea glabra* and *Mucuna pruriens* on cardioprotective activity.

MATERIALS AND METHODS

Docking Software's

Different types of software's like Pose viewer and Auto Dock 4.2 were used for the Molecular Docking investigation of heart related disease causing receptors.

Phytoconstituents /Ligands

Phytoconstituents or Ligands like quercetin 3-β-D-glucoside [2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-methoxy-4H-chromen-4-one,(2R,3S,5S)-2-(hydroxymethyl)tetrahydro-2H pyran-3,4,5-triol]; hesperitin-7-rutinoside [(2S)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-(((3S,6S)-3,4,5-trihydroxy-6-((((2S,4R,5R,6R)-3,4,5-trihydroxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)methyl)tetrahydro-2H-pyran-2-yl)oxy)chroman-4-one];genistein4-β-D-glucopyranoside[5,7-dihydroxy-3-(4-(((3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-2-yl)oxy)phenyl)-4H-chromen-4-one]; and β-sitosterol β-D-glucoside [(2R,3R,4S,5S,6R)-2-(((3S,8S,9S,10R,13R,14S,17R)-17-((2R,5R)-5,6-dimethylheptan-2-yl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a] phenanthren-3-yl)oxy)-6-(hydroxymethyl) tetrahydro-2H-pyran-3,4,5-triol]; were used for molecular docking study.

Potential Targets and Binding site

The 3D structures of cardioprotective drug targets such as PKR1 (5KJY), C-protein (1B09) and G-protein (3N7S) were obtained from PDB database.[9] The active sites in these receptors were determined based on the ligands in the crystallized structures.

Protein preparation

The co-crystal structures of various proteins were obtained from the RCSB protein data bank. Protein preparation wizard (PPrep) of Schrödinger suite has been used to prepare protein. The proteins were preprocessed separately by deleting the substrate co-factor and water molecules with in 5Å distance (water without H bonds), addition of missing loops and amino acids in PDB file using PRIME followed by optimization of hydrogen bonds were carried out to obtain a biologically active protein.

Ligand preparation

The 2D structures of the phytochemical compounds quercetin 3-β-D-glucoside, hesperetin-7-rutinoside, genistein 4-β-D-glucopyranoside and β-sitosterol β-D-glucoside, were converted to 3D structure in maestro 9.5. The 3D structures obtained were converted into structure data format (SDF) files and the activity was performed.

Prediction of Receptor-Ligand interactions

The interactions between the phytoconstituents and the receptors as docked complexes were analyzed by the pose viewer software.[10]

Docking

Docking was executed to acquire a population of probable conformations and orientations for the ligand at the binding site. After preparation of protein and ligands for docking, Virtual screening workflow was initiated, this includes docking of all the prepared ligands at active site of protein using HTVS (High Throughput Virtual Screening)[11], SP (Standard Precision)[12] and XP (Extra Precision) docking algorithms. Conformations with best docking score and pose from HTVS were subjected to SP and XP. Glide XP was finally employed for all docking calculations and was performed in Glide flexible docking. Selection of molecules was based on lowest docking score and interacting amino acids.[13] The average affinity for the best poses was taken as the final affinity value. Various proteins which are target for cardiovascular disease was selected based on the literature survey. The structures of all the receptors were obtained from PDB format. The active site characterization of the enzyme using Q-site finder showed that THR-143, LYS 125, GLY-285, ARG-283, LYS-395, SER-261, THR-287, TYR 142 and LEU 388 are the key amino acids forming active site.

RESULTS

Molecular Docking Results

In present study, Glide SP docking was performed and confirmed the conformation which shows binding affinity towards the cardiac proteins (PDB ID: PKR1 (5KJY), C Protein (1B090, G Protein (3N7S) by docking score and ligand interaction patterns. Molecules with more negative scores were selected as they imply greater

and stronger interaction with the concerned protein. The phytoconstituents/ligands were docked against four different target protein receptors such as PKR1, C Protein and G Protein. SP (Standard Precision) and XP (Extra Precision) docking workflow against cardiac proteins using selected ligands were employed and successfully identified few drug molecules as a potential hits.

PKR1 (5KJY)

Quercetin 3-β-D-Glucoside possess highest binding energy of -8.582, whereas genistein 4'-β-D-glucopyranoside possess least binding energy (-4.386). Hesperitin-7-rutinoside possess less binding energy (-7.037) when compared to the quercetin 3-β-D-glucoside. But the binding energy value (-6.838) of β-sitosterol β-D-glucoside was found to be more than that of genistein 4'-β-D-glucopyranoside. Among all the compounds quercetin 3-β-D-glucoside has highest binding energy when compared to other compounds and it exhibited good binding affinity towards PKR1 receptor. It was shown in table-1 and figure-1.

C Protein (1B09)

The study has revealed that quercetin 3-β-D-glucoside possess peak binding energy of -6.362. Hesperitin-7-rutinoside possess less binding energy of -5.071 which is nearer, to the quercetin 3-β-D-glucoside. The binding energy values of genistein 4'-β-D-glucopyranoside (-4.326) and β-sitosterol β-D-glucoside (-4.-34) was found to be less than that of quercetin 3-β-D-glucoside. The results were indicated in table-2 and figure-2.

G Protein (3N7S)

The binding energy value of isolated compounds with G Protein was found to be different. The docking studies have shown that quercetin 3-β-D-glucoside possess topmost binding energy of -6.362. Hesperitin-7-rutinoside possess binding energy of -4.022. The binding energy values of genistein 4'-β-D-glucopyranoside (-3.486) and β-sitosterol β-D-glucoside (-3.123) was found to be nearly similarly. But the binding energy as well as binding affinity of quercetin 3-β-D-glucoside was found to be more than the other compounds. The results were shown in table-3 and figure-3.

Table-1: Ligands Docking Study with PKR1 (5KJY).

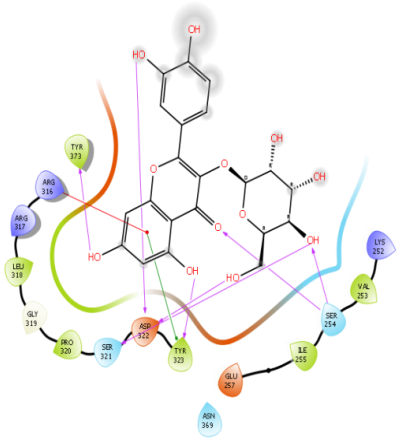
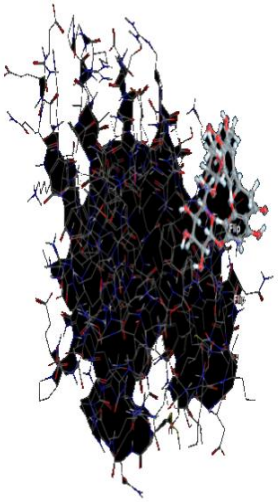
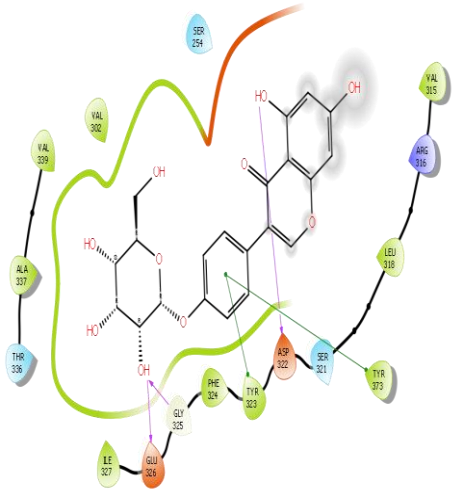
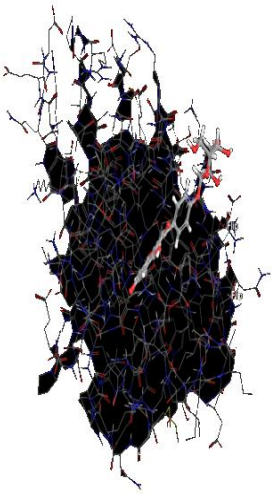
Ligand	Ligand Name	Binding Energy
Quercetin 3-β-D-glucoside	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-methoxy-4H-chromen-4-one, (2R,3S,5S)-2-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol.	-8.582
Hesperitin-7-rutinoside	(2S)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxy-2,3-dihydrochromen-4-one	-7.037
Genistein 4'-β-D-glucopyranoside	5,7-dihydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one,(2R,3S,5R)-2-(hydroxymethyl)-6-methyltetrahydro-2H-pyran-3,4,5-triol.	-4.386
β-Sitosterol β-D-glucoside	(2R,3S,5S)-2-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol, (3S,8S,9S,10 R,13S,14S)-3-methoxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene and 2,6-dimethylheptane and propane	-6.838

Table-2: Ligands Docking Study with C Protein (1B09).

Ligand	Ligand Name	Binding Energy
Quercetin 3-β-D-glucoside	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-methoxy-4H-chromen-4-one, (2R,3S,5S)-2-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol.	-6.362
Hesperitin-7-rutinoside	(2S)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxy-2,3-dihydrochromen-4-one	-5.071
Genistein 4'-β-D-glucopyranoside	5,7-dihydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one,(2R,3S,5R)-2-(hydroxymethyl)-6-methyltetrahydro-2H-pyran-3,4,5-triol.	-4.326
β-Sitosterol β-D-glucoside	(2R,3S,5S)-2-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol, (3S,8S,9S,10 R,13S,14S)-3-methoxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene and 2,6-dimethylheptane and propane	-4.034

Table-3: Ligands Docking Study with G Protein (3N7S).

Ligand	Ligand Name	Binding Energy
Quercetin 3-β-D-glucoside	2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-3-methoxy-4H-chromen-4-one, (2R,3S,5S)-2-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol.	-5.182
Hesperitin-7-rutinoside	(2S)-5-hydroxy-2-(3-hydroxy-4-methoxyphenyl)-7-[(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-[[(2R,3R,4R,5R,6S)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxymethyl]oxan-2-yl]oxy-2,3-dihydrochromen-4-one	-4.022
Genistein 4'-β-D-glucopyranoside	5,7-dihydroxy-3-(4-hydroxyphenyl)-4H-chromen-4-one,(2R,3S,5R)-2-(hydroxymethyl)-6-methyltetrahydro-2H-pyran-3,4,5-triol.	-3.486
β-Sitosterol β-D-glucoside	(2R,3S,5S)-2-(hydroxymethyl)tetrahydro-2H-pyran-3,4,5-triol, (3S,8S,9S,10 R,13S,14S)-3-methoxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthrene and 2,6-dimethylheptane and propane	-3.123

S.No	2 D Structure	3 D Structure
1.	Ligand-I 	
2.	Ligand- II 	

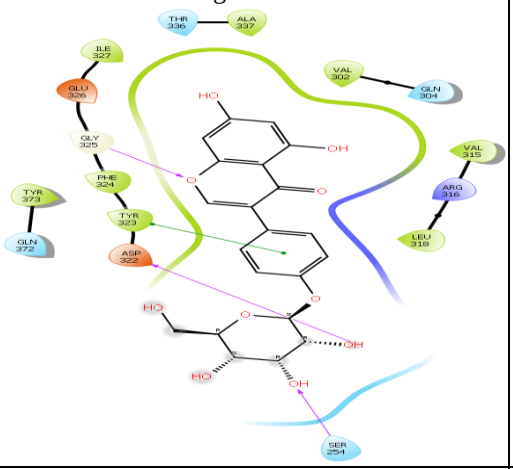
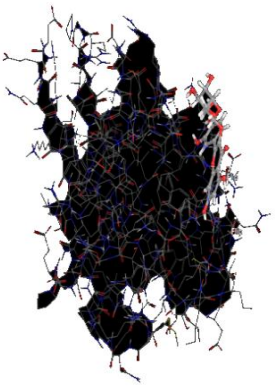
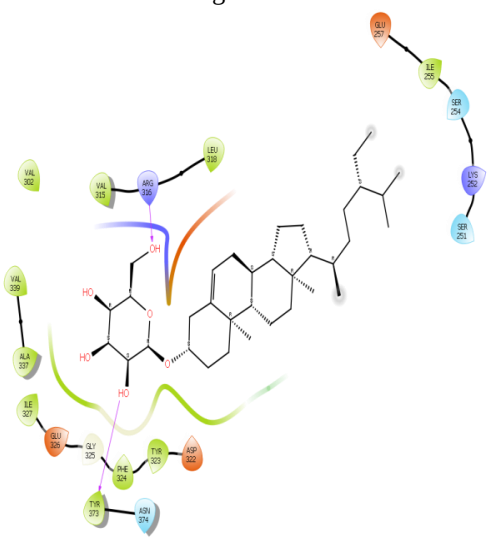
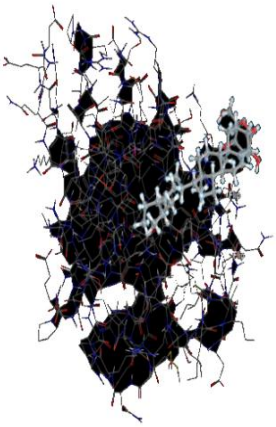
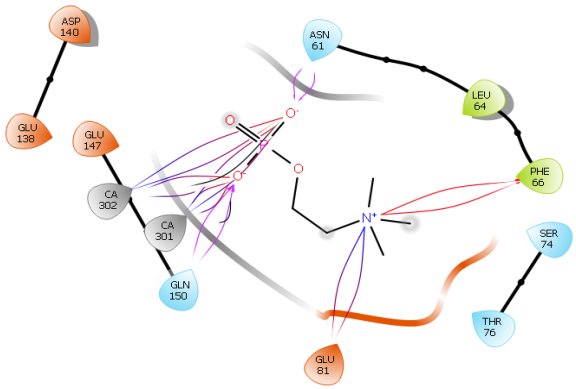
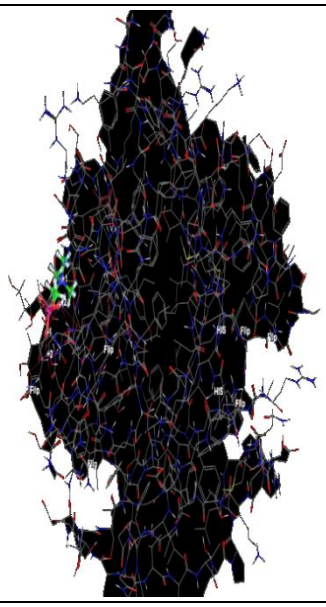
3.	Ligand-III 	
4.	Ligand-IV 	

Figure-I: Receptors and Ligand Interaction of PKR1 (5KJY) Protein data with 4 compounds.

S.No	2D Structure	3D Structure
1.	Ligand-I 	

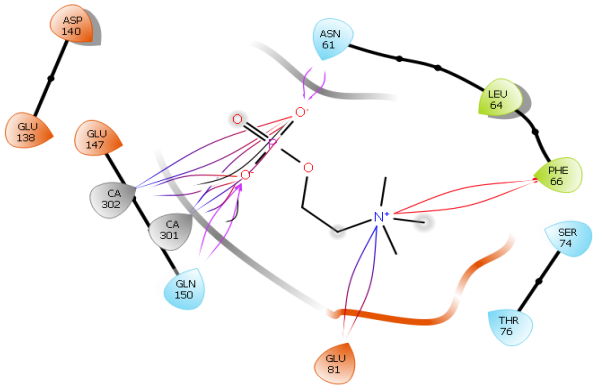
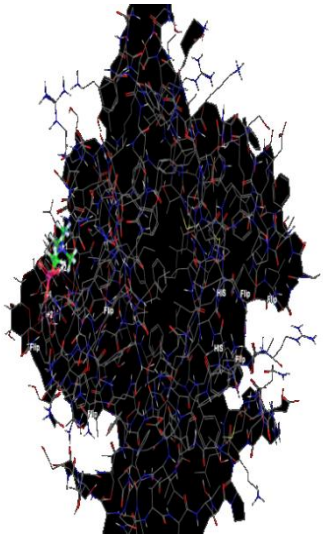
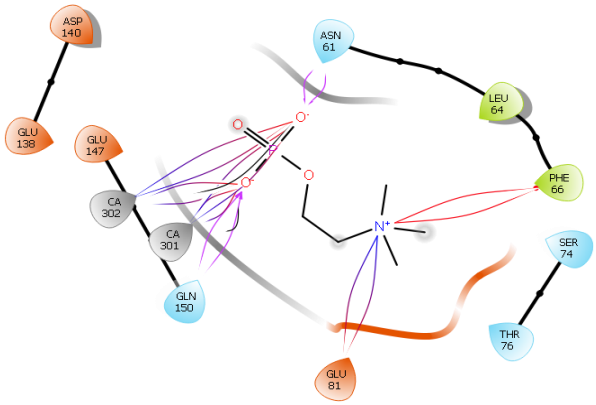
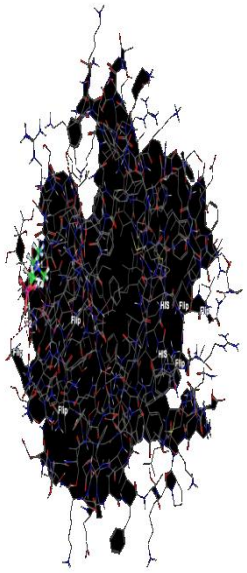
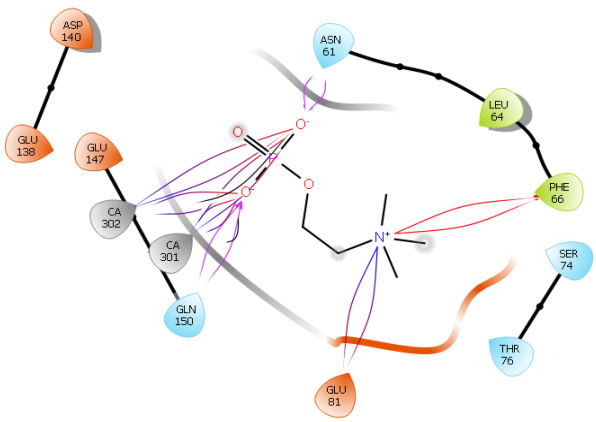
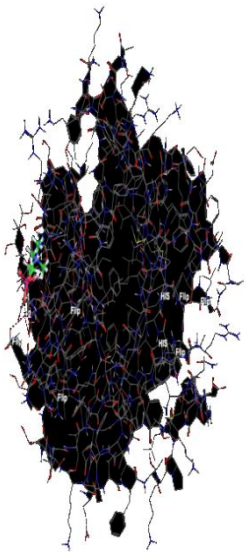
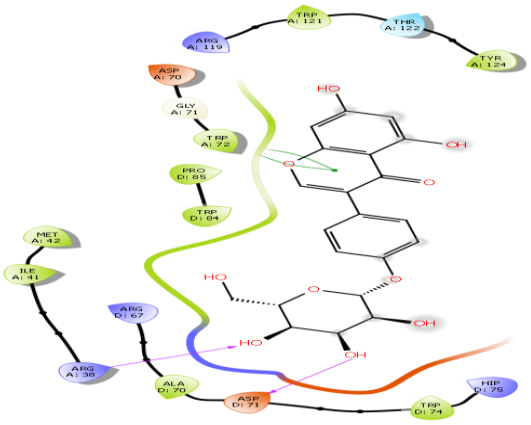
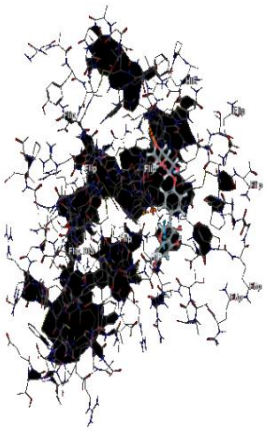
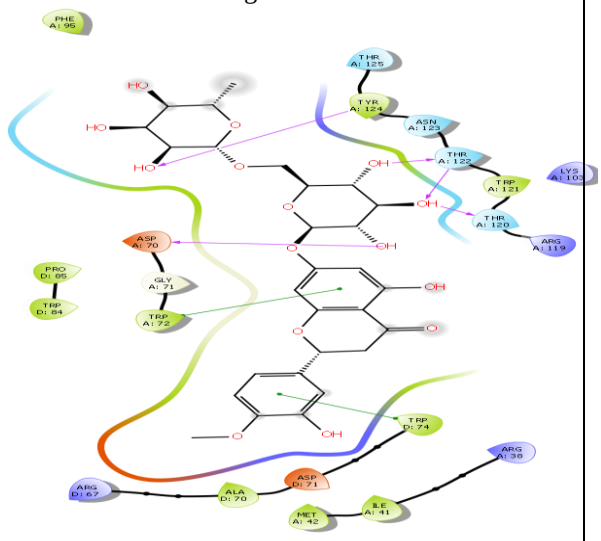
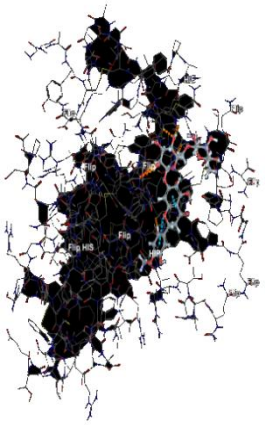
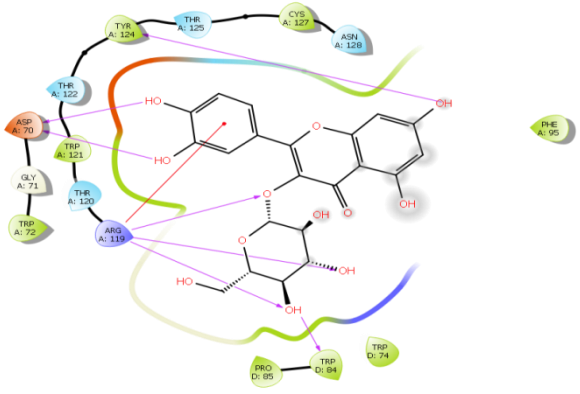
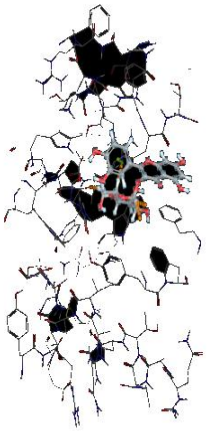
2.	Ligand-II 	
3.	Ligand-III 	
4.	Ligand-IV 	

Figure-II Receptors and Ligand Interaction of C Protein (1B09) data with 4 compounds.

S.No	2D Structure	3D Structure
1.	Ligand-I 	
2.	Ligand-II 	
3.	Ligand-III 	

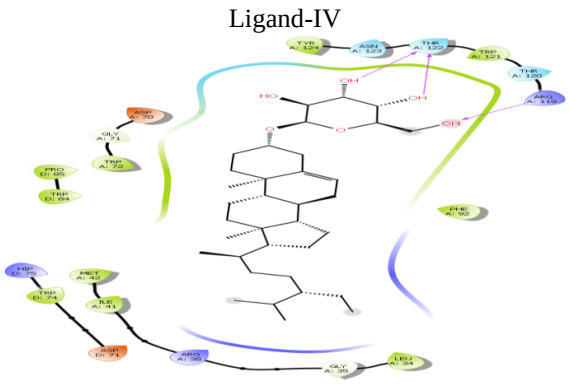
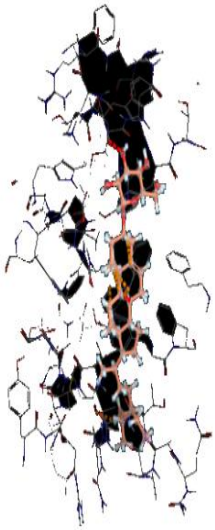
4.	Ligand-IV 	

Figure-III Receptors and Ligand Interaction of G Protein (3N7S) data with 4 compounds.

DISCUSSION

To ensure the interaction between the natural compounds and cardiovascular disease associated targets, it performed molecular docking analysis using Autodock4.2. Docking simulations were carried out by selecting "Autodock" as the docking engine. The entire receptor was defined as a binding site. Docking studies generated key information concerning the orientation of the inhibitors in the binding pocket of the target protein. All the compounds bind successfully to the active site of the target proteins. Docking of proteins with lead molecules were performed individually and binding the energy was calculated. Docking of small molecular compounds into the binding site of a receptor and estimating the binding affinity of the complex is an important part of the structure based drug design process.

In the current study three receptors PKR1 (5KJY), C Protein (1B09), G Protein (3N7S) were selected as drug targets for heart related diseases and their 3D structures were determined from protein data bank and their binding sites were determined. The ligand (quercetin 3-β-D-glucoside) from *Bougainvillea glabra* plant extract showed the highest dock score with receptor PKR1(5KJY) with binding energy -8.582, and hydrogen bond interaction with cardioprotective protein target when compared to other ligands. Similarly with other two receptors (C Protein 1B09 & G Protein 3N7S) it has shown good binding affinity with binding energy value of -6.362 & -5.182 respectively. The binding energy values of remaining ligands (hesperitin-7-rutinoside, genistein 4'-β-D-glucopyranoside and β-sitosterol β-D-glucoside) from *Bougainvillea glabra* and *Mucuna pruriens* had shown less value than that of quercetin 3-β-D-glucoside. These results suggest that our 4 natural compounds potentially interfere with cardiac associated targets, which should prompt further investigations to expose the mechanism of our compounds against heart related diseases. Thus from the docking scores it can be confirmed that the four isolated compounds bind successfully with the drug targets and the compounds may be used as cardiovascular drugs.

CONCLUSION

Based on *in silico* docking result it has been observed that the selected flavonoids, has greater affinity to bind with the receptors. The docking study revealed that the ligand quercetin 3-β-D-glucoside revealed the best binding affinity value with cardioprotective receptor. The binding energy values of remaining ligands have shown less affinity towards all the receptors. This effort will help to recognize the new compounds, which may help to generate important medicinal preparations. In future it may lead to the development of innovative PKR1 (5KJY), C Protein (1B09) & G Protein (3N7S) agonists for cardioprotective diseases.

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