Chapter - 4

DOCKING BASED SCREENING AND VALIDATIONS OF SOME SELECTED NATURAL INHIBITORS AGAINST PDK-1KINASE

4.1 Introduction

The side effects of the currently used drug made us to explore an alternative and traditional approach to finding out new drug compound from the natural origin, compounds which are having anti cancerous activity and also not having any side effects to human normal cell (Kawaii et al., 1999; Pouget et al., 2001). These plant compounds which are also having high binding affinity for many receptors which are over expressed in many cancers could lead to be an alternative treatment for cancer. Natural compounds are far beneficial than synthetic compounds due to less toxicity, more accessibility and being less expensive. Flavonoids are a group of natural compounds with antiviral, anti-oxidative, anti-inflammatory as well as anti-tumoral effects (Sies, 2010) . Flavonoids are structurally characterized by two or more aromatic rings (ARs), are a polyphenols which can be further categorized into flavonols, flavones and isoflavones on the basis of direction of the phenyl ring and state of substitution (Beecher,2003). Compounds from the flavonoid family were thus selected for screening their inhibitory effects on PDK-1 kinase through in silico molecular docking and molecular dynamic simulation study.

4.2 Material and methods

4.2.1 Work Plan

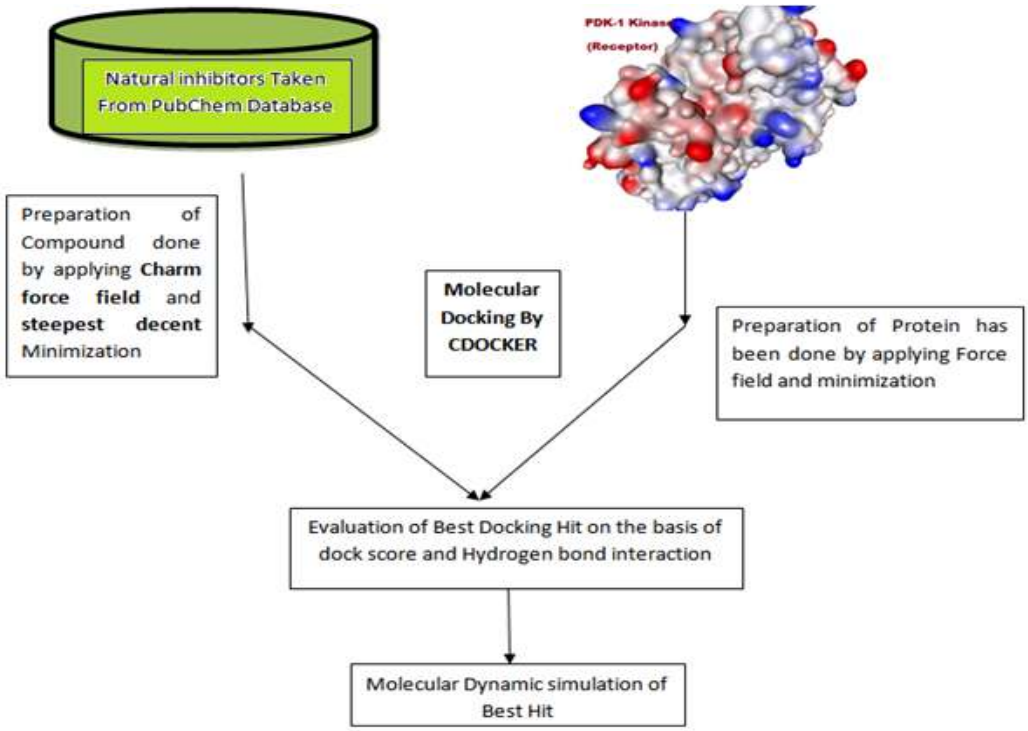


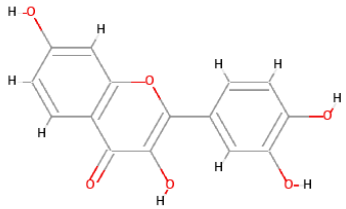
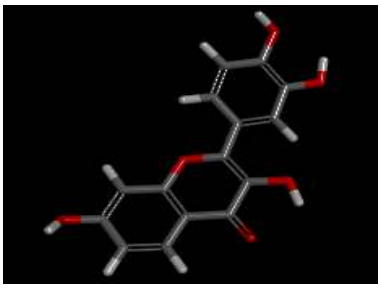
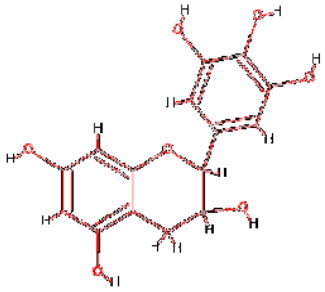
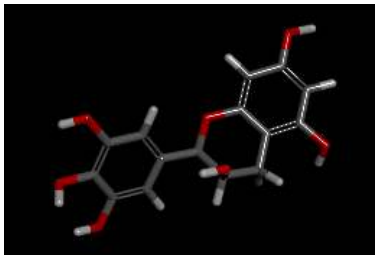
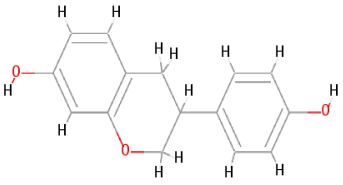
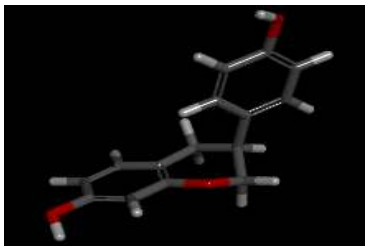
Figure 4.1: Schematic representation of complete work plan

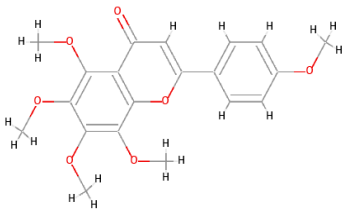
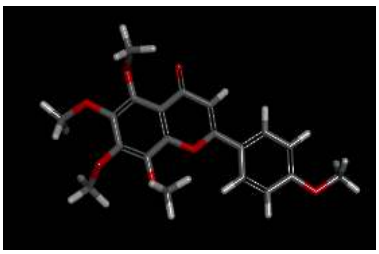
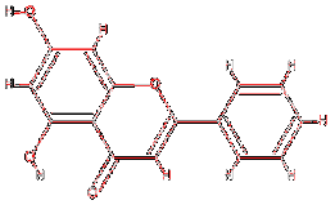
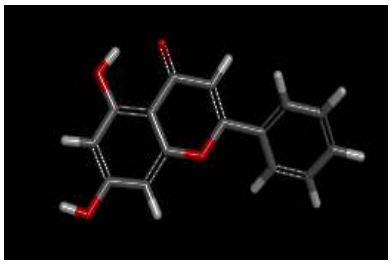
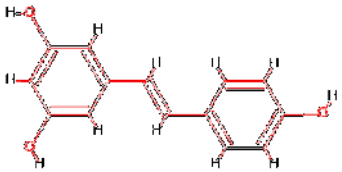
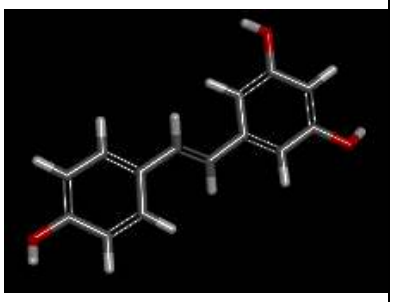
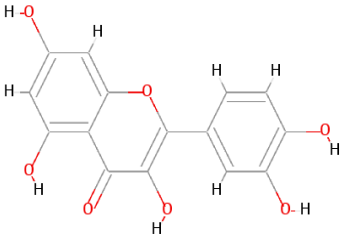
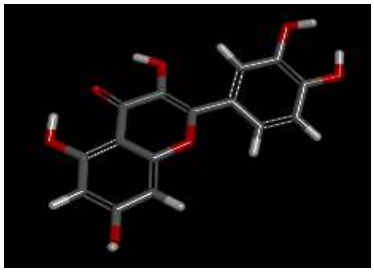
4.2.1 Compounds used for study

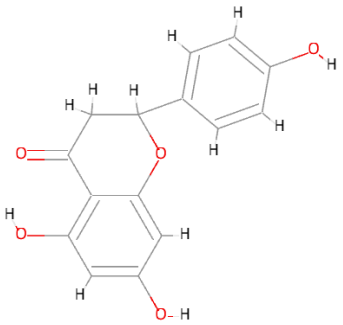
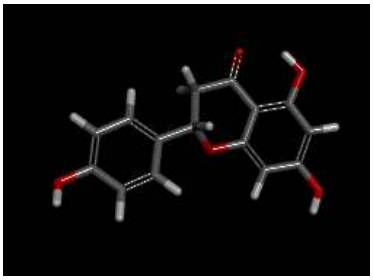
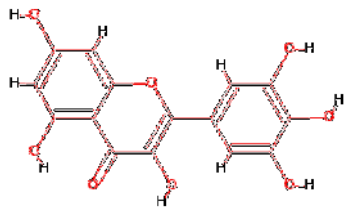
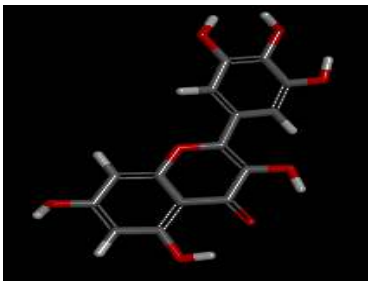
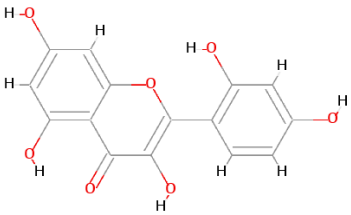
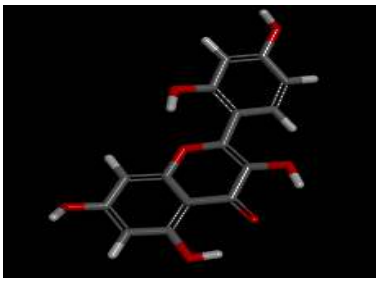
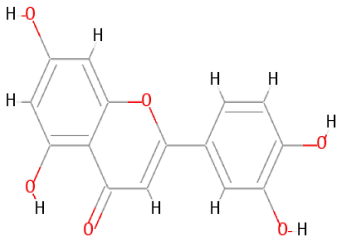
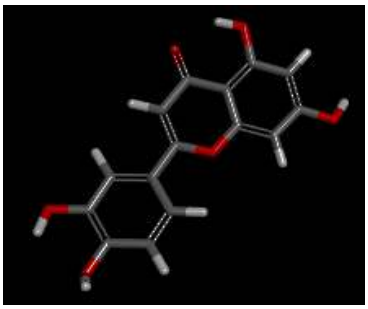
Totally 18 flavonoids were identified from the Pubmed literatures which shows inhibitory effects towards different cancers were selected for the studies. The three dimensional structure of all natural compounds were downloaded in .sdf format using Pubchem database which were further converted in to PDB format using Discovery Studio 3.5 viewer(**Accelrys Inc., San Diego, CA)**. Then CharMM based force field (Books et al., 1983) was applied to them and further, was subjected to

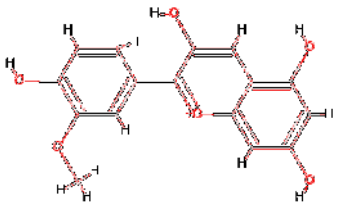
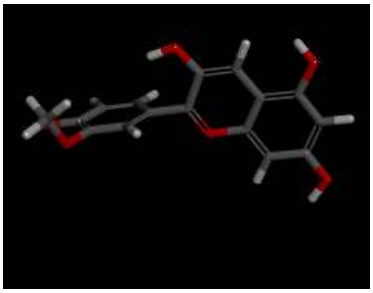
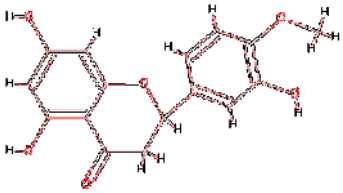
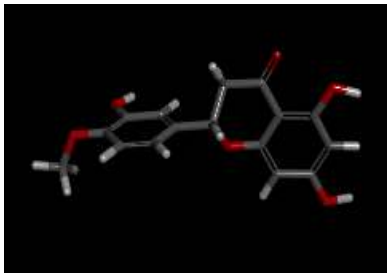
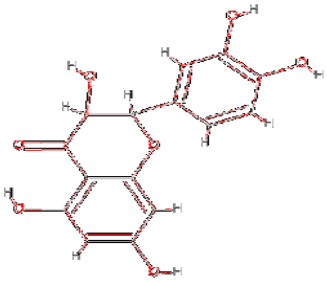
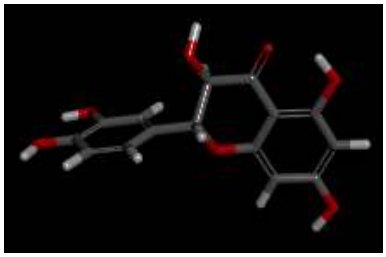
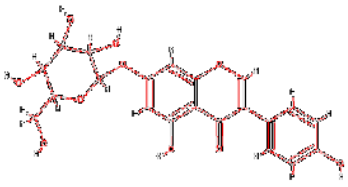
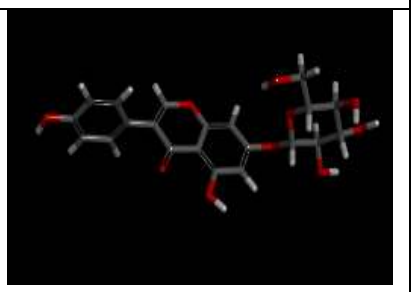
single step minimization using steepest descent method for 500 steps at RMS gradient of 0.01. Final minimized structure was used for docking studies. Detailed list of selected compounds were listed in Table no 4.1:

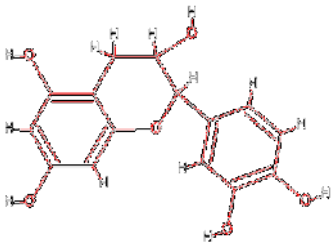
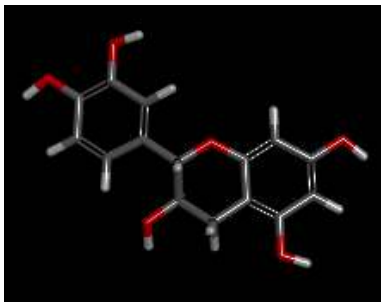
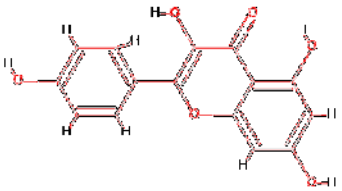
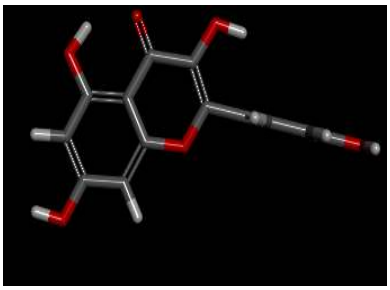
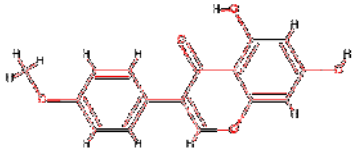
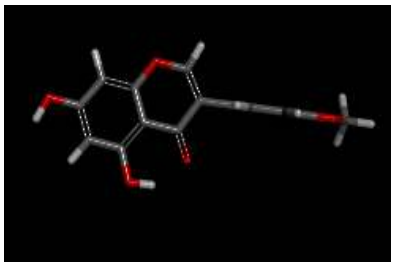
Table No: 4.1: Detailed structures of natural compounds used for study

Inhibitors	2D Structure	3D Structure
Fesetin		
Epigallocatechin		
Equol		

Tangeretin		
Crysin		
Reservetol		
Quercetin		

Narigenin		
Myricetin		
Morin		
Luteolin		

Peonidin		
Hesperetin		
Taxifolin		
Geninstein		

Catechin		
Kaempferol		
Biochanin A		

4.2.2 Molecular docking

4.2.2.1 CDOCKER

Molecular docking was performed by CDOCKER docking method (Wu et.al.,2003) implemented in Discovery Studio 2.5. CDOCKER is a molecular dynamic simulation based docking method. In this docking

method the protein is subjected to keep rigid while the ligands are treated as fully flexible. Final minimization step is used to refine the docking pose. All the docked molecules are analysed on the basis of binding energy and hydrogen bond interactions with active side binding residues and hinge region of amino acids.

4.2.3 Protein preparation

The crystal structure of PDK-1 (PDB id 1UU7) taken in this study was extracted from protein data bank (http://www.rcsb.org/pdb), all the HETATMS were removed. The protein was subjected to two steps energy minimization to remove the bad steric clashes using steepest descent and conjugate gradient methods for 1000 steps at RMS gradient of 0.1 and 0.05 respectively. During the energy minimization process the backbone and side chain were fixed by applying the fixed atom constraint, and only hydrogen atoms were minimized. The CharmM force field (Brooks et al., 1983) was applied to the receptors. Fixed atom constrained was removed after the minimization. The receptor protein is divided into the protein part and crystal ligand part. The protein part was only selected and selection were made to "define selected molecule as receptor" under define and edit binding site sub panel of the Tool panel where in the protein is defined as receptor molecule. By selecting only the ligand part and clicking on "Define sphere from selection" so that the crystal ligand is used to define the binding site of 15 Angstroms on the receptor molecule (Shown in Figure 4.2). This 'input receptor molecule' was used as input parameter in the CDOCKER protocol parameter explorer. The minimized structures of these compounds were used as input ligand in the protocol explorer of CDOCKER. Each of them is given as input in

another parameter meant for 'input ligands' and the protocol were run as many times as the number of inhibitors were selected for the experiment. The various conformations for ligand in this procedure were generated by using molecular dynamics. The generated initial structures for the ligand were further refined using simulated annealing. The CDOCKER energy (-(protein-ligand interaction energies)) of best configuration docked into the receptor of all the selected natural inhibitors, which were calculated and compared with that of interacting residues at active site region with the crystallized inhibitors, PDK-1 kinase protein.

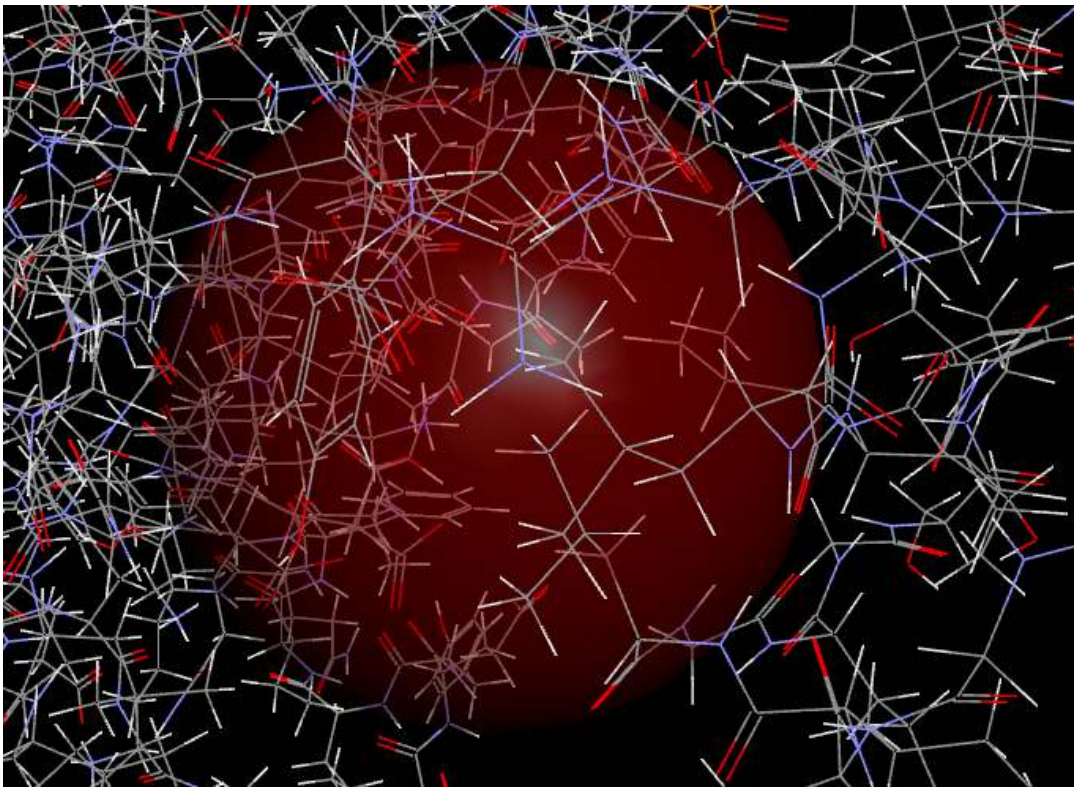


Figure 4.2: Showing the Binding site sphere of PDK-1(PBD ID: 1UU7)

4.2.3 2 D Plot of protein ligand interactions

For study of hydrogen bond as well as the hydrophobic interactions of all docking results, 2D diagram were drawn by Accelrys Discovry Studio 3.5 viewer **(Accelrys Inc., San Diego, CA)**. The two dimensional structure showing the hydrogen bond as well hydrophobic interactions were produced.

4.2.4 Molecular dynamic simulation study of Myricetin in water

MD simulation of the complex was carried out with the GROMACS4.5.4 package using the GROMOS96 43a1 force field (Lindah et al., 2001; Schuttelkopf, and Aalten., 2004) The lowest binding energy (maximum negative) docking conformation generated by CDOCKER was taken as initial conformation for MD simulation. The topology parameters of proteins were created by using the Gromacs program. The topology parameters of Myricetin was built by using the Dundee PRODRG server (Gunsteren et al., 1996).The complex was immersed in an octahedron box of extended simple point charge (SPC) water molecule(Gunsteren et al., 1998). The solvated system (PDK-1, Myrecetin and water) was neutralized by adding 3 Cl ions as the PDK-1 have +3 charge. To discharge the conflicting contacts, two step energy minimization was performed by using the steepest descent method of 10000 steps followed by the conjugate gradient method for 10000 steps. MD simulation studies consist of two phases, equilibration phase and production phases. In the first stage of equilibration, the solute (protein, counterions, and Myricetin) was fixed and the position-restrained dynamics simulation of the system, in which the atom positions of protein were restrained at 300 K for 200 ps. Finally, the full system was subjected to MD production run

at 300 K temperature and 1 bar pressure with isotropic molecule based scaling for 10000 ps. For analysis, the atom coordinates were recorded every 0.5 ps during the entire MD simulation.

4.3 Results and Discussions

4.3.1 Molecular Docking Results

Among the all selected natural flavonoids docked with in the active side of the PDK-1, Myricetin was found to exhibit the most efficient binding with highest docking score. Epigallocatechin, Qercetin and Hesperitin were also effective. All the comparison was made on the basis of dock score given by CDOCKER as well as the hydrogen bond interactions. To verify the precision of the docking program and the docking parameters used for docking, the crystal ligand inhibitor from our reference crystal protein (PDB id:1UU7) was removed and again subjected to molecular docking within the active site of PDK-1 using the same parameters as that taken for docking the natural compounds. It was observed that the crystal ligand had a dock score of -20.7kcal/mol with RMSD value of 0.84. This shows that the original binding mode may be reproduced by CDOCKER docking program. Crystal ligand is used as reference molecule to examine the effectiveness of all other inhibitors. Myricetin, a compound derived from grapes, berries, herbs as well as other plants shows promising response and can be used as a potent inhibitor against PDK-1 for curing cancer. Some more compounds have been revealed by this study which may be used as potent inhibitors against cancer. All the docked inhibitors were bound to the ATP site of PDK-1.The active site of PDK-1 surrounded by a G-loop, a hinge region, and a hydrophobic pocket. Myricetin has the highest dock score of -41.38 kcal/mol followed

by the Epigallocatechin, Querecetin and Hesperitin. In Myricetin, the maximum no of residues are involved in hydrogen bonds, where in total 5 residues are involved in making of six number of hydrogen bonds shown in Figure 4.3(A). The major interactions as shown in the binding site are the important H-bonds with Asp223, Lys111, Glu 166 and other two residues Ala162 and Ser160 in the hinge region. The groups involved in H-bonding were carboxylate, hydroxyl (hydrogen donor), NH (hydrogen donor) and oxygen atoms (hydrogen acceptor) of side chain or backbone of residues. It is also observed that some amino acid residues play an important role in the binding of inhibitors within the active site of PDK-1. V96, A109 and L159 were involved in making hydrophobic contacts in all the cases, and they were presumed to be a key players. (Table 4.2). S160, A162 and T222 were involed in making of maximum number of hydrogen bond followed by D223, K111 and E166. Further, epigallocatechin also had higher dock score.It was observed that Epigallocatechin involved in making of total five hydrogen bonds with five amino acids shown in Figure. 4.2 (B). Quercetin and Hesperetin were involved in making of total six and four hydrogen bonds respectively shown in Figure. 4.2(C) & Figure.4.2 (D). Figure 4.3 represent the surface diagram of Myricetin in the ligand binding sites of PDK-1.

Table 4.2: Top Docking scores of natural compounds with receptor protein PDK-1 (PDB ID:1UU7)

Compounds	Docking Energy (kcal/mol)	Hydrogen bonding residues	Hydrophobic interactions
Myricetin	-41.38	K111,D223,S160,A162, E166	T222,V96,L159,L88,A109,V143,L 212
Epicallacheti n	-33.7	K111,S160,A162,T222, D223	A109,L212,L159,V96,L88,G165,D 166
Qercetin	-33.56	K111,S160,A162,T222	S94,L159,G89,V96,L88,E166, G165,L212,V143,A109
Hesperitin	- 32.46	K111,D223,S160,A162	N210,T222,L159,V143,A109,L88 G165,V96, L212, D210
Resvartrol	-20.12	A162, S160 , T222,N210	L159,V143,A109,E166, L212, V96,G89,L88,D208
Tangeretin	-13.6	No hydrogen bonds formed	V96, L159, A109,E166, D208

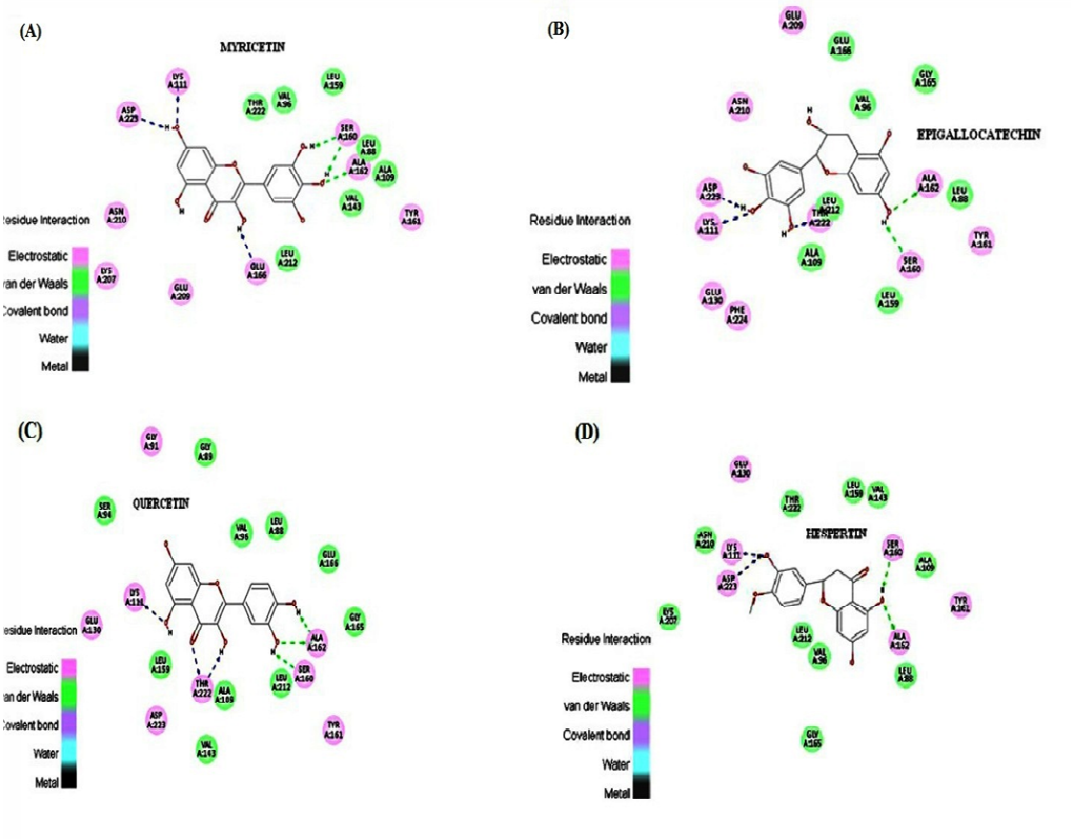


Figure 4.3: (A-D): 2D representation of the top four docked ligands within the active site of PDK-1. Fig 4.3(A) Binding orientation of Myricetin within the active site of PDK-1. Fig 4.3(B) Binding orientation of Epigallocatechin within the active site of PDK-1. Fig 4.3(C) Binding orientation of Quercetin within the active site of PDK-1 Fig 4.3(D) Binding orientation Hespertin of within the active site of PDK-1

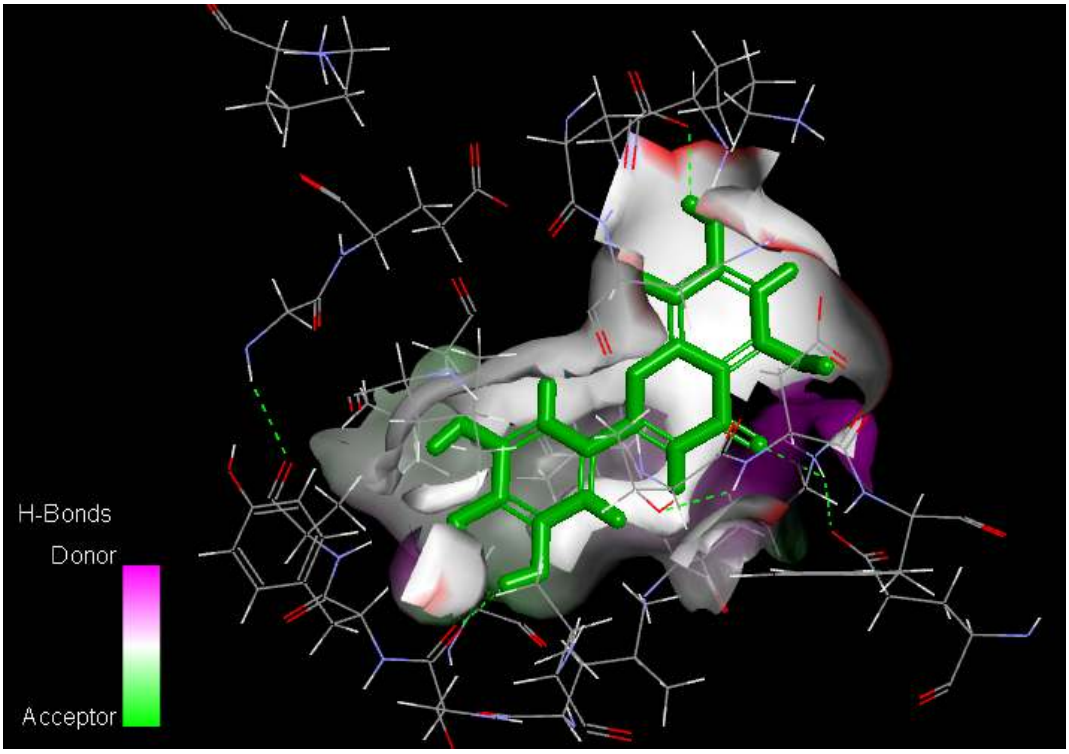


Figure 4.4 Showing the surface diagram of Myricetin in the ligand binding sites of PDK-1

4.3.2. Molecular Dynamic Simulation Results

Finally molecular dynamic simulational study of PDK-1 and myricetin has been revealed intresting results . All-atom MD simulations represent a convenient method to investigate differences in motions of residues/atoms that led to structural and chemical changes. The time dependent behavior of MD trajectories for PDK-1 and myricetin was analysed to include the root mean square deviation (RMSD) for all backbone atoms, coulumbic interction energy as well as van der waal interaction energy. The Root Mean Square Deviation (RMSD) of backbone atoms with respect to the initial conformation was calculated as a function of time to assess the conformational stability of the protein

during the simulations. Figure. 4.5(A) depict that the RMSD trajectories for PDK-1 backbone as well as the Myricetin were always less than 5.0 Å (0.5 nm) during entire simulation, suggesting the stability of simulation system. Figure 4.5 A shows an initial steep rise was observed in the RMSD profile for the first, ~1000 ps and after then subsequently almost a constant profile was achieved for the simulation system of PDK-1 Myricetin complex. While RMSD profile of Myricetin was observed below 0.25 nm with noticeable fluctuation shown in Figure. 4.5 (B). RMSD profile of Myricetin shows stability of compound during entire MD simulation.

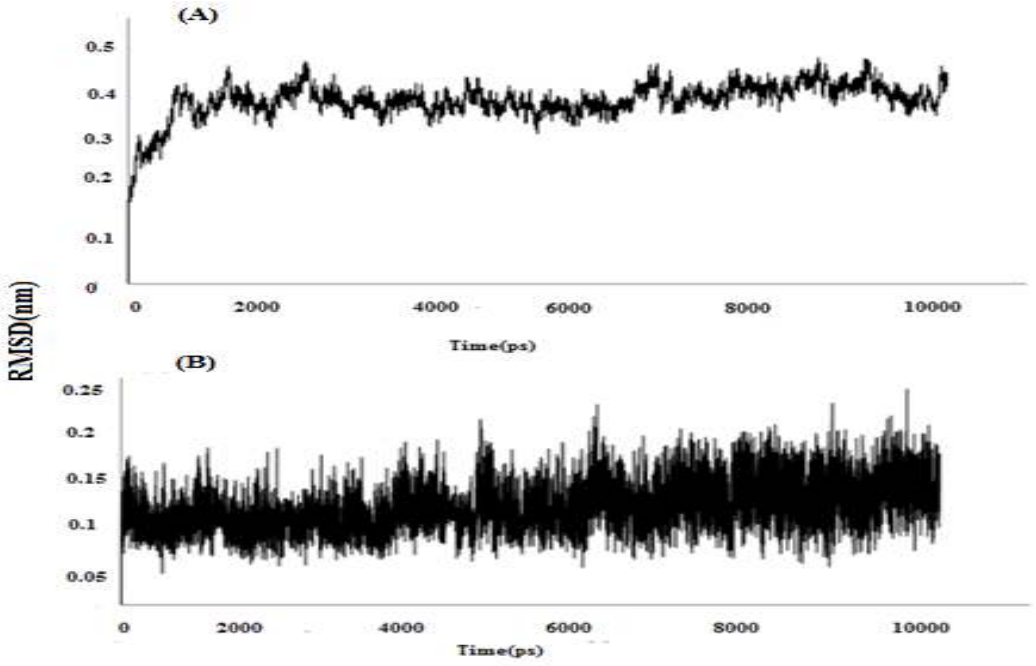


Figure 4.5. RMSD profile of (A) PDK-1 Backbone, (B) Myricetin

To observed in-depth the binding ability PDK-1 and Myricetin during entire MD simulation, the short range (SR) columbic and van der waals (vdw) interaction energies were calculated and displayed (Figure. 4.6 A and 4.6 B). The energy profile of SR columbic interaction energy

between myrecetin and PDK-1 was showed in Figure. 4.6 A. It was observed that SR columbic interaction energy showed slight increase (more negative) during first ~4000 ps after then a decrease (less negative) of ~100 KJ/mol was observed (Figure 4.6(A)). This indicates a decrease in polar interaction after ~4000 ps. In the case of the **vdw** interaction energy, a more stable profile was observed as compared to SR columbic interaction energy throughout the simulation (Figure 4.6(B)). The average value of columbic interaction energy was found -159.68 KJ/mol whereas for vdw interaction energy was -149.25 KJ/mol. The total number of H-bonds (cut off 0.35 nm) which were formed during entire molecular dynamic simulation between Myricetin and PDK-1, was found to be fluctuated from 0 to 6 during entire simulation (Shown in Figure 4.7).

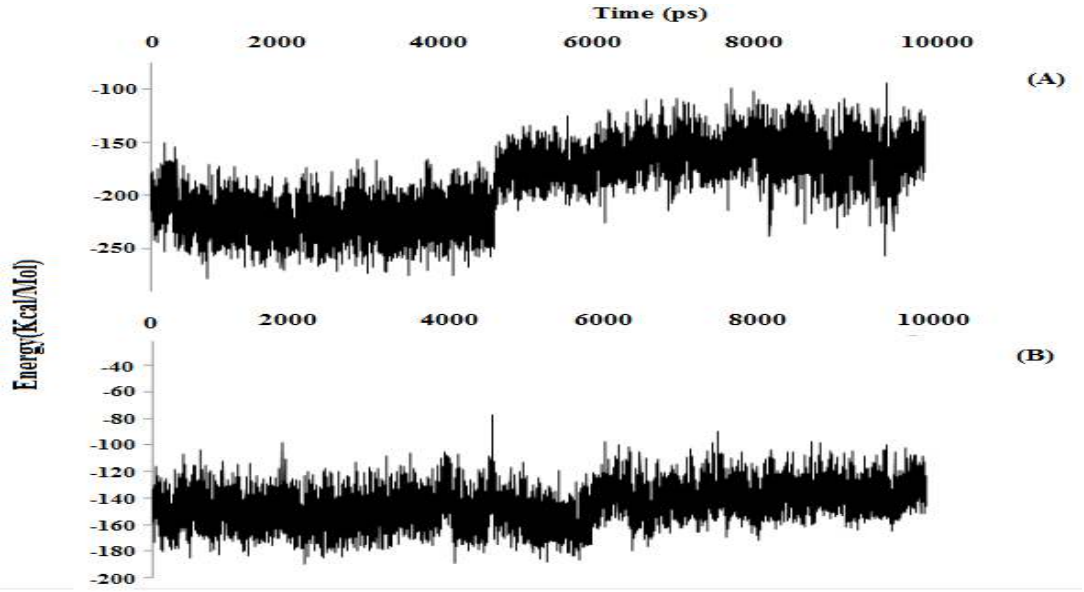


Figure. 4.6: Profile of (A) columbic interaction energy, (B) van der waals (vdw) interaction energy between PDK-1 and Myricetin

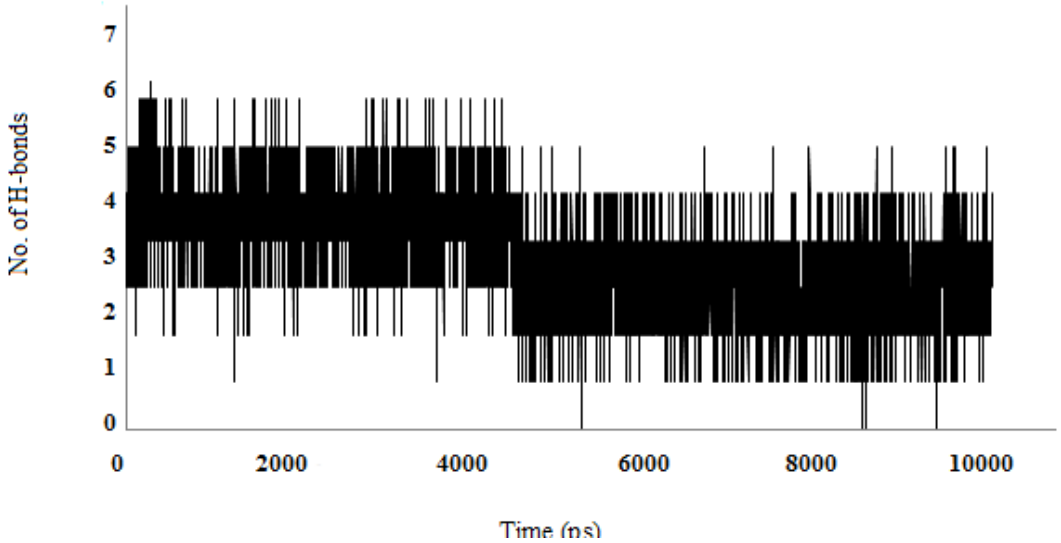


Figure.4.7 No of hydrogen bonds formed during entire simulation (10ns)