INTERNATIONAL JOURNAL OF APPLIED BIOLOGY AND PHARMACEUTICAL TECHNOLOGY

www.ijabpt.com Volume-3, Issue-4, Oct-Dec-2012 Coden : IJABPT Copyrights@2012

ISSN : 0976-4550

Received: 19th Oct-2012 *Revised: 27th Oct-2012* *Accepted: 01st Nov-2012*

Research Article

DOCKING STUDIES OF EMBLICA OFFICINALIS COMPOUNDS WITH DNA POLYMERASE

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ABSTRACT : For understanding the molecular mechanism one can also adopt *in silico* approach where the interactions such as Protein-protein interactions, Protein-compound interactions, DNA-compound interactions, DNA-protein interactions are commonly used. Here we focused on the fate of these interactions for some of the compounds of Emblica officinalis with normal DNA polymerase β. Efficacy of these compounds involves design/taking from the databases of these molecules that are probable to act on the bimolecular target. DNA polymerase beta protein PDB (1DK3) was used to dock with compounds like Quercetin, Myricetin and 3,7,3,4-Tetra Hydroxy Flavone. All these compounds showed best binding studies with DNA polymerase beta.

Keywords: Docking, DNA polymerase beta, Emblica officinalis, Myricetin, Quercetin

INTRODUCTION

A nummber of medicinal plants, traditionally used for thousands of years, are present in a group of herbal preparations of the Indian traditional health care system (Ayurveda) named Rasayana identified for their interesting antioxidant activities. Research in medicinal plants has gained a renewed focus recently (Zhang et al 2004; Suryanarayana et al 2004;2007). The prime reason is that other system of medicine although effective come with a number of side effects that often lead to serious complications. Plant based system of medicine being natural does not pose this serious problems. Emblica officinalis (EO) has a very good usage in Indian indigenous system of medicine. It belongs to family Euphorbiaceae. It is commonly known as Amla or Indian gooseberry. The species is native to India and also grows in tropical and subtropical regions including Srilanka, Pakistan, Bangladesh, South East Asia, China and Malaysia. The main problem of drug industries is in Identification of correct genotype of medicinal plant material. Limitations of morphological and chemical approaches for authentication have created need for newer methods in quality control of botanicals. DNA based marker for EO identification were developed. Random Amplified Polymorphic DNA (RAPD) technique was used to identify a putative marker (1.1 kb) specific for EO (Dnyaneshwar et al 2006). EO contains compounds such as tannins, alkaloids, phenolic compounds, amino acids and carbohydrates (Zhang et al 2003; Habib-ur-Rehman et al 2007). Its fruit juice consists of the highest vitamin C (478.56 mg/100 mL). The fruit when mixed with other fruits, boosted their nutritional quality in terms of vitamin C content (Jain et al 2004). Chemoprevention with food phytochemicals is presently considered as one of the most important strategies to control diseases (Rajeshkumar et al 2003; Khan et al 2002). EO is valued for its unique tannins and flavanoids, which exhibit very powerful antioxidant properties. The inhibition of tumor was done by fruit extract of this plant which has been evaluated on two-stage process of skin carcinogenesis in Swiss albino mice (Veena et al 2006; Bhattacharya et al 2000). Chemopreventive potential of EO fruit extract on 7,12-dimethylbenz(a)anthracene (DMBA) induced skin tumorigenesis in Swiss albino mice was found recently (Sancheti et al 2005). The protection provided by EO was due to its antioxidant capacity and through its modulatory effect on hepatic activation and detoxifying enzymes (Banu et al 2004). Phenolic compounds derived from plant exhibit a number of beneficial effects and can potentially inhibit several stages of carcinogenesis. Efficacy of EO polyphenol fraction (EOP) on the induction of apoptosis in mouse and human carcinoma cell lines and its modulatory effect on N-nitrosodiethylamine (NDEA) induced liver tumors in mice was also investigated (El-Desouky et al 2008). The aqueous extract of the fruits of Terminalia chebula, EO and Terminalia belerica and their equiproportional mixture known as Triphala were evaluated for their invitro antioxidant activity (Deep et al 2005; Kaur et al 2005). Gamma-Radiation induced strand break formation in plasmid DNA (pBR322) was effectively inhibited by Triphala and its constituents (Sandhya et al 2006).

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Though Emblica officinalis has various medicinal applications, but it is the need of hour to explore its medicinal values at molecular level with help of various biotechnological tools and techniques. Further studies should be conducted to elucidate the molecular mechanism of interaction of various plant based drugs with human body in different diseases.

METHODOLOGY

Docking

A docking algorithm is used to estimates the interactions/forces between in the target protein and ligand such as., hydrogen bonding, electrostatic, and van der Waals and it should orient the ligand correctly in the active site cavity. There is a rapid increase in identification of three-dimensional protein structures due to improvements in structure determination techniques such as high throughput X-ray crystallography. Docking in accurate sense is to predict the non-covalent protein–ligand complexes. Theoretically, docking is also known as energy optimization method in order to identify the lowest free energy binding pose of a ligand molecular within the active site cavity. Docking method has two components: conformation (pose) searching and scoring the poses. Addition of protein flexibility is computationally very expensive; therefore most of the well-known docking methods treat the protein structure as rigid or allow only side chain functional groups as flexible. On the other hand, ligand treated as: whole molecule approach (variant 1 and 2) and fragment based (variant 3). A good docking method estimates the forces involved in the protein–ligand recognition viz. electrostatic, vanderwaals and hydrogen bonding and places the ligand appropriately in the active site.

Docking Algorithms used in this Study

A. LigandFit:

LigandFit is a rigid docking method and intended to dock a single ligand or a series of multiple ligand molecules into active site activity. During the docking process, the protein kept keep rigid and whereas ligand remains flexible, allow conformation generation and docked within the binding site.

The 3 key steps:

I. Site search
II. Conformational search
III. Ligand Fitting

Site Search

The site search is used to identify the binding site cavity present in the protein and which will be used for during the docking. During the site search, the entire protein is mapped on to the grid. Grid points are used to identify the occupied and unoccupied grid points by the protein. The site can be defined by two ways and they are,

a. **Shape Based:** An "eraser" algorithm is used to defined based on the cavity/shape of the protein and clear all grid points present in outside the protein.

b. **Docked Ligand:** Sites are defined based on a docked ligand.

Conformational Search

The Monte Carlo method is employed in the conformational search of the ligand. During the search, bond length and bond angles are untouched; only torsion angles (excepted there in a ring) are randomized. Therefore, the ligand molecule(s) should be energy minimized to ensure correct bond lengths and bond angles using LigandFit.

Ligand Fittings

After a new conformation is generated, the fitting is carried out in two steps.

1) The non mass-weighted principle moment of inertia (PMI) of the binding site is compared with non mass-weighted PMI of the ligand according to the following equations (a) and (b).

a) If the value (Fit PMI) is above the threshold or not better than fitting results previously saved, no further docking process will be performed. Another ligand or another conformation of the same ligand will be examined.

b) If the Fit $_{PMI}$ is better than previously saved results, the ligand is positioned into the binding site accordingly to the PMI.

2) Rigid body minimization is applied to the saved conformations of the ligand to optimize their positions and docking scores.

B. CDocker

CDOCKER is known as CHARMm based docking tool and it uses rigid receptor. Initially ligand conformations are generated using high-temperature molecular dynamics with diverse random seeds.

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Random orientations of the conformations are produced by translating the center of the ligand to a specified location within the receptor active site, and performing a series of random rotations. A softened energy is calculated and the orientation is kept if the energy is less than a specified threshold. This process continues until either the desired number of low-energy orientations is found, or the maximum number of bad orientations have been tried. This step can be skipped to use the input orientation only. Each orientation is subjected to simulated annealing molecular dynamics. The temperature is heated up to a high temperature then cooled to the target temperature. A final minimization of the ligand in the rigid receptor using non-softened potential is performed. For each final pose, the CHARMm energy (interaction energy plus ligand strain) and the interaction energy alone are calculated. The poses are sorted by CHARMm energy and the top scoring (most negative, thus favorable to binding) poses are retained. For performance, many of these steps use a nonbond energy grid, rather than the full potential energy terms usually used by CHARMm. This provides a significant time saving at the cost of some accuracy. You can specify whether to use a grid or full potential for the simulation and minimization steps.

RESULTS AND DISCUSSION

In Silico Studies for understanding the Molecular Mechanism

Since the treated rats have shown enhanced polymerase β enzyme (involved in DNA repair) activity, an attempt has been made to study if any of the compounds present in *Emblica officinalis* formulation have an affect over polymerase β enzyme activity. In this regard further *in silico* studies were carried out to study the interactions between the polymerase β enzyme and the ligands by docking approach.

Ligand preparation

Compounds of rasayana that are taken for this study and their possible interaction with DNA polymerase β are shown in the following sections. Compounds used for this study are Quercetin, Myricetin and 3,7,3',4'-Tetrahydroxy flavone.

Quercetin:

Quercetin belongs to antioxidants category and chemically it is 3, 5, 7, 3', 4'- Pentahydroxyflavone. Different physiochemical parameters of Quercetin are as follows

1.	Chemical Formula	:	$C_{15}H_{10}O_7$
2.	IUPAC Name	:	2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one
3.	water solubility	:	10.0 mg/L
4.	logP	:	4.40
5.	pKa	:	7.30

Myricetin:

Different physiochemical parameters of Myricetin are as follows

1.	water solubility	:	3.
2.	logP	:	1.85
3.	logS	:	-3ALOGPS
4.	pKa	:	7.61
5.	polar surface area	:	147.68
6.	rotatable bond count	:	1
7.	Weight Average	:	318.2351
8.	Monoisotopic	:	318.037567296
9.	Chemical Formula	:	C15H10O8

3,7,3',4'-Tetrahydroxy flavone:

Different physiochemical parameters of 3,7,3',4'-Tetrahydroxy flavone are as follows water solubility 1.54e-02 g/lALOGPS

logP	:	3.650	ALOGPS
logP	:	4.240	
logS	:	-4.40	ALOGPS
pKa	:	0	
Polar surface area	:	55.840	
Rotatable bond count	:	6.0	

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These structures of three molecules were built in discovery studio and charmM forceield is used to type the molecule. These molecules are subjected to the energy minimization using Smart Minimizer, which minimizes using charmM.

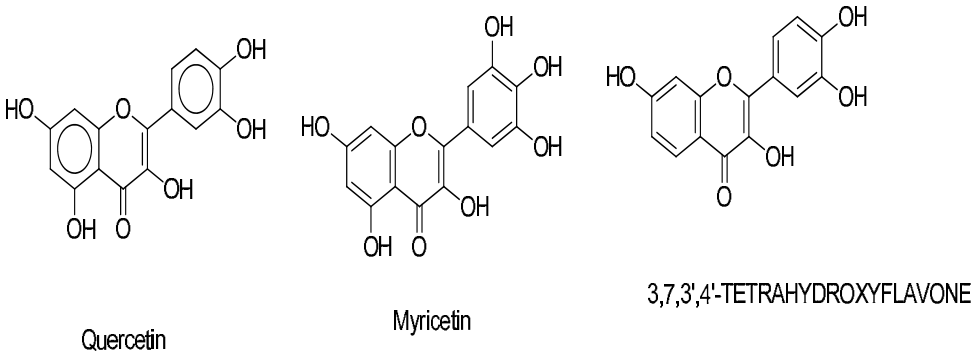

Figure 1: 2D-Structures of ligands

Protein Selection and Preparation:

In mammalians, DNA polymerase β is one of the 19 known cellular Polymerases which is smallest in size. It has no proof reading activity and plays a role in DNA-repair. It is a 39KDa single elongated polypeptide comprising 335 amino acid residues with two globular regions. The amino terminus (8 KDa) is joined to the polymerase domain (31KDa) with a hinge region. Hinge region is protease sensitive. The 8 KDa domain carries deoxyribose phosphate lyase (5'-dRpase) activity as well as single stranded DNA binding activity. The larger fragment 31 KDa domain binds to double stranded region at the 3'-OH end to be extended and carries polymerase catalytic function.

Protein Preparation for Docking

3D-Structure of DNA Polymerase β with the PDB ID of 1DK3 was selected from PDB. The protein was typed with CharmM force field and hydrogen's were minimized using smart minimizer. Minimized protein was subjected for binding site analysis. From the binding site analysis shows that the first site is located further beyond Lys35, between helices 1 and 4 and neighboring Leu19, Leu22, Glu26 or Lys72. The other possible sites that are related are Lys60, Leu62 and Ala70, further in the direction of Gly64 & Gly66. This sphere of DNA polymerase was docked for interaction studies with different ligands of *Emblica officinalis* formulation.

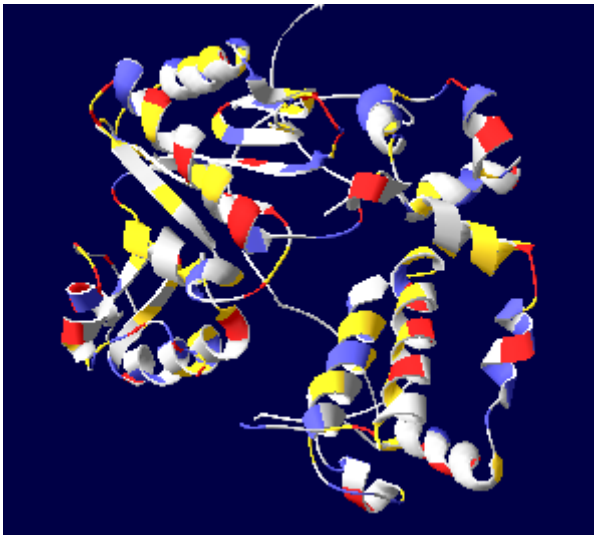

Figure 2: Three Dimensional Structure of DNA Polymerase β

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Docking between DNA Polymerase β and Compounds of *Emblica officinalis* formulation

Protein-Ligand docking was done by using the CDocker protocol. Different conformational poses were generated and poses were checked for the interactions with the binding site by running the CDocker protocol. Affinity between DNA polymerase β (1DK3) and Quercetin, Quercetin analogue Myricetin and 3,7,3',4'-Tetrahydroxyflavone were studied. Active site of DNA Polymerase β contains arg 258, phe 272 and Tyr 296 and the palm contains asp 192 and asp 190, where the catalytic reaction between DNA polymerase and damaged DNA strand occurs. Here we performed the interaction studies between the major compounds of rasayana and the DNA Polymerase β. These ligands poses with least free energy were selected; lowest free energy molecular pose state is most stable pose.

***In silico* interaction studies between DNA Polymerase β and compounds of *Emblica officinalis* formulation (Quercetin and its analogues Myricetin and 3,7,3',4'-Tetrahydroxy flavone)**

In the present study, which is evident from these experimental results DNA polymerase β which is showing more activity in treated rats is taken as a target protein and the details of the target protein is mentioned below.

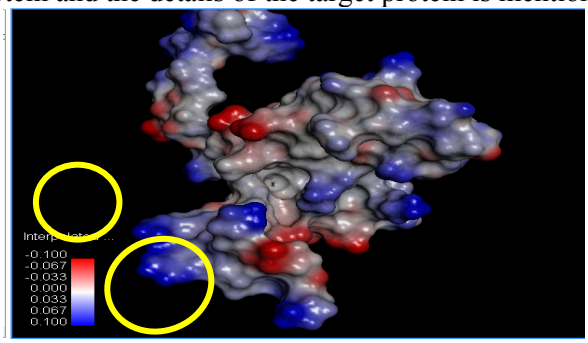

Figure 3: Different binding sites of DNA Polymerase β obtained by accelrys discovery studio software

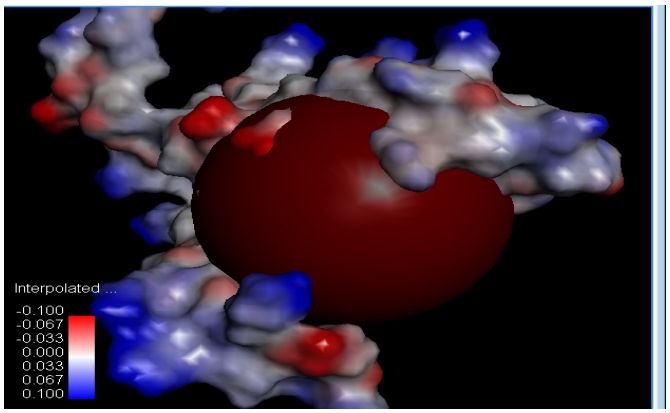

Figure 4: Sphere of selection for the binding Site 1 of the Protein DNA Polymerase β

From the above pictures, one site is active site and followed by the binding sites that are most probable binding sites.

Docking Studies

The docking studies were carried out using CDocker algorithm of Accelrys Discovery studio software to analyze the key interactions between DNA Polymerase β and selected compounds. The best docked pose selected based on the CDOCKER ENERGY.

Quercetin

The best predicted bounding pose of Quercetin showing interactions with key amino acids of DNA Polymerase β (Figure V). The first hydrogen bond interaction was observed between 3'-hydroxyl group of phenyl and carboxyl group of GLU26 residue (OH---O, 2.097 Å) and 3'-hydroxyl group also forming interaction with hydroxyl group of the TYR39 residue (OH---O, 2.52 Å). The another hydrogen bond interaction was observed between 4'-hydroxyl group of the phenyl and carboxyl group of GLU26 residue (O---OH, 2.19 Å). Chromen-4-one moiety of the Quercetin occupies the hydrophobic cavity created by ALA38, ALA42, ALA43, VAL45, ILE46, ALA47, and LEU69.

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Table 1 : Cdocker_Energies for top 10 poses of Quercetin

Pose number	CDocker_Energy	CDocker_Interaction_Energy
1	27.6207	32.0072
2	27.5684	31.8835
3	27.4871	31.9043
4	27.4869	31.5709
5	27.4628	31.7687
6	27.4519	31.6991
7	27.4439	31.8159
8	27.3367	31.272
9	27.2519	31.7092
10	27.0717	31.1299

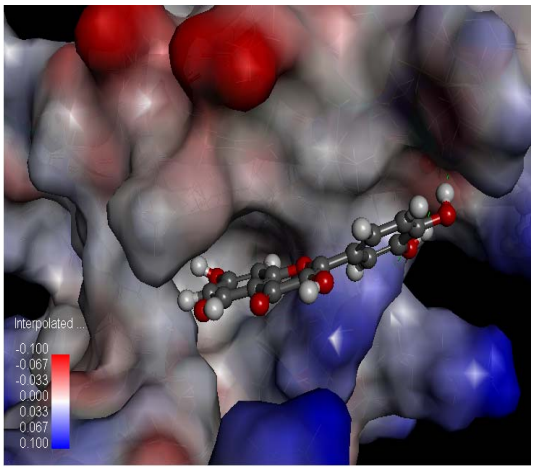

Figure 5: Interaction between DNA polymerase β (1DK3) and Quercetin

Myricetin

The best predicted bounding pose of Myricetin also showing interactions with key amino acids of DNA Polymerase β (Figure 6). The first hydrogen bond interaction was observed between 3'-hydroxyl group of phenyl and carboxyl group of GLU26 residue (OH---O, 2.10 Å) and 3'-hydroxyl group also forming interaction with hydroxyl group of the TYR39 residue (OH---O, 2.42 Å). The another hydrogen bond interaction was observed between 4'-hydroxyl group of the phenyl and carboxyl group of GLU26 residue (O---OH, 2.29 Å). chromen-4-one moiety of the Myricetin occupies the hydrophobic cavity created by ALA38, ALA42, ALA43, VAL45, ILE46, ALA47, and LEU69.

Table 2: Cdocker Energies for top 10 poses of Myricetin

Pose number	CDocker_Energy	CDocker_Interaction_Energy
1	29.4207	32.7002
2	28.5684	31.8851
3	27.4871	31.0043
4	27.4869	31.5709
5	26.8959	31.0083
6	26.4355	28.9905
7	26.4097	29.3005
8	25.8127	28.4897
9	24.6402	28.6034
10	25.1717	31.0299

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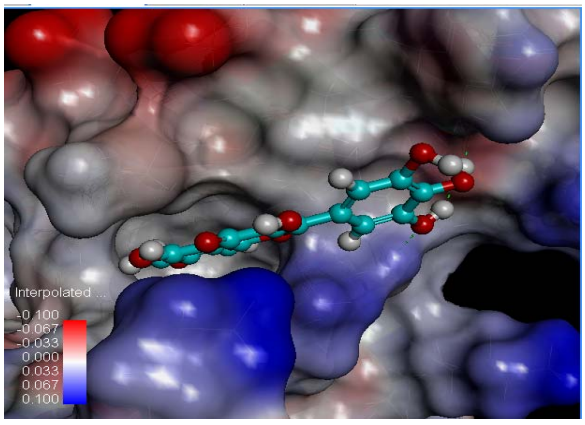

Figure 6: Interaction between DNA polymerase β (1DK3) and Myricetin

3,7,3',4'-Tetrahydroxyflavone

The best predicted bounding pose of 3,7,3',4'-Tetrahydroxyflavone showing same interactions with key amino acids of DNA Polymerase β (Figure 7). The first hydrogen bond interaction was observed between 3'-hydroxyl group of phenyl and carboxyl group of GLU26 residue (OH---O, 2.117 Å) and 3'-hydroxyl group also forming interaction with hydroxyl group of the TYR39 residue (OH---O, 2.48 Å). The another hydrogen bond interaction was observed between 4'-hydroxyl group of the phenyl and carboxyl group of GLU26 residue (O---OH, 2.09 Å). chromen-4-one moiety of the 3,7,3',4'-Tetrahydroxyflavone occupies the hydrophobic cavity created by ALA38, ALA42, ALA43, VAL45, ILE46, ALA47, and LEU69.

Table 3: Cdocker Energies for top 10 poses of 3,7,3',4'-Tetrahydroxyflavone

Pose number	CDocker_Energy	CDocker_Interaction_Energy
1	28.6207	32.1172
2	26.3367	30.261
3	27.3367	31.272
4	27.4628	31.7687
5	27.4519	31.6991
6	24.6402	28.6034
7	24.6402	29.9066
8	24.4032	30.2657
9	24.2527	30.0106
10	24.0417	30.1199

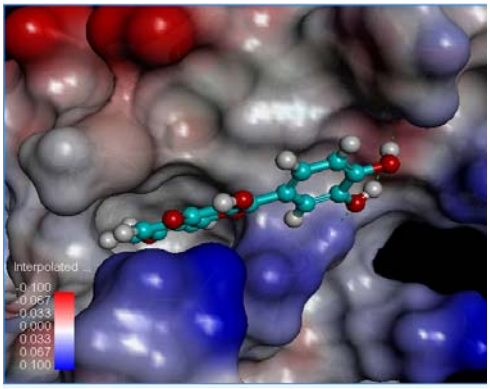

Figure 7: Interaction between DNA polymerase β (1DK3) and 3,7,3',4'-Tetrahydroxyflavone

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CONCLUSION

The key constituents (Quercetin, Myricetin,3,7,3'4' tetrahydroxy flavone) of the *Emblica officinalis* formulation have shown stable interaction with DNA polymerase β enzyme on *in silico* analysis. Both *in vivo* and *in silico* analyses have shown an enhanced DNA Polymerase β enzyme activity on administration of test item. The *in silico* studies have shown that major constituents of amalaki rasayana form stable interactions with the DNA Polymerase β enzyme. The results of *in silico* analysis which are in well coordination with those of *in vivo* studies further emphasize on the ability of amalaki rasayana on DNA damage repair and its possible application as an antiaging formulation.

ACKNOWLEDGEMENT

Authors are thankful to Department of Biotechnology, JNTUH, Hyderabad for providing the software.

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