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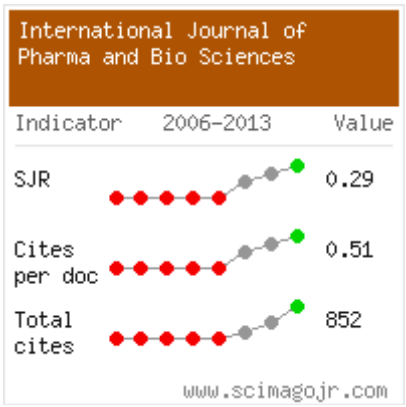

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Int J Pharm Bio Sci 2015 April; 6(2): (B) 752 - 759

Research Article **Bioinformatics**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

VIRTUAL SCREENING OF POMEGRANATE (*Punica granatum*) CHEMICAL CONSTITUENTS FOR KINASE DOMAIN OF HUMAN HER2 AS A DRUG TARGET FOR COLON CANCER

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ABSTRACT

The entire world is now looking for natural and medicinal plants as a supplement for chemical drugs. The popularity of herbal medicine is very common in developing countries, many scientists and researchers are concentrating more on herbal plants and fruits. We all know that cancer is one of the most dreadful diseases. One among the cancer is Colorectal cancer (CRC), the fourth most common cancer in men and the third in women worldwide. The Kinase domain of Human HER2 is one of the important therapeutic drug targets for colon cancer and it belongs to ErbB family In this current study, we have carried out computational biology work in virtual screening on pomegranate chemical constitutes for colon cancer. Results show that compounds such as Pelletierine, Mannite and N-methylisopelletierine are almost docked in all protein cavities with different dock scores in each site. Arg residues present in the drug target protein play an important role in receptor ligand interaction with compounds. But clinical trials and experimental results are needed to be carried out for each individual compounds to prove its bioefficay

KEYWORDS: **Cancer, computational biology, pomegranate, virtual screening, bioefficay.**

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INTRODUCTION

Cancer is an abnormal disease, which affect the normal cell growth in the body. The cascade expression of multiple genes and protein paves complications to cure the disease. There are few important, crucial proteins as the primary source for either inducing or suppressing the gene and protein expression[1]. Colorectal cancer (CRC) is the fourth most common cancer in men and the third in women worldwide. It is a frequent cancer, with more than 1 million new cases every year and a poor survival rate[2]. The Kinase domain of Human HER2 is one of the important therapeutic drug targets for colon cancer. Nonsignificant or aberrant signaling of ErbB family members in human paves the way for cancer. Hence, it needs to be controlled by natural therapy or by treating with natural medications[3]. Herbal medicinal plants are alternative substitutes for chemical drugs and is found to have tremendous applications. Recently, many scientists from all over the world, are finding an alternative practice to use medicinal plants for curing many dread full diseases, research says. Also, majority of the world population depends on herbal health care practice[4]. We have also worked on the antimicrobial activity of diethyl phthalate, present in the extract of the leaves of *L.Coramendalica* as identified from GC-MS[5]. Many traditional healing herbs, fruits and plant parts have been reported to have medicinal value, especially in the rural areas and that these can be used to prevent and cure several human diseases[4]. One such important fruit is *Punica granatum* L. (Pomegranate). The pomegranate, *Punica granatum* L., is the predominant member of two species comprising the Punicaceae family and highly distinctive fruit. The edible part of the fruit contains polysaccharides, acids, minerals, sugars, vitamins, and polyphenols, the aim of many studies on this plant lead to identify the therapeutic constituents[6]. Julie Jurenka, in his reviews describes about the therapeutic applications of pomegranate and its uses in Ayurvedic medicine. *Punica granatum* has many medicinal values, in earlier days it was used as a blood tonic, antiparasitic agent, and to heal ulcers, aphtae and diarrhea. The same author described about the practice of *Punica granatum* as a remedy for diabetes in India[7]. Although pomegranate has extensive potent medicinal property, it may be attributable to several mechanisms. A recent review states, that diverse chemical compounds present in pomegranate is effective for treating inflammation and cancer. Research has focused on its antioxidant, anticarcinogenic, and anti-inflammatory properties[8]. This pomegranate fruit constituent of flavonoids, anthocyanidins, estrogenic flavonols, ellagic acid ellagitannins, flavones and punicic acid[9-11]. This fruit is rich in polyphenol contents that seems to indicate the most therapeutically beneficial pomegranate, polyphenols can help to maintain cardiovascular health[10, 12]. In other studies by Aviram states[12], that it has a potent antioxidant compound found to reduce platelet aggregation and naturally lower blood pressure, factors that prevent both heart attacks and strokes. Pomegranates are not only good for our heart and blood vessels but they have been shown to inhibit various dreadfull cancer, such as breast cancer, prostate cancer, colon cancer, leukemia and to prevent vascular changes that promote tumor growth in lab animals[13,14]. On the other hand, in-*vitro* studies on pomegranate extracts, on cancer cells shows good inhibition for HT-29 colon cancer cell line, extracts have a therapeutic effect and interrupts cell-cell signaling process in cancer[15]. In the current study the structure of important constituents, of pomegranate are retrieved from different chemical databases. In addition, we have performed molecular interaction commonly known as docking to screen the lead for colon cancer.

MATERIALS AND METHODS

Retrieval of protein from protein database

The structure of the drug target protein Kinase domain of Human HER2 (erbB2) and its Xray crystallographic structure with 2.25Å was retrieved from protein data bank. With its

Identification number as 3PP0, commonly known as a PDB ID and complexes with chemical structure2-{2-[4-({5-chloro-6-[3-(trifluoromethyl)phenoxy]pyridin-3-yl}amino)-5H-pyrrolo[3,2-d]pyrimidin-5- yl]ethoxy}ethanol .

Protein preparation

The raw protein from the protein databank with the PDB ID 3PP0 human protein Kinase domain HER2 (erbB2) is further prepared for docking studies. Initially, all other chemical moieties, present in protein are removed. This is followed by energy minimization using the tool smart minimize. The bad steric clashes are removed with 1000 steps at RMS gradient of 0.1 and 0.03 respectively. Before that, the suitable force field CHARMm available with Accelrys life science software (Momany FA *et al.,* 1992)[16] was applied.

Natural compound retrieval

The structure of chemical constituents present in pomegranate such as Gallic acid, Pelletierine, Mannite, n-methylisopelletierine, pelargonidin, Punicalin, Punicalagin, ellagic acid cyanidin was retrieved from a chemical compound databases. The list of compounds with their corresponding ID is shown in the Table 1.

Table 1
Chemical Constituents present in pomegranate

	Chemical Constituents	ID
1	Gallic acid	CID 370
2	Pelletierine	CID 92987
3	Mannite	CID 6251
4	N-methylisopelletierine	CAS 18747-42-7
5	Pelargonidin	CAS 7690-51-9
6	Punicalin	CAS 65995-64-4
7	Punicalagin	CAS 65995-63-3
8	Ellagic acid	CID 5281855
9	Cyanidin	CID 128861

Docking

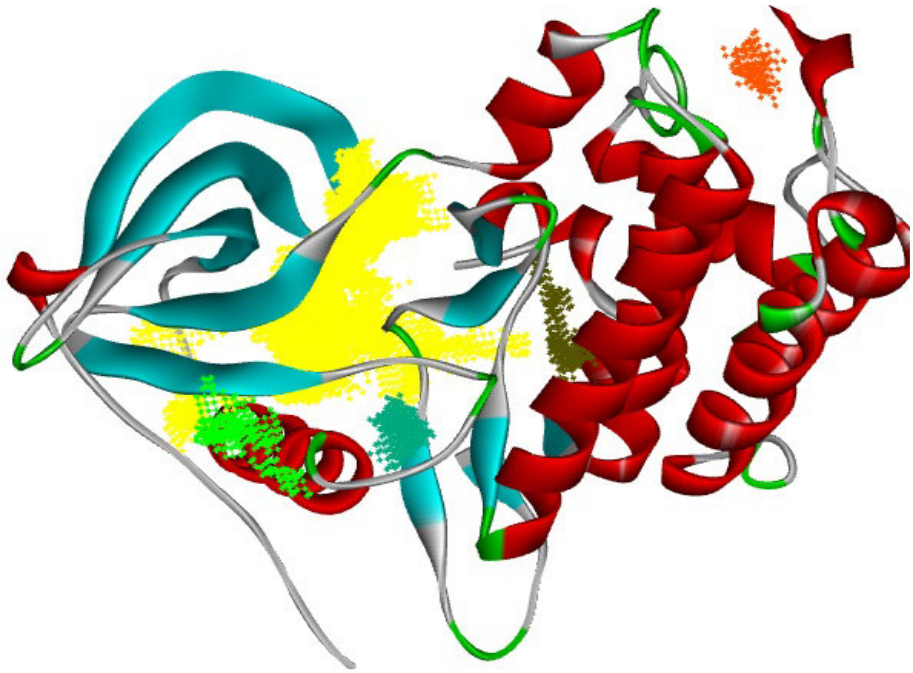
Structure based drug design is the method of performing docking using the known protein docking with known ligands. This has been the frequently used method of analyzing the receptor-ligand interaction between the active site of the protein and the chemical molecules. In this current study the PDB ID 3PP0 human protein Kinase domain HER2 (erbB2) is docked with Pomegranate chemical constituents using ligandfit protocol available through Acclerys Discovery Studio 2.1.

RESULTS AND DISCUSSION

Protein and its binding site

The theory of flood filling algorithm is used to find the different cavities of the protein; this program was found to be inbuilt in acclerys for defining the active site of the proteins.

Figure 1
Binding site of protein Kinase domain HER2 (erbB2)



In this current study, we try to find the docking of compounds with all the sites of the protein. Usually the general theoretical concept in structure based drug designing is docking of all the chemical compounds, leads, or ligands to one such a particular large cavity of the protein. Nevertheless, here we docked with all the sites of the protein to ensure that, any site of binding will block the mechanism to reduce the effect of the disease. The figure 1 shows the binding site of protein Kinase domain HER2 (erbB2) in different colors and table 2 describes about the various sites and its volume, point count, coordinates and partition for docking.

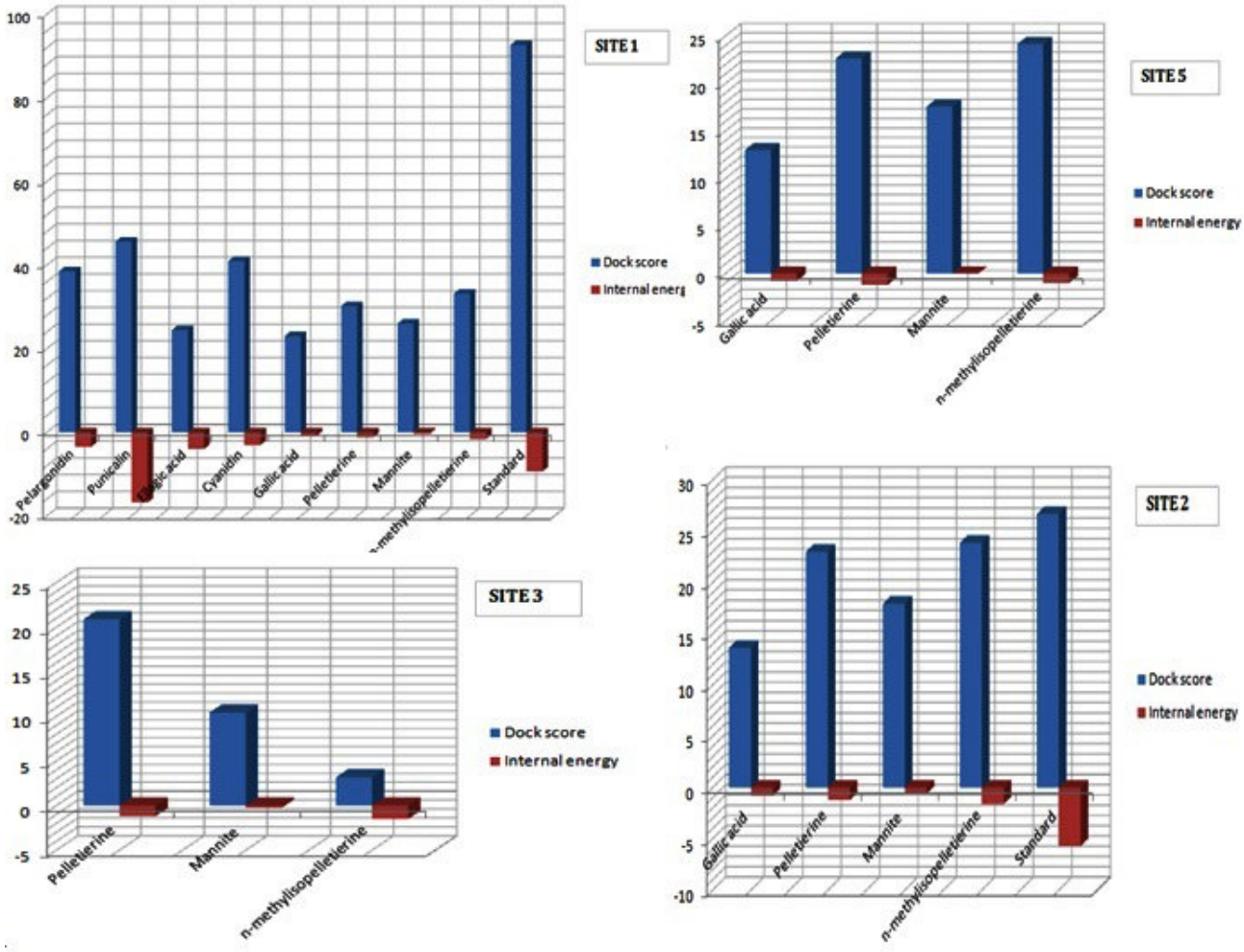
Table 2
Binding site parameters of protein Kinase domain HER2 (erbB2)

Site	Partition	Volume in Å	X,Y,Z	Point Count
1	3	484.25	13.115, 19.658, 21.117	3874
2	1	29.25	29.115, 23.00, 23.117	234
3	1	28.25	23.115, 25.908 ,29.867	226
4	1	23.875	4.365, 24.908, 33.367	191
5	1	13.75	5.365, 10.658 ,50.36	110

Receptor-Ligand Interaction

The concept of structure based drug designing is applied, when both lead compounds and drug target protein are known for molecular docking. It is an iterative process to dock the lead compounds with the specific site of the drug target protein. The active site of the protein is automatically generated using the flood-filling algorithm as shown in the table 2. The site 1 has three partitions due to large volume 484.25Å with point count of 3874 in equal grid spacing of 0.5 (X),0.5(Y),0.5(Z) direction respectively. A series of pomegranate containing chemical constituents were docked with a sphere site being defined as 13.115 (X), 19.658 (Y), -21.117 (Z) using "2 500 120, 4 1200 300, 6 1500 350, 10 2000 500, 25 3000 750" Number of Monte Carlo Trials.

Figure 2
Docking and internal energy of the compounds in various binding sites



The Same protocol was followed for the other two sites, *viz* site 2, site 3, site 4 and site 5 respectively. Figure 2 shows the dock score and internal energy of the compounds of all the sites. It was observed that, one or more than one compound shows molecular interaction to the binding site aminoacid. But no molecular interaction was found in the site 4, these results convey directly that there is no favorable binding of the compounds at the site 4.

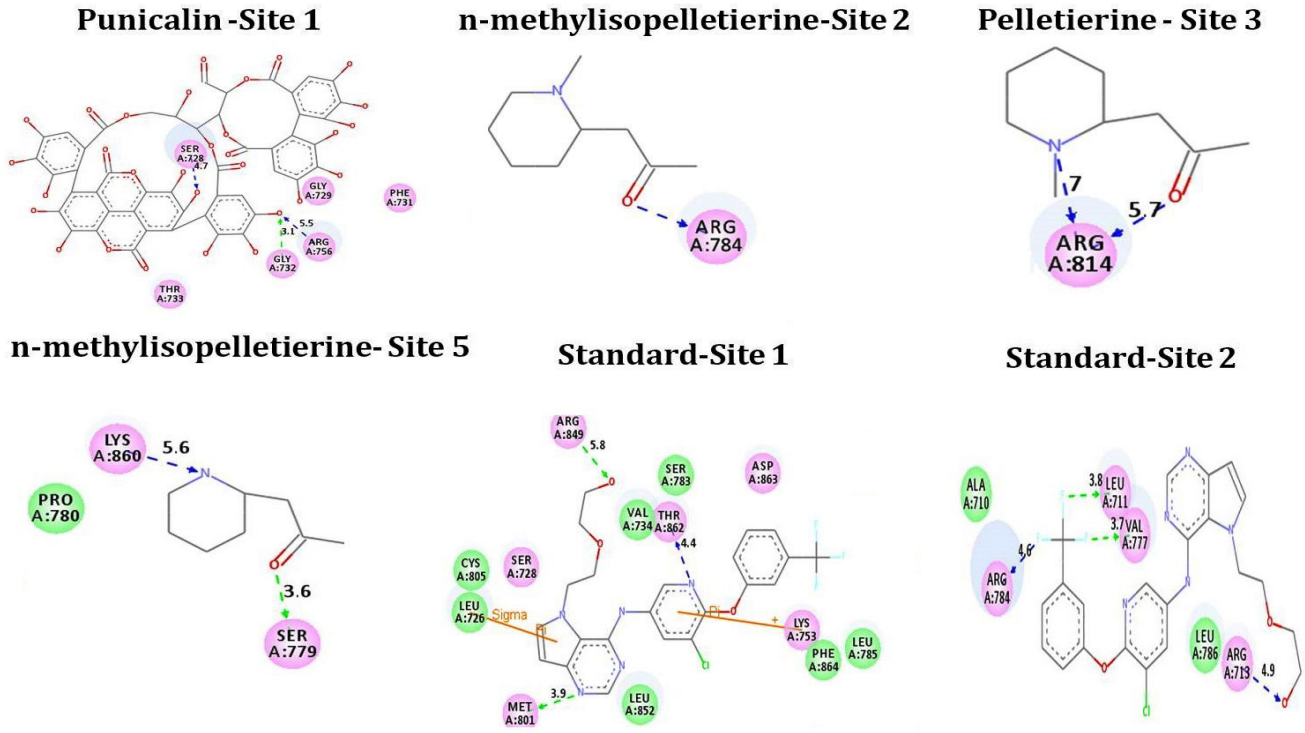
Table 3
Dock score and internal energy of compounds with binding site of drug target protein

Compounds	Dock score in Kcal/mol	Internal energy
	Site 1	
Pelargonidin	38.515	-3.457
Punicalin	45.615	-16.801
Ellagic acid	24.456	-4.005
Cyanidin	40.952	-3.04
Gallic acid	23.011	-0.799
Pelletierine	30.187	-1.208
Mannite	25.982	-0.361
n-methylisopelletierine	33.189	-1.629

Standard	92.64	-9.363
	Site 2	
Gallic acid	13.635	-0.788
Pelletierine	22.996	-1.217
Mannite	17.942	-0.575
n-methylisopelletierine	23.878	-1.592
Standard	26.696	-5.683
	Site 3	
Pelletierine	20.87	-1.194
Mannite	10.428	-0.23
n-methylisopelletierine	3.172	-1.502
	Site 4	
	No docking	
	Site 5	
Gallic acid	12.984	-0.788
Pelletierine	22.629	-1.206
Mannite	17.559	-0.015
n-methylisopelletierine	24.14	-1.001

Pelletierine, Mannite and N-methylisopelletierine are mostly present in the bark of the pomegranate. Table 3 shows receptor –ligand interaction in all the sites of the protein except in site 4. Here we have used, the standard as complex compound that is already bound to the PDB identifi.tion number .i.e., 2-{2-[4-({5-chloro-6-[3-(trifluoromethyl) phenoxy] pyridin-3-yl} amino)-5H-pyrrolo [3,2-d]pyrimidin-5- yl]ethoxy}ethanol. It is seen from the figure 2 that for site 1, the standard compound shows highest dock score that other compounds, but the role of this standard compound is not observed at other sites. Hence, this may due to specific binding of the compound to the respective amino acid. Perhaps pomegranate shows broad binding to all the sites. The table 3 also shows the dock score and internal energy of compounds that bind to respective sites of the drug target protein and figure 3 shows receptor-ligand interaction.

Figure 3
Receptor-ligand interaction



CONCLUSION

Hence, we conclude that pomegranate chemical constituents have a good potent interaction with all the binding cavities of the drug target protein of colon cancer. Pomegranate (*Punica granatum*), has already been reported by many experimental studies on diabetics, where only the extract of the fruit or bark was studied for the diabetics, ulcer, parasitic and cancer. Hope this theoretical paper will reveal the importance of individual chemical constituents that are present in Pomegranate (*Punica granatum*) is effective to treat the colon cancer and it can serve as a drug candidate in the future, but clinical trials and experimental results are needed to be carried out for each individual compound to prove its bioefficay.

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