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Research Article

A Recepto-Informatics Study of the Natural Nutraceuticals having Potential Anticancer Efficacy for Breast Cancer

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Abstract

Background: Breast cancer is a serious global health concern causing the highest mortality rate in females. Available synthetic drugs to treat breast cancer are marred by extreme toxicity issues and suggest some alternate route to address the dreadful disease. The present study is an *insilico* effort to identify the antitumor potential of specific plant metabolites.

Materials and Methods: The structure of the Human Estrogen Receptor (HER), a potential target of breast cancer was chosen as target molecules, retrieved from the Protein Data Bank (PDB) and the structures of flavonoid compounds have been collected from PubChem database. Molecular docking and drug similarity studies were performed for these natural compounds to assess and analyze the anti-breast cancer action.

Results: Replacement of pharmacophore group in genistein with chlorate group and beta glycoside in daidzein was shown to possess more affinity as compared to that of standard drugs Tamoxifen, Toremifene and Raloxifene. The interaction studies suggest that the compound could serve as probable lead molecules in drug development. Both the compounds also exhibited the highest binding affinity with human ER more significant than 8.0 Kcal/mol.

Conclusion: The results of this study can be implemented to design novel anti-cancerous drugs in the coming future. The interaction studies of the standard drug with Breast cancer markers serve as a tool to synthesize new compounds of desired efficacy against the deadly disease.

Keywords: Dietary Phenols; Human Estrogen Receptor; Tamoxifen; Breast Cancer; Calycosin; Daidzein; Geinstein; Naringenin

Introduction

Breast cancer is one of the most leading causes of death in women across the world accounting for approximately 15% of all female cancer deaths in the United States [1]. Healthy breast cells and most breast cancer cells possess receptors for estrogen and progesterone circulating in the blood [2]. The binding of hormones with their respective receptors induces growth response in the form of signal cascade culminating in cell proliferation and growth. Moreover, both estrogen and progesterone function following oncogenes and tumor suppressor genes resulting in cancer progression [3].

Various phytochemicals have been shown to kill plethora of cancer cells [4] successfully. Moreover, recent research has shown that increasing the consumption of vegetables and fruit might help in checking the risk of breast cancer [5,6]. For example, it is demonstrated that the intake of soy food is related to more prolonged survival and low recurrence among breast cancer patients, thus establishing the relationship of soy food with breast cancer. In general, phytochemicals may be classified into several classes; among these, both flavonoids and isoflavones have been found to demonstrate strong anti-cancer properties. Among these, dietary phytoestrogen is a versatile family of naturally occurring non-steroidal plant-based compounds that can act as an alternative of estrogen. Due to their structural similarity with estrogens, phytoestrogen/isoflavones can be exploited for their estrogenic or anti-estrogenic effects [7,8]. It is hypothesized that this structural resemblance may induce competition with estrogen for its binding to the human estrogen receptor (HER). Depending on the type of HER on the cells, these chemicals may activate or suppresses the activity of estrogen, thereby in some cases decreasing the health risks associated with excess intake of estrogen. Thus, some isoflavones can reverse the effect of estrogen, a hormone correlated to an augmented risk of breast and other hormone-dependent cancers [9]. They act rather like Tamoxifen, a drug commonly used to treat and prevent breast cancer.

Soy food is a vibrant and dietary source of isoflavones. Genistein, naringenin, calycosin and daidzein are principal isoflavones present in soy food [10]. Most of the compounds possess anti-can-

cer properties and have been used in several pre-clinical studies [11], although these studies are not being translated into clinical settings due to lack of highly potent and specific lead molecule. The study was aimed to identify a lead molecule with a better affinity to HER. In the present study, we performed insilico analysis on some bioactive phytoestrogen as well as their derivatives and evaluated their interactions with HER and assessed their molecular interactions using autodock.

Methodology

Retrieval of atomic structure and analog preparation

All the software used for the analysis is freely available for academic use. The PDB (www.rcsb.org) [12] is a worldwide repository for the processing and distribution of 3D biological macromolecular structure data. The protein structure of HER alpha (PDB ID 3ERT) was downloaded from PDB.

Drug targets

A total of 18 flavonoids were identified from the Pubmed literature search that showed inhibitory effects on breast cancer cells. The 3-D structure of Tamoxifen and other dietary phenols i.e. Daidzein, Genistein, Calycosin, Naringenin as well as their derivatives, were downloaded in. sdf format using Pubchem and converted to PDB format using Pymol and further used for docking studies.

Active site identification of the human estrogen receptor

The catalytic sites of HER along with area and volume of binding pocket was predicted with Computed Atlas of Surface Topography of Proteins (CASTP) program (http://cast.engr.uic.edu) [13].

Molecular docking

The present study was confined to human estrogen receptor-α, an anti-breast cancer drug (Tamoxifen, Toremifene and Raloxifene), some dietary phenols (Daidzein, Genistein, Calycosin and Naringenin) and their derivatives. Molecular docking was performed to calculate the extent of drug-receptor binding energy. The docking experiments were performed using AutoDock 4.2 (The Scripps Research Institute, www.scripps.edu, assistance from AutoDockTools (ADT), an additional program that allows the user to correlate with AutoDock from a Graphic User Interface (GUI). AutoDock is a set of automated docking tools intended to forecast how small molecules/ligands, such as substrates or drug candidates, bind to a receptor/protein of known 3D arrangement. For its input and output, Vina uses the PDBQT molecular structure file format. PDBQT files can be generated (interactively or in batch mode) and viewed using MGL tools. Pymol has been selected for the analysis of docking results [14,15].

Preparing the ligand and macromolecule files for AutoDock

The PDB files obtained from the World Wide Web repository are often far from perfect for docking study and present with potential problems like missing hydrogen atoms, multiple molecules, added waters, and related issues. Using the GUI of ADT, files were prepared.

AutoDock and Vina need receptor and ligand representations in a specific pdbqt format which is a modified protein data bank format containing atomic charges, atom type definitions and for ligands all the topological information [16,17]. These file preparations are carried out by the plugin using scripts from the AutoDock Tools package.

The Macromolecule file

The downloaded PDB file of HER-α (PDB ID 3ERT) was first read in ADT, added waters removed and polar hydrogen were added. Kollman charges were added. Finally, the file was saved with. pdbqt extension (where 'q' represents charge).

The Ligand File

In a parallel process, the ligand files were examined in ADT, all hydrogen added, charges added, and non-polar hydrogen merged and saved with pdbqt extension. ADT then automatically determined the best root. The ligand files were then saved with. pdbqt extension (q representing charge).

The Grid Parameter File

Both AutoDock and Vina use 3D rectangular boxes (Grid box) for defining the binding site. In the plugin, the box center can by defined by providing explicit coordinates (Grid parameter file). The grid volume was large enough to allow the ligand to rotate freely, even with its most fully extended conformation. The grid parameter involves drawing a zone comprising of X, Y, and Z-axis in 3D to cover the whole molecule and allows for ligand binding. The parameters used for the docking process via AutoDock Vina were as follows: Centre x = 22.397, centre y = 5.635, centre z = 21.98, size x = 40, size y = 40, size z = 40, exhaustiveness = 8. The parameters required to create such a grid were stored in the Grid Parameter File with. gpf extension.

The Docking Parameter File

The docking parameter file, which instructs AutoDock about the ligand to move, the map files to use, and other properties defined for the ligand was created. AutoDock's search methods include the Monte Carlo simulated annealing (SA) method, the Genetic Algorithm (GA), local search (LS), and the hybrid genetic algorithm with local search (GALS).

Running Autodock vina

Finally, the Auto Dock vina program was run and resultant files (in pdbqt format) so developed were checked in Pymol visualizer for its 3D orientations and the binding energies of the docked ligand-protein complexes were observed.

ADMET prediction of flavonoid compounds

Absorption, distribution, metabolism, excretion and toxicity (ADMET) properties were predicted with the help of Discovery Studio 4.1 (Accelrys, San Diego, CA, USA). Here we have used seven mathematical parameters to predict quantitatively. Table 2 showing the threshold ADMET values (rules/keys). Based on a level

against benchmarks, properties of the flavonoid compounds can be predicted (Prija Ponnan., *et al.* 2013).

BBB

BBB is one of the essential parameters of the ADMET study. In the circulatory system, BBB is a physical barrier that prohibits many substances from traveling into the CNS. Drugs after interacting with molecular targets in the CNS, must cross the BBB to be used as therapeutic agents. On the other hand, medications recommended for peripheral targets, low or no BBB penetration might be required to avoid side effects of CNS.

Human Intestinal Absorption level (HIA):

For oral drug development, the prediction of HIA is a significant concern for selection as a candidate drug molecule. Drugs that are mostly absorbed by the intestine and later excreted are thought as useful for cancer treatment.

Solubility level

Other concerns in the drug development industry face are their solubility in an aqueous medium. The potent drug should be miscible to be effective in the treatment of disease.

Plasma protein binding (PPB)

It is widely accepted the fact that only free drugs can cross membranes and bind to the proposed molecular target (Smith DA., *et al.* 2001). Drugs can bind a plethora of cells and proteins present in the blood, including RBC, WBC, platelets, albumins, glycoproteins, lipoproteins and globulins.

Polar surface area (PSA)

PSA of the molecule can be defined as the area of its van der Waal's surface that arises from the oxygen, nitrogen, or hydrogen atoms attached to oxygen or nitrogen atoms. PSA in the range of 80-100 (Å2) is advisable for drug-protein interaction (Clark DE, 1999).

Result and Discussion

There is a strong correlation between levels of estrogen with the risk of breast cancer. The estrogen present in high concentration in the breast cancer cells binds to ER to stimulate to show an effect such as genesis, cell apoptosis and malignant development. Two forms of ER present in the breast cancer cell are Estrogen Receptor alpha (ER-α) and Estrogen Receptor Beta (ER-β). Uterus, vagina, mammary gland and pituitary gland show maximum expression of ER-α. Abnormal expression of ER-α plays a significant role in breast cancer development affecting around 70% of the primary breast cancer population [18-20]. ER-α plays a critical role in transcription of DNA necessary for gland development; besides, it also acts as an essential factor for the downstream signaling pathway [21]. It also regulates cell proliferation and differentiation through the paracrine process. Thus, ER inhibition offers a practical approach to the prevention and treatment of breast cancer [22]. Some of the potent anticancer drugs which are frequently used such as Tamoxifen, Raloxifene and Toremifene either interfere with estrogen production or estrogen action [23]. Unfortunately, these drugs are associated with several toxic manifestations, including blood clotting, stroke, cancer of uterine and cataracts [24,25]. Thus, there is a constant need to search and identify novel drugs of phytochemical origin with less toxic effects.

HER-α has been exploited as a primary therapeutic target for breast cancer. The 3D structure of HER-α retrieved from the PDB (PDB ID: 3ERT) determined by X-ray crystallography at a resolution of 1.60 (Å) was visualized in Pymol. The HER-α is composed of 595 amino acids and comprises of three domains: a modulating N-terminal domain, a DNA-binding domain and a C-terminal ligand-binding domain (LBD). The N-terminal modulating domain, also known as the A/B or AF-1 domain has a ligand-independent transactivation function. The C-terminus contains a ligand-dependent transactivation domain, also known as E/F or AF-2 domain, which overlaps with the ligand-binding domain. The C-terminal region possesses a DNA binding domain and is highly conserved (96%) as compared to ERs. AF-1 and AF-2 activate transcription independently and synergistically and act in a promoter- and cell-specific manner. AF-1 seems to provide a significant transactivation function in differentiated cells.

Human Estrogen Receptor catalytic site predictions were analyzed using the CASTP program. The best ligand binding site was observed to be a pocket of volume 1178.9Å3 and area of 901.1Å2 which consists of 36 residues, Pro 324, Pro 325, Met343, Leu346, Thr347, Leu349, Ala350, Glu353, His356, Met357, Trp383, Leu384, Ile386, Leu387, Met388, Gly390, Leu391, Arg394, Phe404, Gly420, Met421, Ileu424, Cys449, Gly521, His524, Leu525, Tyr526, Met528, Lys529, Cys530, Lys531, Asn532, Val533, Val534, Pro535 and Leu539. The predicted 36 active residues were used as the catalytic sites for 18 natural flavonoid compounds used for docking studies. The results of the interaction between the active site residues of target HER-α protein and 18 flavonoid compounds are shown in Table 1. By analyzing the docking interactions, Naringenin (-8.0 to -6.5) and calycosin 7-O-glucoside (-7.6 to -7.0) were found to show the highest binding energy (Table 1) when compared with other compounds which are having the activation energy of < 7.0 kcal/mol. It was also found that commercially available anti-breast cancer drugs viz. Tamoxifen, Tormifene and Raloxifene that targets estrogen receptor exhibited binding energy in the range of -5.9 to -7.1 Kcal/mol (Table 1).

The identification of a closely interacting functional group of the ligand with the receptor was achieved by employing the Pymol visualization tool. The isoflavones used in the study were predominantly from plant family Leguminosae, which includes the soybean [26]. In the present study, the HER was found to interact with Biochanin A through 12 amino acid residues, namely M343, M388, M421, L346, L387, L391, L525, A350, R394, H524, E353, G521and G420 (Figure 2 A-B, Table 1). The interaction with Calycosin 7-O-glucoside through 12 amino acid residues, namely A350, L346, L384, L387, L391, L525, L529, R394, C530, V533,

M343, M421, M522, Y526, W383, F404 and T 347 (Figure 2 C-D, Table 1). The compound Naringenin interacts through 13 amino acid residues, namely, M388, M421, L346, L349, L387, L391, L525, A350, E353, G521, G420, H524 and I424 (Figure 2 E-F, Table 1). Prunin (Naringenin 7-O-glucoside), the glycoside of naringenin, interacts through 09 amino acid residues, namely M357, I386, G390, P324, P325, E353, H356, L387 and K449 (Figure 2 G-H, Table 1). The compound daidzein 7-O-beta-D-glucoside interacts through 17 amino acid residues namely V533, C530, M343, M388, M528, T347, F404, R394, A350, E383, G353, K529, L346, L349, L384, L 387 and L525 (Figure 2 I-J, Table 1). The commercially available drug Tamoxifen was shown to bind to the CAS (catalytic active site) of HER via 16 amino acid residues, namely W383, A350, F404, R394, G353, G 420, T 347, M 343, M 421, G 521, L346, L349, L384, L387, L391 and L525. It seems that the amino acid residues A350, L391, L525, R394, and H524 play a critical role in the binding of the drug to its CAS (Figure 2 K-L, Table 1).

Seven carbon atoms of Biochanin A namely, C12, C13, C16, C17, C18, C19 and C21, were predicted to be involved in hydrophobic interactions with amino acid residues G420, G521, A350, L381, L525, M388, and M421. On the other hand, seven carbon atoms and four O atoms of Naringenin namely, C10, C13, C16, C17, C18, C19 and C20 were found to be involved in hydrophobic interactions with amino acid residues L349, L381, L525, G521, G420, M388, I424 and A350 of the receptor. In the case of Prunin, six carbon atoms namely C11, C12, C13, C14, C15 and C16, were found to make hydrophobic interaction with amino acid residues, I386, L387, K449, P324, G390, M357, H356 of HER-α. Fourteen carbon atoms of Calycosin O-beta-D-glucoside namely C11, C14, C16, C18, C20, C21, C22, C23, C24, C25, C26, C28, C29 and C32 were predicted to be involved in hydrophobic interactions with amino acid residues L525, L346, L391, K529, W526, T386, M343, A350 and R394 of the receptor whereas daidzein 7-O-beta glucoside has interaction via 16 carbons namely C12, C13, C14, C16, C17, C18, C19, C20, C21, C22, C23, C24, C27, C29 and C30.

Further insight into the nature and number of bonds formed, it was revealed that various types of interactions took part in the binding of these ligands to HER. The nature of bonding involves hydrogen bond, polar bond, hydrophobic interactions and cation-pi or pi-pi interactions. Accordingly, it was determined that three oxygen atoms namely, O5, O2 and O3 of Biochanin A, were found to involved in polar bond through amino acid residue R394, E353, Y133, L346, L387 and H524 of HER. Further, in Naringenin three Oxygen atoms namely, O3, O4 and O5, were observed to make the polar bond with the amino acid residues E353, L346, 387 and H524 of the HER. This is in harmony with another study where a catalytic triad residue of HER has been reported to make polar interaction with a specific inhibitor. In the case of Prunin, O3, O4 and O6 were found to form polar bonds with the amino acid residues P325 and E353, while Genistein interacts with amino acid residues E353, 346, L387, H524 and R391 through its O2, O4 and O5 atoms. Calycosin 7-O-glucoside was shown to make polar bonds through two oxygen atoms O3 and O7 to the amino acid residues C530 and T347.

The docked structure was further analyzed with the Pymol visualizing software for the exact location of the pharmacophoric part of the ligand. The hydroxyl (-OH) and carbonyl (-CO) group were identified as principal participating groups, involved in the direct contact. Earlier studies demonstrated that complete interaction with the catalytic triad residues and with Leu525 appears essential for full drug activity. His524 or Leu525 as established by mutation studies are pivotal for ligand interaction and recognition while Arg394 acts as a crucial ligand-binding residue [27]. For Biochanin A and Naringenin, the 4'-hydroxyl group contributes a hydrogen bond with the receptor's glutamate (Glu353). In the case of Biochanin A, the same -OH group is additionally recognized by the side chain of the conserved arginine (Arg394), which correctly orients and braces the ligand at the binding pocket of the receptor. Histidine (His524) is a third crucial catalytic residue that interacts via its delta nitrogen, with the -OH group of the B ring of Naringenin or the internal O atom group attached to B ring of Biochanin A (Figure 2, A-B) [27]. It appears that all hydroxyl groups from these two ligands were actively engaged in H bonding. Two additional H bonds with Leu346 and Leu387 were shown by Biochanin A as well as Naringenin; however, H-bonding with critical Arg397 residue was absent from Naringenin. Uniquely, Glu353 makes two H-bonds with two O atoms of Prunin O-glucosidic ring, one through its main chain -COO group and other with its side chain -COO group. Prunin does not show bonding with any other residue of the catalytic triad. This can be attributed to the orientation of interaction between drug and target.

Figure 1: Images of isoflavonoids and their analogs. (A) Daidzein, (B) 3'-Hydroxydaidzein, (C) 6- Hydroxydaidzein, (D) 8- Hydroxydaidzein, (E) Daidzein 7-O-glucoside, (F) 8-prenyldaidzein, (G) Genistein, (H) 8-chlorogenistein, (I) 3-hydroxygenistein, (J) 2-hydroxygenistein, (K) Formononetin, (L) Formononetin glucoside, (M) Calycosin, (N) Calycosin 7-O-glucoside, (O) Naringenin, (P) 8-prenylnaringenin, (Q) Naringenin 7-O-glucoside, (R) Biochanin A and (S) Tamoxifen.

Prunin interacts with critical amino acids in an opposite orientation than that observed with binding in Biochanin A and Naringenin, with H-bonds shown by its O-glucosidic ring only. Similarly, when the O-beta-D-glucoside ring is introduced in Calycosin, a new set of interactions takes place, exhibiting no contact with the characteristic catalytic triad. Two H-bonding takes place, one between 3' hydroxyl of glucosidic ring and N of Cys530, with another between O7 atom of A ring and side-chain hydroxyl of Thr347. Thus, the introduction of the O-glucosidic ring eliminates the interaction with the catalytic triad and lowers the binding stability.

Earlier studies reported that attenuation of estrogenic potency achieved to various degrees by replacing the 7-OH of daidzein with alkoxy group (with more prolonged and bulkier substitution having more significant effects) [28]. Modification on the 7-O position offers a spectacular increase in binding property, signifying the versatility of the daidzein structural motif, which could present an additional pharmacological task that requires the best balance of hormonal activities of the compounds.

S. No.	Ligand Molecules	Molecular formula	Binding energy (Kcal/mol)	Interacting Amino Acids
1.	Biochanin A	$C16H_{12}O_5$	-(7.7-6.6)	M388, M421, L346, L387, L391, L525, A350, R394, H 524, E353, G521, G420
2	Calycosin 7-o-Glucoside	$C_{22}H_{22}0_{10}$	-(7.6-7.0)	A350, L346, L391, L525, L529, R394, C530, M343, Y526, W383, F404, T347
3	Naringenin	$C_{15}H_{12}0_5$	-(8.0-6.5)	M388, M421, L346, L349, L391, L 525, A350, E353, G521, G420, H524, L387, I424
4.	Prunin	$C_{21}H_{22}O_{10}$	-(7.8-6.9)	M357, I386, G390, P324, P325, E353, H356, L387, C449
5.	Daidzein 7-O-β-D Glucoside	$C_{21}H_{20}O_9$	-(8.0-7.3)	V533, C530, M343, M388, M528, T347, R394, A350, K529, L349, L384, L525, L346, L391, E353, L387, W383
6.	Tamoxifen	$C_{26}H_{29}NO$	-(6.8-5.9)	W383, A350, F404, R394, G353, T347, L346, L349, L384, L387, L525, M421, M343, M421, M388, G521

Table 1: Showing interacting amino acids of a few flavonoids/Tamoxifen.

S. No.	Compound/Drugs	Mol. Formula	Binding Energy (Kcal/mol)
1.	Daidzein	$C_{15}H_{10}O_4$	-(6.8-6.5)
2.	3'-Hydroxydaidzein	$C_{15}H_{10}O_5$	-(6.8-6.2)
3.	6-Hydroxydaidzein (Demethyltexasin)	$C_{15}H_{10}O_5$	-(7.1-6.5)
4.	8-Hydroxydaidzein	$C_{15}H_{10}O_5$	-(7.1-6.7)
5.	Daidzein 7-O-beta-D-glucoside	$C_{21}H_{20}O_9$	-(8.0-7.3)
6.	8-prenyldaidzein	$C_{20}H_{18}O_4$	-(7.6-7.1)
7.	Genistein	$C_{15}H_{10}O_5$	-(7.4-6.5)
8.	8-chloro Genistein	$C_{15}H_9ClO_5$	-(7.5-6.5)
9.	3-hydroxy Genistein	$C_{15}H_{10}O_6$	-(6.9-6.3)
10.	2-hydroxy Genistein	$C_{15}H_{10}O_6$	-(7.1-6.5)
11.	Formononetin	$C_{16}H_{12}O_4$	-(6.6-5.9)
12.	Formononetin glucoside	$C_{22}H_{22}O_9$	-(7.4-6.8)
13.	Calycosin	$C_{16}H_{12}O_5$	-(6.9-6.1)
14.	Calycosin 7-O-glucoside	$C_{22}H_{22}O_{10}$	-(7.6-7.0)
15.	Naringenin	$C_{15}H_{12}O_5$	-(8.0-6.5)
16.	8-prenyl naringenin	$C_{20}H_{20}O_5$	-(7.2-6.7)
17.	Naringenin 7-O-glucoside (Prunin)	$C_{21}H_{22}O_{10}$	-(7.8-6.9)
18.	Biochanin A	$C_{16}H_{12}O_5$	-(7.7-6.6)
19.	Tamoxifen	$C_{26}H_{29}NO$	-(6.8-5.9)
20.	Tormifene	$C_{26}H_{28}ClNO$	-(7.1-6.6)
21.	Raloxifene	$C_{28}H_{27}NO_4S$	-(7.0-6.5)

Table 1B: Binding energy of different dietary flavonoids and drugs (Tamoxifen, Tormifene, and Raloxifene) with human estrogen receptor α.

Previous molecular modeling studies with ER ligands suggested that more favorable interaction energy associates with partially antagonistic rather than purely agonistic activity, which conforms to the present observations as well as the behavior in HER‑-responsive systems [29,30]. Several synthetic ligands to HER-α have been developed due to the central role of estrogen signaling in various diseases from cancer to aging [31-33]. The crystal structure of HER-α LBD complex bound to the non-steroidal ligand shows that the hydrophobic interactions primarily rule the accommodation of diverse LBD structures [34]. However, structures of human HER-β bound to genistein and rat HER-β bound to Raloxifene emphasize the importance of hydrogen bond on the reverse sides of the particular ligands [35].

Figure 2: Image showing interacting amino acids of ERT with (A-B) Biochanin A, (C-D) Calycosin 7-O β-D glucoside, (E-F) Naringenin, (G-H) Prunin, (I-J) Daidzein-7-O-glucoside and (K-L) Tamoxifen.

Thus, both H-bond and hydrophobic interactions contribute to the binding affinity and stability of the complex. Daidzein 7-O-β-D Glucoside and naringenin exhibited best binding affinity and therefore can be taken as lead molecule for further improvements. Since the two phytochemicals are ubiquitously present in soy food in our daily diet, requirements can save women from developing breast cancer.

ADMET						
S. No.	**Name of compound**	**BBB**	**HIA**	**Sol.**	**PPB**	**PSA (Å^2)**
1	Biochanin A	3	0	3	1	76.791
2	Calycosin7oglucoside	4	3	3	2	157.098
3	Genistein	3	0	3	2	88.677
4	Naringenin	3	0	3	2	88.677
5	Prunin	4	3	5	0	92.192

Table 2: ADMET prediction with the help of Discovery Studio 4.1 by employing parameters like BBB, HIA, Sol, HTL, CYP2D6, PPB and PSA.

In the ADMET study, we choose seven parameters. Out of 18 compounds, we selected a few parent compounds for the ADMET study. The value from the ADMET study for BBB of Biochanin A is 3 which infer, the compound does not cross BBB (table 2). Biochanin A, genistein and naringenin have shown good absorption as HIA, while the absorption of calycosin7-O-glucoside and prunin found low. Solubility levels predicted from ADMET of the test compound are good.

PPB of calycosin7-O-glucoside, genistein and naringenin are more than 95% while Biochanin A and prunin have shown 80-90%. PSA of Biochanin A, genistein, naringenin and prunin are in the range of 70-80Å2, which predicts as a preferable binding predicted from the *insilico* study.

S. No	Blood-Brain Barrier (BBB)	
1.	Level	Description
	0	Very High
	1	High
	2	Medium
	3	Low
	4	Very low
	5	Warning
2.	Human Intestinal Absorption Level (HIA)	
	0	Good absorption
	1	Moderate absorption
	2	Low absorption
	3	Very low absorption
3.	Solubility Level (Sol)	
	0	Extremely low
	1	Very low but possible
	2	Low
	3	Good
	4	Optimal
	5	Too soluble
	6	warning
4.	Plasma protein binding (PPB)	
	0	Binding (< 90%)
	1	Binding (≥ 90%)
	2	Binding (≥ 95%)
5.	Polar surface area (PSA)	
	70-80 (Å^2)	Most preferable
	80-100 (Å^2)	Preferable
	Greater than 120 (Å^2)	Not preferable

Table 3: Thumb rule for the prediction of different parameters by the ADMET with the help of Discovery Studio 4.1.

Conclusion

The type and number of interactions between protein and its ligand play a crucial role in the drug design based on structure. In the current work, computational approaches have been exploited to identify the mechanism of interactions and binding affinity between HER and few phytochemicals. Our data suggested that at least some of the flavonoid derivatives (Prunin, Diadzein 7-O-β-D Glucoside, Naringenin and Daidzein) possess a high binding affinity for breast cancer receptors than the well-established drugs available in the market. This can open a new vista in the development of a prescription for the treatment of breast cancer. The present analysis also shows that Leu346, Leu384, Leu387, Phe404 and Leu525 are the most critical residues of potential drug targets.

Reported natural compounds from this study could be exploited as the model for designing therapeutic lead compounds which could results in considerable reductions in the therapeutics development period. From ADMET study of few selected compounds indicated that it could not cross BBB while using it as a formulation. *Invitro* study of Biochanin A and naringenin has shown to be very promising on a few cell lines of breast cancer (data not shown). This study needs experimental validation and clinical trials to ascertain phytochemical property as a more potent drug for the management of different types of cancers in general and breast cancer in particular.

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Authors Contribution

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Disclosure

There is no conflict of interests.

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