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IN SILICO APPROACH OF POTENTIAL PHYTOCHEMICAL INHIBITOR FROM EUPHORBIA HIRTA AND FICUS RELIGIOSA FOR THE TREATMENT OF BREAST CANCER BY MOLECULAR DOCKING

Pharma

Dhiraj Kumar* Department of pharmaceutical Sciences of Technology, Birla Institute of Technology, Mesra, Ranchi, India. *Corresponding Author

Sanjana Bhagat Department of pharmaceutical Sciences of Technology, Birla Institute of Technology, Mesra, Ranchi, India.

ABSTRACT

Breast cancer in women is the most common most cancers and in 2018 there have been around 2 million new cases recorded. The highest rate of breast most cancers are stated in Belgium observed by way of Luxembourg. It is the 2nd most frequent cancer, Lung most cancers being the first. If the cancer tumour is located only in the breast, the survival rate would be 99%. If the tumour has huge lymph nodes round the survival rate would be 85% and if the tumour had extended to far away parts, the survival rate would come down to 27%. Mammary gland is an essential organ in mammals which has potential to secrete, synthesize and deliver milk to the babies for nourishment, enhancement and protection. Generally, most cancers are named after the body part in which it originated; thus, breast cancer refers to the erratic enchancment and proliferation of cells that originate in the breast tissue. There are some types of tumours that may also develop inside more thana few areas of the breast. Most tumours are the outcome of benign(non-cancerous) alters within the breast. The estrogen receptors (ER) in regular and diseased states are enormous for the enhancement of applicable therapeutic strategies. Two most important forms of ER exist, ERα and ERβ,which are encoded by separate genes. Estrogens play a central function in breast cancer enhancement with ER popularity being the on the whole significant predictor of breast cancer prognosis. The powerful lead molecule binding mode, residue-interaction patterns and docking energy were examined by using molecular docking and binding free energy studies. The lead compounds and 3ERTcomplex structural stability. The drug-likeness properties of lead compounds were anticipated ADME analysis.

KEYWORDS

Estrogen protein; Breast cancer; Virtual screening; ADME

INTRODUCTION

Cancer could be a chronic abnormal cell disorder or a lethal disease demonstrated by immortality and unrestricted cellular division. Cancer cells could also be invasive, aggressive and metastatic and usually spread into various organs. breast cancer affects the function of normal mammary epithelial cells and is extremely heterogeneous in nature. Breast cancer is one amongst the leading causes of disease among female individuals throughout the world [Jemal et al., 2011]. In the year 2012 alone over 144, 937 breast cancer cases were reported with 48.45%mortality. The incidence of breast cancer cases increased dramatically in patients at the people of 50–64 [Nandakumar, 2001]. In cities, one breast cancer case was reported in every 22 women and in the geographical region this concentration slashed to 1 in 60 women. However, breast cancer cases have also been said amongst male individuals rarely. In the United States, extra than 1500 new cases are reported each year [Giordano et al., 2002]. In western countries, this most cancers are one of the second most leading reasons of death worldwide. In Asian countries, more than 60% breast cancer cases are identified as estrogen receptor alpha effective (ERα) cancers. In normal mammary gland development, ERα plays a big role in breast cancer development, however, ERa specifically mediate the proliferation of estrogen-induced cells in an autocrine mode of action in ERa breast most cancers cell strains in vitro[Tan et al.,2009].

The hyper production of estrogen is one of the main causes for the development of breast cancer. It used to be reported that estrogen receptor is a receptor of nuclear and successfully activated via binding of 17b- estradiol ligand and also described as estrogen. Naturally human population incorporates ER-a and ER-b estrogen receptors, of these two receptors, ER-a is usually expressed in the mammary gland and uterus. In women, estrogen receptors play a vital position in apoptosis, inflammation, homeostasis, differentiation, metabolism, maturation and proliferation in breast most cancers [Bai and Gust, 2009]. The receptor, ER-a is nicely recognised to take section immune surveillance, resistance to apoptosis, metastasis and cell growth [Jiang et al., 2006]. The hyper activity of estrogen hormone might also potentially lead to the multiplication of the ER-a in the mammalian cells which lead to protection and growth of types of breast cancers, and additionally holds various molecular ambitions for the investigation of most cancers. In current years, hormonal treatment, chemotherapy, radiotherapy form a range of combinations which should be relatively organized for every character therapy. Approximately, 60% of the premenopausal women and about 75% of the post-menopausal women have suffered by using estrogen-dependent breast cancer, and ER-a activity was once successfully inhibited by most cancers remedy [Peng et al., 2009]. In the case of breast cancer, endocrine therapy is widely endorsed for metastatic/recurrent breast most cancers or the establishing stage. The presence of ER-a receptor suggests that virtual screening (VS) may be used as an effective tool to identify and display screen the potential compounds from Euphorbia hirta and Ficus religiosa.

Euphorbia hirta: a vital medicinal herb, belongs to genus Euphorbia, family Euphorbiaceae. the entire plant is in many instances utilized to cure a range of diseases, mainly gastrointestinal disorders (including intestinal parasites, diarrhea, peptic ulcers, heartburn, vomiting, and amoebic dysentery), afflictions of the skin and mucous membranes (including warts, scabies, tinea, thrush, aphthae, fungal afflictions, and measles), and respiratory system disorders (including asthma, bronchitis, hay fever, laryngeal spasms, emphysema, coughs, and colds).

E. hirta mostly incorporates flavonoids, terpenoids, phenols, essential oil, and other compounds. One kind of the important parts of E. hirta is flavonoids which include **quercetin, quercitrin, quercitol**.

Ficus religiosa: A variety of Ficus species have been substantially used in traditional medicine, and their barks, fruits, leaves, adventitious roots, latex, seeds possess pharmacological things to do and are medicinally used in different forms as well as in aggregate with different herbs.

It has been gaining recognition due to the fact of its potential health benefits, such as prevention and cure of cancer, respiratory disorder, sexual disorder, gastric problems, inflammation, neurological disorders, diabetes, and cardiovascular problems. Ficus extract is recognised to possess antimutagenic and antimalignant effect, and it has been attributed to the presence of polyphenols phytosterols, tannins, furanocoumarins, hydrocarbons, aliphatic alcohols, unstable components, and a couple of secondary metabolites-like flavonoids mainly **kaempeferol, quercetin, and myricentin**.

MATERIALS AND METHODS

Construction Of Phytochemical Library

Text mining evaluation of some plants through the usage of server DLAD4U (Disease List Automatically Derived For you), PubTator and Carrot2 servers resulted in 2 plants (Euphorbia hirta and Ficus religiosa) with potential anticancer activity. Hence the phytochemicals of these plants may additionally have anti-cancer properties. Therefore, to find out anti-cancer phytochemicals towards Estrogen receptor, a library of 15 phytochemicals was once built from two plants through searching the scientific literature and PubChem(Fig 1).

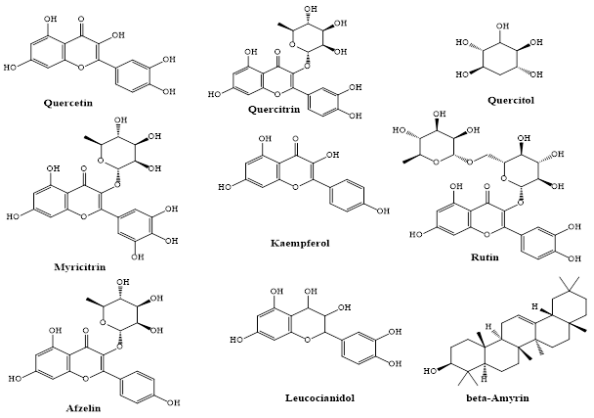


Fig:1 Linear Chemical Structure Of The Active Compounds Investigated Usually As ER Ligands And For Chemopreventing And Treating Breast Cancer

Preparation Of Receptor

The crystal shape of Human Estrogen Receptor (Protein Data Bank) Code: 3ERT,Resolution:1.9Å) was downloaded from the Protein Data Bank (RCSB PDB, http://www.rcsb.org).In this structure, the Human Estrogen Receptor is co-crystalized with the selective inhibitor 4-HydroxyTamoxifen.Human Estrogen Receptor and the inhibitor 4-HydroxyTamoxifen were extracted using Auto-Dock-Tools. The Human Estrogen Receptor crystal shape was once organized for docking simulation through putting off water molecules, hetero atoms and adding polar hydrogens with Auto-Dock-Tools.

Preparation Of Ligands

The 3D structure of each phytochemical used to be downloaded from PubChem (https://pubchem.ncbi.nlm.nih.gov) in SDF layout and then converted into PDB format the usage of Open Babel software. The 3D structure of Reference molecules 4-HydroxyTamoxifen which was once co-crystallized with protein 3ERT used to be downloaded from ChemSpider.

Rigid Docking

Computational Docking is carried out to obtain a population of feasible orientations and conformations for the ligand at the binding site. Firstly, docking was once performed with reference molecules of respective proteins to validate the docking protocol. The grid center for docking was set X= 30.282, Y= -1.913 and Z= 24.207 with dimensions of the grid box 40 × 40× 40Å for 3ERT.Aftervalidation of the docking protocol, virtual screening was once performed by rigid molecular docking into the active site of proteins. Throughout the virtual screening, the ligand molecules were flexible and macromolecules were once kept as rigid. Finally, the end result of binding energy was once extracted from the software. The best confirmation of the compounds which had lower binding energy as compared to the reference molecule was once chosen for similar analysis. Molecular interactions between protein-ligand complexes, which includes hydrogen bonds and the bond lengths, had been analyzed and depicted via using BIOVIA Discovery Studio visualizer.

Drug Likness Activity And ADMET Prediction

The drug-likeness analysis was carried out to recognize any cytotoxicity to humans by Swiss-ADME an open-source software. The pharmacological significance of a ligand is evaluated on various properties like drug bioavailability, drug-likeness or ADMET etc. These properties are calculated the use of certain physicochemical and structural properties. Therefore, all ligands had been evaluated for their druglike nature under Lipinski's rules of five with the aid of Swiss-ADME. Absorption, Distribution, Metabolism, Excretion, and Toxicity (ADMET) are pharmacokinetic properties of a ligand that deals mainly with its Absorption Distribution, Metabolism, Excretion, and Toxicity. The admet server was used for inspecting the ADMET pharmacokinetic properties and mutagenic properties of screened compounds respectively.

Visualization

The evaluation of 2D Hydrogen-bond interactions of the complicated receptor-ligand structure used to be carried out through BIOVIA Discovery Studio visualizer program to identify the interactions of an amino acid of a receptor with a ligand. BIOVIA Discovery Studio depicts hydrophobic bonds, hydrogen bonds, and their bond lengths in each docking pose in the form of graphical representation. Thus, lower binding energy of screened compounds suggests a greater affinity for Estrogen Receptor. These compounds were namely: These compounds were namely: **Quercitrin, Myricitrin, Kaempferol, afzelin, Beta-sitosterols, Beta-Amyrin**.

RESULTS

Virtual Screening

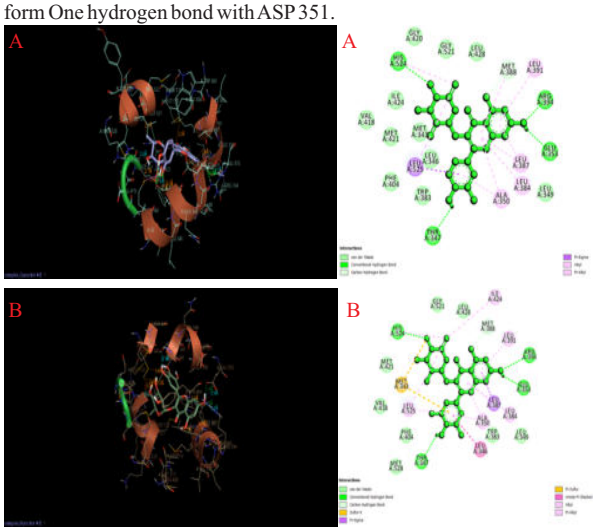
Before the screening, the docking protocol used to be validated by means of re-docking the reference molecules into the binding pocket of the active site of 3ERT structure. The result confirmed that the docked reference molecules have been partially superimposed with the co-crystallized reference molecule. Thus, the protocol used to be regarded well sufficient for reproducing the docking outcomes similar to the X-ray crystal structure and was observed for digital screening. Virtual screening resulted in the top 6 phytochemicals from every goal displaying extensively lower binding energy. The binding energy of phytochemicals with 3ERT was -11.53 for Reference, -8.92 for Quercitrin, -7.76 for Myricitrin, -7.54 for Kaempferol, -9.2 for afzelin, -11.45 for Beta-sitosterols, -8.32 for Beta amyrin. Thus, lower binding energy of screened compounds shows a higher affinity for 3ERT enzyme [Table 1].

Drug-likeness And Admet Prediction

The results of Swiss-ADME software shows the molecular weight of phytochemicals are under 500, LogP value is under 5, the numbers of bond acceptors are under 10, and several other hydrogen bonds are donors under 10 phytochemicals were found to agree with Lipinski's rule of 5. Hence the screened phytochemicals follow Lipinski's rule of 5. However, three compounds show 2 and one compounds show 3 and five compounds show 1 violation of Lipinski's rule (Table 2). Table 1 illustrates the relative ADMET profiles of the screened phytochemicals as compared references. Log S refers to the solubility of the ligand that ideally stages between -8.25 and 0.86. All the hit phytochemicals are displaying Log S values between these ranges. Among all the screened ligands, Beta-Amyrin Log S value (-8.25) and Quercitol has the most Log S value (-0.86). The BBB permeability value for all the hit phytochemicals was comparable to the reference molecule. The BBB permeability value for all the hit phytochemicals was once related to the reference molecule. The perfect vary of BBB values for a perfect drug candidate stage between -3.0 and 1.2. All the phytochemicals have the BBB value under this best range.

Visualization

The 2D & 3Dinteractions of the screened ligands, as well as reference compounds, in the active sites of the receptor, were visualized by using BIOVIA Discovery Studio& Flare software (Figure 2 & 3). The reference molecule Hydroxy Tamoxifen of 3ERT shows interaction with the several residues and forms one hydrogen bond with ARG394, and yields the binding energy is -11.53 kcal mol-1.Quercitrin forms Three hydrogen bonds with THR 347,GLY 428, LEU348. Myricitrin forms Two hydrogen bonds with GLU353,ARG 394. Kaempferol forms Three hydrogen bonds with GLU 353,MET388,THR347. Afzelin forms One hydrogen bonds with LEU 348.Beta-sitosterols form One hydrogen bond with ASP 351.



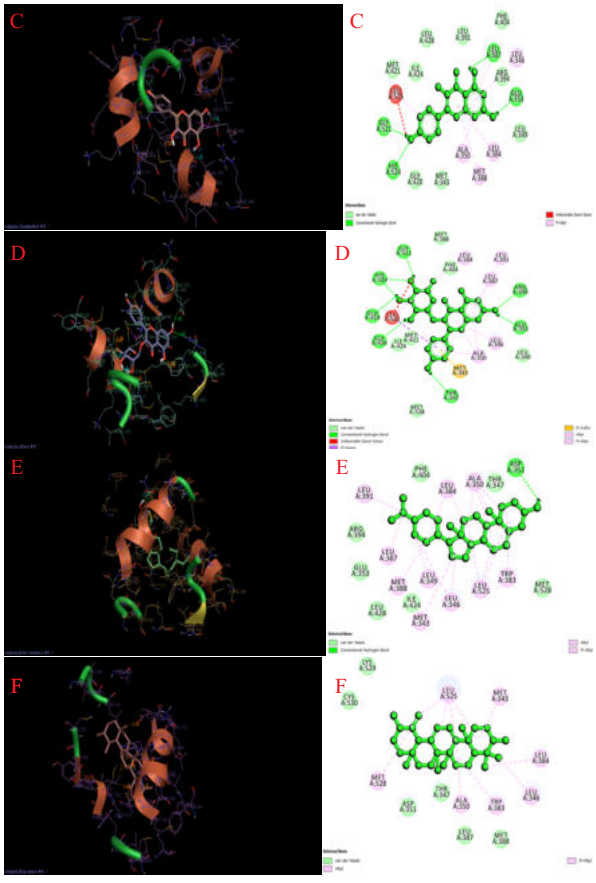


Figure 2. The three-dimensional & two-dimensional protein structure of the ER-ligand complex: (a) Quercitrin (b) Myricitrin (c) Kaempferol (d) afzelin (e) Beta-sitosterols (f) Beta-Amyrin.

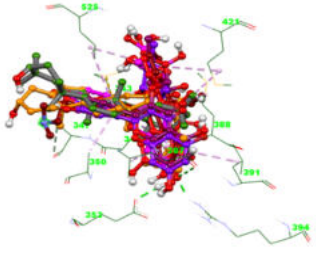


Fig 3. Putative binding orientation of Quercitrin, Myricitrin, Kaempferol, afzelin, Beta-sitosterols, Beta-Amyrin and co-crystal ligand within the active pocket of Estrogen Receptor

Table 1. Binding Energy Of Different Identified Phytocompounds And Drugs (tamoxifen) With Human Estrogen Receptor.

S. No.	Compound/Drugs	Mol. Formula	Binding Energy (Kcal/mol)	Inhibition Constant(kI)
1	Quercetin	$C_{15}H_{10}O_7$	-6.72	11.88um
2	Quercitrin	$C_{21}H_{20}O_{11}$	-8.92	288.22nm
3	Quercitol	$C_6H_{12}O_5$	-4.48	520.53um
4	Myricitrin	$C_{21}H_{20}O_{12}$	-7.76	2.07um
5	Kaempferol	$C_{15}H_{10}O_6$	-7.54	2.99um
6	Rutin	$C_{27}H_{30}O_{16}$	-6.48	17.85um
7	afzelin	$C_{21}H_{20}O_{10}$	-9.2	179.15nm
8	Leucocyanidin	$C_{15}H_{14}O_7$	-7.02	7.21um
9	Beta-sitosterols	$C_{20}H_{24}N_2O_3$	4.06nm	4.37um
10	Beta-Amyrin	$C_{30}H_{50}O$	793.94nM	382.12nM
11	Alfa-Amyrin	$C_{30}H_{50}O$	4.58uM	2.01uM
12	Eligic acid	$C_{14}H_6O_8$	3.91uM	1.19uM
13	Mycritin	$C_{21}H_{24}N_2O_2$	7.8uM	917.0nM
14	**Hydroxy Tamoxifen**	$C_{26}H_{29}NO_2$	3.52nM	161.89nM

Table2. ADMET Properties Of Screened Phytochemicals

ADMET Properties									
S. No	Molecule	HBD	HBA	LogS	BBB	HIA	NOR	TPSA (Å²)	Rule of 5 violation
1	Quercetin	5	7	-3.16	No	High	1	131.36	0
2	Quercitrin	7	11	-4.44	No	Low	3	190.28	2
3	Quercitol	5	5	-0.86	No	Low	0	101.15	0
4	Myricitrin	8	12	-3.20	No	Low	3	210.51	2
5	Kaempferol	4	6	-3.31	No	High	1	111.13	0
6	Rutin	10	16	-3.30	No	Low	6	269.43	3
7	afzelin	6	10	-3.47	No	Low	3	170.05	1
8	Leucocyanidin	6	7	-1.60	No	High	1	130.61	1
9	Beta-sitosterols	6	7	-1.60	No	High	1	130.61	1
10	Beta-Amyrin	1	1	-8.25	No	Low	0	20.23	1
11	Alfa-Amyrin	0	4	-1.51	Yes	High	6	52.60	0
12	Eligic acid	4	5	-1.64	No	High	1	97.99	0
13	Mycritin	8	12	-3.20	No	Low	3	210.51	2
14	Hydroxy Tamoxifen	1	3	-5.84	Yes	High	9	32.70	1

DISCUSSION

Currently, Breast cancer has become a huge challenge for each and every country. As we know that very few drugs are available for the prevention of the disease, we can use some natural products which can be useful to prevent the disease with minimum / no side effects. Our study is primarily based on specific targeting of Estrogen receptors to find novel compounds that can be used as a new drug against Breast cancer. We prepared a phytochemicals library from 2 medicinal plants. These phytochemicals were subjected to molecular docking against Estrogen enzymes. Based on molecular docking study, we observed six phytocompounds namely Quercitrin, Myricitrin, Kaempferol, afzelin, Beta-sitosterols, Beta-Amyrin showing better and significant binding energy against these receptors. Afzelin& Beta-sitosterols have maximum phytochemicals which show binding with Estrogen. Moreover, ADMET outcomes show that these compounds have an on-carcinogen property for humans. As a recommendation since there are few effective drugs against Breast cancer, many such medicinal plants are available which have anticancer, antibacterial and antifungal activity. The phytochemicals of these medicinal plants (Euphorbia hirta and Ficus religiosa)can be used towards Breast cancer.

CONCLUSIONS

The existing study was carried out to find novel inhibitor molecules against Estrogen Receptor. Consequently, a library of 14 phytochemicals was once analyzed by molecular docking techniques. The results of the top 6 ligands had been in contrast with reference molecules of protein which demonstrates that these phytochemicals can bind more efficiently and act as inhibitors. Thus, we conclude that these phytochemicals can be utilized as viable anti-Breast cancer candidates. These novel molecules may want to be utilized for further innovation and improvement of anticancer compounds towards Breast cancer.

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