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In silico screening of phytochemicals to explore potent anti-breast cancer inhibitors against estrogen receptors

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Abstract
Breast cancer is the second most common cancer across the globe, there is a need for the development of effective therapeutic agents. Current computational studies play a significant role in identifying new leads for disease treatment. This study was performed to screen the effective bioactive molecules against estrogen receptors. A dataset of plant-based natural anti-breast cancer compounds was selected. Molecular docking was performed to estimate the spatial affinity of target compounds for the active sites of the estrogen receptor. The *In silico* ADMET studies were performed for the lead molecules. Results showed that Genistein, Daidzein and Panaxadiol are having the best docking score and good binding affinity than other ligands. Hence, Genistein, Daidzein and Panaxadiol can be considered as a better drug candidate for anti-breast cancer inhibitors against estrogen receptors which can be explored further.

Keywords: ADMET, breast cancer, estrogen receptor, molecular docking, phytocompounds

1. Introduction

Breast cancer is strangely common among the population across the globe. An estimated 2.1 million cancer cases accounted for in 2018, found to be a fifth leading cause among cancer mortality worldwide. A ratio of persons suffering from breast cancer to healthy population was found to be 1 out of every nine women among the developed nations and 1 in every 20 persons in less developed nations as reported in 2018 (Dolatkhah, *et al*., 2020) [9]. Different types of breast cancers exist, few may have hormone receptors like estrogen or progesterone (others may have both) and are called ER+ or PR+ breast cancer, respectively. The main driver among, the majority of breast cancer cases is the estrogen receptor ER since it is found to exist among 75% of overall breast cancer cases (Masoud and Pagès, 2017) [17].

For the normal female physiology, reproduction and behavior, the steroid hormone estrogen is essential, because of its effects on cellular processes together with cell proliferation and cell survival. The nuclear estrogen receptors (ERα and ERβ) facilitate these effects. The ERα and ERβ estrogen receptors are encrypted by separate genes, positioned on different chromosomes. ERα-positive cells make a vital involvement in mammary development. On the contrary, normal development happens for the mammary glands of ERβ mice. When the two receptors are co-expressed in breast cancer cell lines, ERβ functions as an adversary of ERα, harming the ability of estrogen to arouse proliferation. Minimum 70% of the breast cancers are categorized as ER-positive breast cancers and meddling with estrogen action has been a linchpin of breast cancer therapeutics for over a century (Musgrove and Sutherland, 2009) [18].

Herbal medicine has turned out to be a very safe, non-toxic, and easily accessible source of compounds used in cancer treatment. Phytocompounds are thought to counteract the effects of diseases in a body due to the possession of various biomolecular characteristics (Khan, *et al*., 2020) [12]. Exploration of human breast cancer inhibitors executes a vital part in the drug discovery method. Based on previous *in silico* studies, a review shows that 131 phytocompounds were selected from 51 plant families (Prabhavathi, *et al*., 2020) [23]. These plant families comprises Apocynaceae (Richard, *et al*., 2015; Omogbadegun, 2013) [25, 19]. And Euphorbiaceae family (Dasaroju and Gottumukkala, 2014) [7]. Has got 4.58% of plant compounds from each family respectively, followed by Lamiaceae has got 6.10% phytocompounds (Akhtar and Swamy, 2018; Kim, *et al*., 2016; Preethi and Padma, 2016; Woźniak, *et al*., 2015; Wang, *et al*., 2012) [1, 13, 24, 28, 27]. From Asteraceae family, 9.92% of plants compounds (Omogbadegun, 2013; Csupor-Löffler, *et al*., 2011) [19, 5]. And rest of the compounds are grouped as other plant families (Lee, *et al*., 2017; Levitsky and Dembitsky, 2015; Pierpaoli, *et al*., 2015; Gladys, *et al*., 2013; Bhoopat, *et al*., 2011) [14, 15, 20, 10, 3]. From these plants, the active phytocompounds were identified from a varied set of medicinal plants as anti-breast cancer agents.

Virtual screening thousands of compounds is made possible using the *in silico* approaches, in a very reasonable time. This drastically reduces the costs involved in the identification of hits and further increasing the probabilities of finding the anticipated drug candidates. One of the widely popular and systematic structure-based *in silico* methodology is molecular docking studies. Molecular docking is among one of the most popular and successful structure-based *in silico* methods, which help predict the interactions occurring between molecules and biological targets. It helps to find the interactions happening among the molecules and biological targets. This methodology is normally achieved by first predicting the molecular orientation of a ligand within a receptor, and then assessing their complementarity through the use of a scoring function (Pinzi and Rastelli, 2019) [22]. This study aims at screening a set of phytocompounds which could inhibit ER. The study

undertakes virtual ligand screening to bring forth a set of phytochemicals capable of inhibiting ER targets and thus implementing a multi-target approach. To meet that goal, the study focused on ERα and ERβ which are responsible for breast cancer and a set of phytochemicals were virtually screened against the ER through a structure-based drug design approach.

2. Material and methods

2.1 Ligand preparation

For the current study, biologically active medicinal plant phytocompounds for anticancer property collected from the previous literature review are considered. The 40 phytocompounds were selected as ligands for anti-breast cancer agents which were screened by in silico approach (Prabhavathi, *et al*., 2020) [23]. Ligand preparation of whole 40 phytocompounds is carried out. The three dimensional (3D) structure of the phytocompounds was downloaded in sdf format using the PubChem database (http;//pubchem.ncbi.nlm.nih.gov) for docking studies. Ligand Optimization was performed using energy minimization parameter Universal Force Field (UFF) with a conjugate gradient optimization algorithm at 200 steps, Energy minimization was carried out to attain the lowest free energy by open babael in PyRx (Dallakyan and Olson, 2015) [6]. And converted them into PDBQT formats for molecular docking.

2.2 Protein structure preparation

The protein structure of human ERα (PDB Code: 3ERT) and ERβ (PDB Code: 1QKM) downloaded from protein databank (http://www.rcsb.org/) in pdb format (.pdb) were selected as breast cancer target proteins. The 3D crystal structure of human ERα accession number 3ERT (Shiau, *et al*., 1998) [26] in complex with ligand 4-hydroxytamoxifen and ERβ with accession number 1QKM (Pike, *et al*., 1999) [21] in complex with partial agonist genistein was retrieved from protein data bank (http://www.rcsb.org), the protein structures were refined for docking study. The complexes bound to the receptor molecule, all the heteroatoms and the non-essential water molecules were removed using BIOVIA Discovery Studio Visualizer (BIOVIA, 2017) [4]. And saved as PDB format. These processed protein structures are then converted to PDBQT file by selecting make macromolecules using the PyRx tool (Dallakyan and Olson, 2015) [6]. The additional configuration parameters were also set to their defaults. The missing amino acids in the target were added. The potential ligand binding domain region were recognized for docking studies.

2.3 Setting grid parameters

Active binding sites are the regions of the proteins where ligand molecules bind and inhibit the disease. Hence Protein-Ligand binding sites are the most selected binding sites for docking studies (Yusuf, *et al*., 2018) [30]. As a result, in the present work, the grid box was set to study the protein-ligand interaction of ERα and ERβ proteins. The grid box was set at the binding region of 4-hydroxytamoxifen of ERα with a grid volume of 29.41, 23.36 and 26.15 Å for X, Y and Z dimensions correspondingly, and the grid centre was fixed to 29.70, -0.60 and 26.05 Å for X, Y and Z axis respectively. The binding domain of ERβ with partial agonist genistein was preferred to set the grid box and, the grid volume was adjusted to 25.53, 20.043 and 24.07 Å for X, Y and Z dimension and the grid centre was set to 26.46, 9.53 and 113.38 Å for X, Y and Z.

2.4 Molecular docking study

The molecular docking study was carried out with the processed phytocompounds and ERα and ERβ receptor using Autodock PyRx docking tool (Dallakyan and Olson, 2015) [6]. Molecular docking energy evaluation was done with the help of Dock score function. The ligand-receptor interaction energy (kcal/mol) was obtained after docking the ligands into the active site of 3ERT and 1QKM. The docked ligand-receptor complex was assessed on the lowest negative binding energy (kcal/mol) values.

2.5 Post docking analysis and validation

Post docking analysis was carried out for the virtual screened phytocompounds against the active conformation of ERα and ERβ receptor. The docked protein-ligand complex was analyzed using Discovery Studio v19.1.0.18287 (BIOVIA, 2017) [4]. For the best-docked poses (Guedes, *et al*., 2014) [11]. Based on the docking score of ligands against binding target and clinically used drug, the interaction analysis involves the study of non-covalent interactions like Van der Walls, hydrogen bonds, charge interactions and hydrophobic interactions. The results were validated to compare the interaction of amino acids involved in docking studies with the clinically used drugs of ER breast cancer, Tamoxifen, Raloxifene.

2.6 ADMET prediction

Absorption, Distribution, Metabolism, Excreation and Toxicity (ADMET) properties of ligand were analyzed using admetSAR, an online web server (http;//immd.ecust.edu, cn: 8000/). ADMET properties of the ligand were evaluated to determine their activity inside the body at the early phase of the drug discovery process. By using admetSAR tool Blood-Brain Barrier (BBB) penetration, Human Intestinal Absorption (HIA), Caco-2 permeability, renal organic cation (ROC) transporter, CYP enzymes, Human ether-a-go-go-Related Gene (hERG) inhibition, AMES mutagenesis, Carcinogens toxicity, and LogS values obtained are analyzed (Yang, *et al*., 2019) [29].

3. Results and Discussion

3.1 Molecular docking studies

The ERα and ERβ receptor are targeted by forty one phytocompounds. Phytocompounds were subjected to Molecular docking simulations studies to evaluate the potential inhibitions. The docking study was performed using the docking algorithm inbuilt with Auto Dock Vina in PyRx software (Dallakyan and Olson, 2015) [6]. The predicted docking score or binding energy and hydrogen bonding are strongly focused on docking analysis. The values of binding free energy of the phytocompounds at the active site of ERα and ERβ receptor are reported in Table 1.

Table 1: The Binding energy of different phytochemicals against ERα (3ERT) and ERβ (1QKM) target.

Sl. No.	Phytocompounds PubChem Id	The binding energy of ERα (kcal/mol)	The binding energy of ERβ (kcal/mol)
1	Strophanthidin/6185	-7.9	-6.3
2	Acronycine/345512	-7.4	-7.9
3	Amooranin/11503749	-8.8	-2.1
4	Galangin/5281616	-8.1	-8.4
5	Pinocembrin/68071	-8.6	-8.7
6	1-Acetoxychavicol acetate/119104	-6.8	-6.9
7	Arctigenin/64981	-7.4	-5.0
8	Hellebrigenin 3-acetate/267436	-7.5	-4.6
9	Emetine/10219	-8.2	-6.6
10	Holacanthone/158475	-8.0	-7.1
11	Glaucarubinone/441796	-8.1	-7.4
12	Berberine/2353	-7.4	-7.7
13	Kaempferol/5280863	-8.2	-8.7
14	Daidzein/5281708	-9.0	-9.7
15	Genistein/5280961	-9.1	-9.7
16	Licoagrochalcone A/11099375	-8.7	-5.8
17	Boeravinone G/11537442	-6.8	-8.5
18	Boeravinone H/16745324	-7.1	-7.5
19	Indicine-N-oxide/280564	-7.2	-6.3
20	Camptothecin/24360	-8.7	-5.9
21	Harringtonine/276389	-7.3	-7.1
22	Colchicine/6167	-8.0	-6.7
23	Medicagenic acid/65048	-8.7	-4.4
24	Asiatic acid/119034	-8.6	-5.7
25	Lapachol/3884	-7.8	-7.6
26	5-Methoxypodophyllotoxin/3035544	-7.3	-7.6
27	Lignans/443013	-7.4	-7.2
28	Alpha-Peltatin/92129	-7.8	-8.1
29	Podophyllotoxin/10607	-7.7	-7.7
30	Epicatechin/72276	-8.3	-7.9
31	Apigenin/5280443	-8.5	-8.9
32	Oleanolic acid/10494	-8.7	-5.5
33	Withaferin A/265237	-8.9	-3.0
34	Rosmarinic acid/5281792	-7.9	-8.0
35	Tylocrebine/246845	-6.8	-6.9
36	Panaxadiol/73498	-8.5	-11.1
37	Ursolic acid/64945	-8.8	-5.1
38	Taxodione/73588	-7.8	-7.3
39	Digallic acid/341	-7.3	-8.2
40	Quercetin/5280343	-7.9	-8.1
41	Hydroxytyrosol/82755	-5.7	-5.8
43.	Ref. Drug Tamoxifen/2733526	-9.5	-4.0
44	Ref. Drug. Raloxifene/5035	-9.9	-7.3

Comparative docking analyses were carried out to find potential hit phytocompounds with the lowest binding energy values than the clinically approved reference drugs. Forty one phytocompounds are compared with reference drugs. The phytocompounds Genistein -9.1 kcal/mol, Daidzein -9.0 kcal/mol, Withaferin A -8.9 kcal/mol, Ursolic acid and Amooranin as exhibited -8.8 kcal/mol have shown lowest binding energy values very close to the binding energy values of the clinically used reference drugs Tamoxifen-9.5 kcal/mol and Raloxifene -9.9 kcal/mol. The rest of the phytocompounds have exhibited the binding energy value ranging from -5.7 to -8.7 kcal/mol for the ER α receptor. Similarly, for ERβ receptor, 21 phytocompounds have shown the lowest binding energy value than the reference drug Raloxifene -7.3 kcal/mol, The phytocompound panaxadiol with binding energy -11.1 kcal/mol as shown the highest negative binding energy fallowed by Genistein and Daidzein with -9.7 kcal/mol binding energy values than the reference drug Raloxifene -7.3 kcal/mol with ERβ as reported in Table 1. This Comparative Computational docking analyses of selected phytocompounds with the ERα and ERβ receptor target gives an insight into the efficacy of phytocompounds over clinically approved reference drug molecules.

3.2 Interaction analysis of the hit phytocompounds

The hit phytocompounds are validated through non-covalent interactions analysis between the hit compounds and target receptors. The docking score and stability of the hit phytocompounds and their interactions at the binding site of target ERα and ERβ receptor are recognized by common amino acid residue forming interactions reported in Table 2 and 3. Their best-docked pose is represented in Figure 1 and 2 for ERα and Figure 3 and 4 for the ERβ receptor. Further, these interactions were compared to reference drug Tamoxifen and Raloxifene to assist in understanding the putative mechanism of action.

Table 2: Molecular interactions between ERα receptor (3ERT) and hit phytocompounds.

Sl. No.	Phytocompounds / Pubchem Id	Binding energy kcal/mol	Hydrogen bond (HB) interaction (Å)		Hydrophobic Interaction Alkyl / Pi alky	Pi -Sigma	Pi - Sulfur	Van der Walls interaction
1.	Genistein/5280961	-9.1	Leu346 Arg394 Glu353 Leu387	2.35 1.95 3.05 2.99 2.87	Ala350, Ltu391, Met388, Leu 525	Ile424	Met421	Leu349, Thr347, Met343, Glu419, Gly420, His524, Gly521, Leo384, Leu428
2.	Daidzein/5281708	-9.0	Glu353	2.26	Leu525, Leu387, Met388, Leu384, Leu391, Ala350	Ile424	Met421	Gly420, Met343, Leu346, Leu349, Arg394 Leu428, Gly521
3.	Withaferin A/265237	-8.9	--	--	Leu525, Trp383, Leu387, Lru384, Ala350	-	-	Val533, Pro535, Cys530, Lys529, Met528, Thr347, Met343, Leu346, Asp351, Leu536.
4.	Ref. Drug Tamoxifen/2733526	-9.5	-	-	Leu387, Leu346, Met421, Leu525, Ala350, Met388.	-	-	Glu420, His524, Glu419, Arg394, Glu353, Leu349, Met528, Leu391, Phe404, Trp383, Gly521, Ile424, Met343, Leu384, Leu428, The347
5.	Ref. Drug. Raloxifene/5035	-9.9	Gly521 Arg394 Leu387	2.87 2.34 2.42	Lys529, Ala350, Leu346, Leu525, Leu384.	Leu525	Met343, Met421	Tyr526, Thr347, Ile424, Gly420, His524, Met522, Trp383, Leu349, Glu353, Leu391, Met388

Table 3: Molecular interactions between ERβ receptor (1QKM) and hit phytocompounds

Sl. No.	Phytocompounds / Pubchem Id	Binding energy kcal/mol	Hydrogen bond (HB) interaction (Å)		Hydrophobic Interaction Alkyl / Pi alky	Pi - Sigma	Pi - Sulfur	Van der Walls interactions
1.	Panaxadiol/73498	-11.1	Arg346	2/34	Met295, His475, Leu476	-	-	Met473, Met479, Met336, Ile373, Ile376, Leu380, Met340, Phe377, Leu343, Phe356, Gly472, Leu298, Thr299, Ala302, Leu339, Leu301, Ala357, Glo305
2.	Daidzein/5281708	-9.7	-	-	Leu343, Leu339, Ala302, Lei298, Leu476	Met336	-	Arg346, Leu380, Met340, Ile376, Ile373, Gly472, Glu305, Leu301, Thr299, Met295, Met479
3.	Genistein/5280961	-9.7	Glu305, Leu338, His475	3.07, 2.83 2.89 1.76	Leu343, Ala302, Leu298, Leu476. Leu339, Phe356.	Met336	-	Thr299, Met479, Gly472, Met295, Ile373, Leu 380, Ile376, Phe377, Leu301, Met340, Arg346
4.	Ref. Drug Tamoxifen/2733526	-4.0	.	-	Arg284, Pro285, Met296	-	-	Val485, Ala292, Ser293, Ser297, Lys300.
5.	Ref. Drug. Raloxifene/5035	-7.3	Arg346	2.56	Pro358, His279, Pro277, Trp345.	-	-	Ile355, Glu305, Met309, Leu339, Val338, Lys401, Gly342, Tyr397, Ile348, Asp349

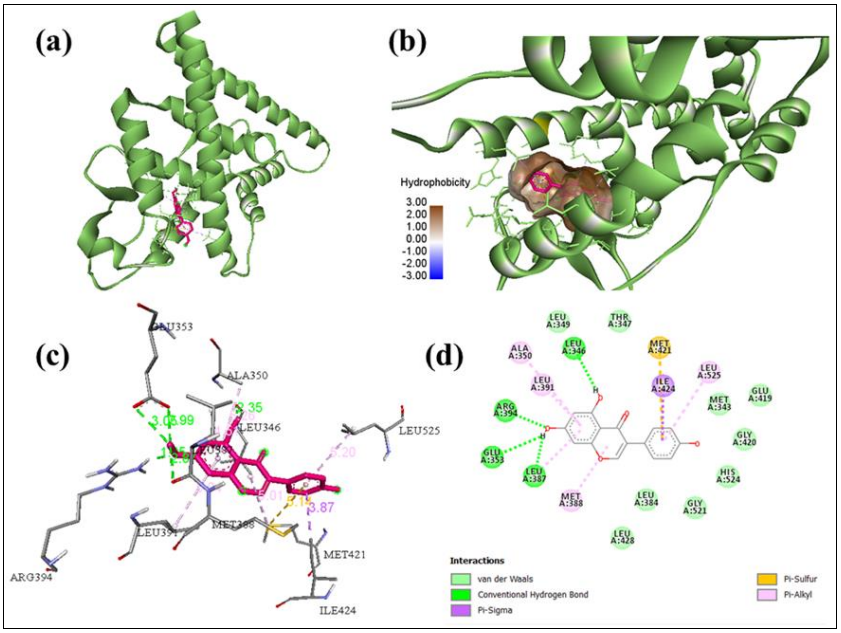

Fig 1: Docked poses of ERα (3ERT) with Genistein/5280961 (a) Genistein docked to ERα; (b) Hydrophobicity surface at the active binding site of ERα with Genistein; (c) 3D stick diagram of surrounding ER-α amino acids with Genistein; (d) 2D view of surrounding ERα amino acids with Genistein.

The hit phytocompounds Genistein (-9.1 kcal/mol), Daidzein (-9.0 kcal/mol) and Withaferin A (-8.9 kcal/mol) stability in the binding site of ERα receptor is also recognized by common amino acids residue forming interactions like conventional hydrogen bond, hydrophobic interaction, Pi Sigma, Pi Sulfur, and van der Walls interactions are reported in Table 2, in comparison with the reference drug Tamoxifen and Raloxifene. The best interaction poses for Genistein, with ERα receptor is shown in Figure1. Genistein docked well with the target binding pocket of the ERα receptor with the binding energy of -9.1 kcal/mol determines the ability of Genistein to inhibit ER-α receptor. The conventional hydrogen bonds with the amino acids Leu346, Arg394, Glu353 and Leu387 at a distance of 2.35, 1.95, 3.05, 2.99 and 2.87 interrelated with the high binding affinity of Genistein with ERα receptor. The high binding affinity of Genistein with ERα receptor may be due to the bond length distance difference of amino acid residue in comparison with reference drug Tamoxifen and Raloxifene with ERα complex, hydrogen bond-forming amino acids are Gly521, Arg394 and Leu387 and the bond length distances are 2.87, 2.34 and 2.42 Aº. In both of the complexes (ERα - Genistein and ERα -Raloxifene complex), Arg394 and Leu387 amino acids have formed H-bonds. Hydrophobic interactions with amino acids Ala350, Leu 391, Met388 and Leu 525 and van der Walls interactions with Met473, Met479, Met336, Ile373, Ile376, Leu380, Met340, Phe377, Leu343, Phe356, Gly472, Leu298, Thr299, Ala302, Leu339, Leu301, Ala357 and Glo305 of Genistein with ERα are reported in Table 2 has also contributed to the low binding energy.

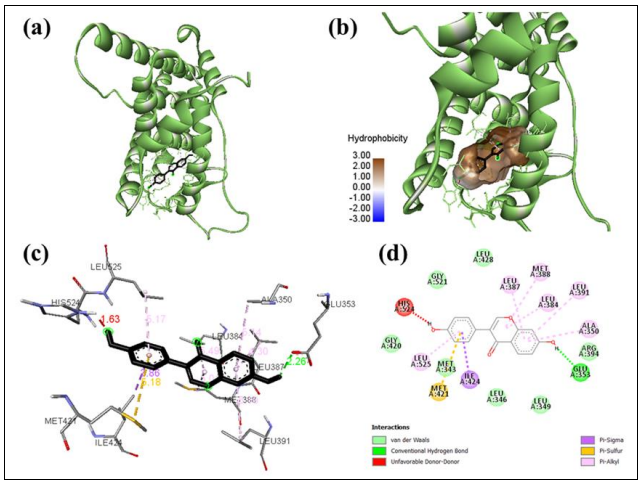

Fig 2: Docked poses of ERα (3ERT) with Daidzein/5281708: (a) Daidzein docked to ERα; (b) Hydrophobicity surface at the active binding site of ERα with Daidzein; (c) 3D stick diagram of surrounding ERα amino acids with Daidzein; (d) 2D view of surrounding ERα amino acids with Daidzein.

The best interaction poses for Daidzein with ERα is shown in Figure 2. Daidzein docked well with the ERα with a binding free energy value of -9.0 kcal/mol, as reported in Table 1. Conventional hydrogen bonds with amino acid Glu353 at a distance of 2.26 Å with binding affinity close to the reference drug. Hydrophobic interactions with amino acids Leu525, Leu387, Met388, Leu384, Leu391 and Ala350 Van der Walls interactions with Gly420, Met343, Leu346, Leu349, Arg394 Leu428 and Gly521 are reported in Table 2, Pi-Sigma and Pi-sulfor interactions formed by the amino acids Ile424 and Met421 and dominant ligand-protein association in protein binding site also contributed to low binding energy (Barratt, *et al*., 2005) [2].

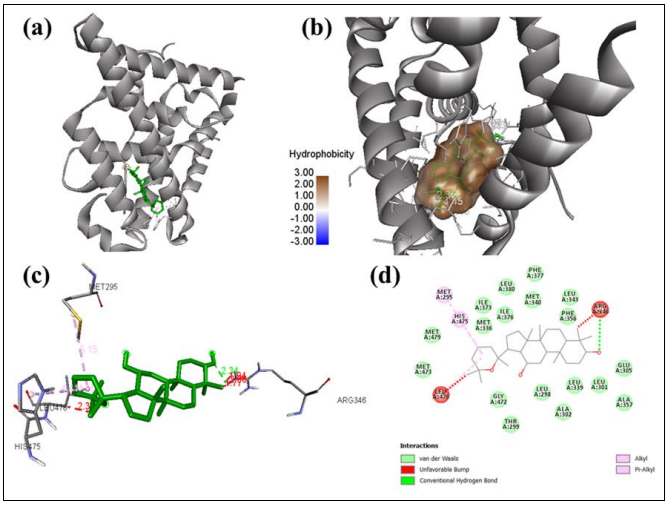

Fig 3: Docked poses of ERβ (1QKM) with Panaxadiol /73498: (a) Panaxadiol docked to ER β; (b) Hydrophobicity surface at the active binding site of ER β with Panaxadiol; (c) 3D stick diagram of surrounding ER β amino acids with Panaxadiol; (d) 2D view of surrounding ER β amino acids with Panaxadiol.

The two hit phytocompounds Panaxadiol (-11.1 kcal/mol), and Genistein (-9.7 kcal/mol) stability in the binding site of the ERβ receptor is recognized by common amino acids residue forming interactions like conventional hydrogen bond, hydrophobic interaction, Pi Sigma, Carbon-Hydrogen bond and van der Walls interactions are reported in Table 3 in comparison with the reference drug Tamoxifen and Raloxifene. The best interaction poses for Panaxadiol with ERβ receptor is as shown in Figure 3. As reported in Table 1, Panaxadiol represented the lowest binding energy of -11.1 kcal/mol with the ability to inhibit the ERβ receptor compared to other phytocompounds and reference drug. The presence of conventional hydrogen bonds with Arg346 showed an interatomic distance of 2.34 Å, representing a high binding affinity of the docked complex (Daze and Hof, 2016) [8]. Other interactions such as Pi-alkyl interactions with amino acids Met295, His475 and Leu476 and strong van der Walls interactions with Met473, Met479, Met336, Ile373, Ile376, Leu380, Met340, Phe377, Leu343, Phe356, Gly472, Leu298, Thr299, Ala302, Leu339, Leu301, Ala357 and Glu305 of Genistein with ERα are reported in Table 2 has also contributed to the low binding energy.

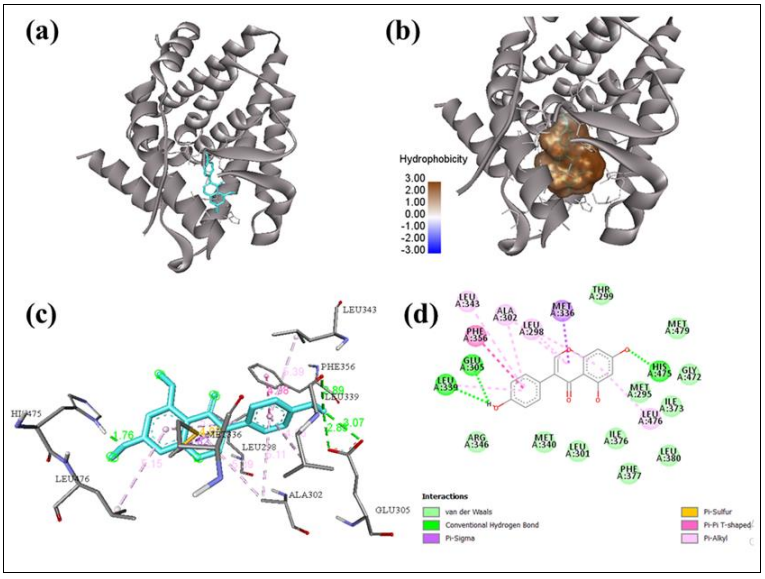

Fig 4: Docked poses of ERβ (1QKM) with Genistein/5280961: (a) Daidzein docked to ERα; (b) Hydrophobicity surface at the active binding site of ERα with Daidzein; (c) 3D stick diagram of surrounding ERα amino acids with Daidzein; (d) 2D view of surrounding ERα amino acids with Daidzein.

The ERβ Genistein docked complex shows a negative free binding energy of - 9.7 kcal/mol reported in Table 1, suggesting the possibility of a stable interaction between the drug and the receptor and docked image is represented in Figure 4. The hydrogen bond was recognized with amino acids Glu305, Leu338 and His475 at a distance of 3.07, 2.83 2.89 and 1.76Å between the Genistein and the binding site of the ERβ. The additional stabilizing energy related to the binding force of ligand is linked with other bond types like alkyl and pi-alkyl bonds with amino acids Leu343, Ala302, Leu298, and Leu476. Leu339 and Phe356 and assists to improve the hydrophobic force of the ligand in the binding domain of the ERβ receptor. Binding interaction was also associated with the presence of van der Walls interactions with the amino acids Thr299, Met479, Gly472, Met295, Ile373, Leu380, Ile376, Phe377, Leu301, Met340 and Arg346. The docked complex stability of the phytocompound with the receptor is also associated with the Pi-sigma interaction of amino acid Met336,

This Comparative computational docking analyses of selected phytocompounds with the ERα and ERβ target gives an insight into the efficacy of phytocompounds over clinically used reference drug molecules. The reported docking results found that Panaxadiol Exhibits the best binding interaction among the 41 phytocompounds for ERβ and Genistein for both ERα and ERβ receptor. These lead compounds was then assessed for studies.

3.3 ADMET evaluation studies

Docking analyses showed Panaxadiol as the lead inhibitor for ER-β receptor and Genistein as the common inhibitor for both ER-α and ER-β target. Both Panaxadiol and Genistein was checked for ADMET profile using admetSAR software. The admetSAR results were shown in Table 4. Both Panaxadiol and Genistein was found to be capable of passing through BBB with a probabaility score of 0.87 and 0.67 and able to absorb by intestine with probability scores of 1.00 and 0.98 respectively. In addition, Panaxadiol is an inhibitor of P-glycoprotein and an efflux transporter on BBB (score < 0.7) whereas Genistein is a non-inhibitor of P-glycoprotein and an efflux transporter on BBB (score <0.6). This suggest that the Panaxadiol penetrate into the brain after absorption. Bothe Panaxadiol and Genistein are non-inhibitor of ROC transporter. Panaxadiol exhibited low CYP enzymes Inhibitory promiscuity (CYP IP), whereas Genistein as shown high CYP IP. ROC transporter and CYP enzymes, two important biomarkers assessing potential effects of the Panaxadiol and Genistein on liver and renal functions accordingly, with probability score > 0.87 and > 0.90 respectively.

Table 4: Predicted ADMET profile of lead compounds

ADMET	Panaxadiol		Genistein	
	Results	Probability	Results	Probability
BBB	BBB+	0.87	BBB+	0.67
HIA	HIA+	1.00	HIA+	0.98
Caco-2	Caco2+	0.70	Caco2+	0.70
P-gp subtrate	Substrate	0.73	Substrate	0.50
P-gp Inhibitor	Inhibitor	0.61	Non-inhibitor	0.78
ROC transporter	Non-inhibitor	0.87	Non-inhibitor	0.90
CYP IP (Inhibitory promiscuity)	Low	0.91	High	0.80
hERG (Human ether-a-go-go-Related Gene) a prediction of arrhythmias	Weak -inhibitor	0.96	Weak Inhibitor	0.95
AMES Toxicity	Non-toxic	0.77	Non toxic	0.96
Carcinogen	Non -carcinogen	0.91	Non-carcinogen	0.92
Biodegradation	Not ready biodegradable	0.99	Not ready biodegradable	0.85
Acute Oral Toxicity	III	0.50	II	0.58
Aqueous Solubility(logS)	-4.30	-	-3.09	-

Drug toxicity is a great concern to the medical world. The toxicity prediction also indicated that both Panaxadiol and Genistein are the weak inhibitor of hERG, non-carcinogenic and non-AMES toxic (score > 0.77).Bothe phytocompounds were predicted to have acute oral toxicity category 3 and 2 which are considered as nontoxic for oral toxicity with the probability scores of 0.50 and 0.58 respectively. Panaxadiol showed higher solubility with log S value of -4.30 than the Genistein and the log S value is -3.09, which effects the movement of both Panaxadiol and Genistein from the site of administration into the blood. The toxicity studies also indicated that both Panaxadiol and Genistein has less concerns of inducing arrhythmias and cancers through mutagenesis (Lin, *et al*., 2014) [16]. The admetSAR results were shown in table 4.

4. Conclusion

The potential phytocompound inhibitors for target ERα and ERβ are screened by Molecular docking and ADMET study. Molecular docking studies showed the binding affinity of Genistein, Daidzein with ERα and the binding affinity of Panaxadiol, Genistein, with ERβ respectively by ensuring lowest binding energy with best geometrical arrangement among the forty one phytocompounds data set used. The ligand Genistein, Daidzein and Panaxadiol in the catalytic site of ERα and ERβ are surrounded by a non-covalent interactions with hydrophobic region. Non-covalent and Hydrophobic interactions affect the lead compound for best orientation; this plays a critical role in the inhibition activity of the lead compounds. The toxicity studies indicated that Genistein and Panaxadiol have less concerns about inducing arrhythmias and cancers through mutagenesis. These preliminary encouraging results could offer a new framework toward the discovery of novel potent anti-breast cancer agent. Further this lead molecule can be considered for *in vitro* and *in vivo* studies to know the inhibitory action of lead compound.

5. References

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