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Molecular docking and *in-silico* study of natural antagonists of ER-alpha receptor: Potential candidates against breast cancer

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Abstract: Breast cancer is affecting the women in both developing and non-developing countries in a dangerous ratio. Estrogen receptor alpha (ER-α) controls a number of physiological processes. Its over expression is seen in breast cancer. Estrogen positive breast cancer can be treated by hormone therapy, that's why we focused on anti-estrogen natural molecules. A library of 5022 phytochemicals was searched, and selected top molecules based on their drug scoring. Fulvestrant was used as the reference drug. 4 molecules showed more drug scoring than the standard and these include Kushenol K, Flavobin, Kushenol N and CHEMBL66996 in descending order. All these molecules were further assessed for their other biological activities and found better than the standard. Therefore, these compounds may be used effectively as antagonists of ER-alpha receptor and as potential candidates against breast cancer.

Keywords: Breast cancer, anti-estrogen, ER-α, natural molecules

INTRODUCTION

Breast cancer is very dangerous disease which leads the person to the mouth of death. Out of every ninety women, ten women might be the victim of breast cancer. In Pakistan, the most frequently diagnosed cancer among females is also breast cancer, accounting for nearly one in nine female patients. Its incidence in Pakistan is 2.5 times higher than that in neighboring countries like Iran and India (Asif *et al*., 2014). ER-alpha stimulated breast cancer is caused due to over-expression of ER-alpha. Fulvestrant is an FDA approved anti-estrogen drug, which target the ER-alpha, and it causes its down-regulation. Fulvestrant competitively and reversibly binds to estrogen receptors in cancer cells and attain its antagonist effects against estrogen (Schiff *et al*., 2003).

Prevention is better than the cure, so it can be prevented by healthy life style, diet, and exercise. It can be done by screening of monthly chest palpation, and second way is by mammography after every three years. Breast cancer develops from the uncontrolled growth of breast cells. The symptoms of breast cancer include a noticeable piece of mass in breast or excretion of liquid from nipples (National Comprehensive Cancer Network, 2003). Risk factors include excess assemblage of fat, lack of physical activity or exercise, excess use of alcohol. The most occurring cancer in the woman is breast cancer (Ahmed *et al*., 2014).

Estrogen receptor alpha (ER-α) controls a number of physiological processes. Its over expression is seen in breast cancer (Ali and Coombes, 2000). Breast cancer is progressed by, or mainly affected by steroid hormones especially estrogen, because of their interaction with specific target cell receptors, so estrogen antagonist therapy is considered to be maximum useful for patients with Estrogen positive breast cancer (Schiff *et al*., 2003).

Medicinal plants and their extracts are source of medicines from the history. Phytochemicals occur naturally in plants and control many diseases, including cancer (Fatima *et al*., 2014).

Now, in this era, technology is running the world, everything is progressing day by day, it has given the opportunity to researchers or scientists to do more work in less time and in economical way. Molecular docking is a process in which we can check the compatibility between the target which can be any enzyme/protein and the ligand molecules, before its in vitro synthesis (Zhichen *et al*., 2015). Docking can be performed through computer programs like Swiss Dock, DSX online, dog scoring sever and Chimera.

Aim of this study was to do *in silico* screening of the effective bioactive compounds which may be the potential inhibitors of ER-α and may act as a drug which may be effective in preventing the breast cancer.

MATERIALS AND METHODS

A library of 5022 phytochemicals was searched, the phytochemicals having anticancer potential were identified, then they were docked with estrogen receptor alpha (PDB ID 1HCQ.A), by using the software Swiss dock (http://www.swissdock.ch/).

Preparation of target

The structure of estrogen receptor alpha was taken from Protein Data Bank (PDB), by using the ID (3ERT). For

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the refinement of structure and find its different molecular properties, for the removal of water, for finding its residues, and any information related to its structure and to make it optimized, we used Chimera software.

Preparation of phytochemicals database

All selected phytochemicals of different plants were downloaded from Pubchem (https://pubchem.ncbi.nlm.nih.gov/), and MP3D (http://bioinform.info/). 3D structure of each ligand used in this study was saved in '.cif" format by using Chimera software.

Compatibility test

To check the compatibility of ligand molecules, we used the VAMMPIRE-LORD online: http://vammpire.pharmchem.uni-frankfurt.de/.

ADMET properties

We also found out the ADMET properties of finalized molecules by using MedChem Designer: http://medchem-designer.software.informer.com/.

Molecular docking

Binding pockets of estrogen receptor alpha was found by using DOgsite Scorer server online: http://dogsite.zbh.uni-hamburg.de/.

Table 1: Properties of the selected active pocket of target ER-alpha based upon top scores

Name	Volume	Surface	Lipo Surface	Depth	Drug Score
P0	1217.86	1460.90	1112.95	20.82	0.82

Table 2: Pubchem ID, zinc entry and other physical properties of finalized molecules

Sr #	Pubchem	Zinc entry	Molecular weight (g/mol)	Molecular formula	Hydrogen bond Donor	Hydrogen bond acceptor	Rotate-able bond
1	44428630	13817014	472.527	$C_{26}H_{32}O_8$	5	8	8
2	381851	4245081	454.412	$C_{26}H_{30}O_7$	1	3	2
3	31553	2033589	482.436	$C_{25}H_{22}O_{10}$	5	10	4
4	5315615	899870	360.314	$C_{18}H_{16}O_8$	5	8	7
5	104741	3995807	606.770	$C_{32}H_{47}F_5O_3S$	2	9	14

Table 3: Docking results and other drug related properties of top four finalized molecules

Sr #	Pubchem	Name	Simple fitness	Full fitness	Inter full	Intra full	Surface full	Energy	Delta G (kcal/mol)	Cluster
1	44428630	Kushenol N	14.8617	-1107.3	-65.964	55.7349	203.251	14.8617	-8.724	0
2	381851	Kushenol K	6.51553	-1132.2	-56.134	-1331.0	205.588	6.51553	-9.318	0
3	31553	Flavobin	25.8513	-1073.0	-64.136	99.1061	202.357	25.8513	-8.230	0
4	5315615	CHEMBL 66966	3.22647	-1131.2	-108.09	68.5052	203.058	3.22647	-8.410	0
5	104741	Fulvestrant	21.1353	-1103.8	-91.853	93.3239	203.226	21.1353	-10.06	0

Table 4: Drug scoring results based on several properties of finalized molecules

Sr #	Pubchem ID	Name	PCS	Number/rank	Tors_score	Sas_score	RMSD	Drug score
1	44438630	Kushenol N	1.195	0/1	0.000	-10.713	None	1810
2	381851	Kushenol K	1.038	0/1	0.000	-13.373	None	1396
3	31553	Flavobin	1.022	0/1	0.000	-11.530	None	1545
4	5315615	CHEMBL66996	1.384	0/1	0.000	5.337	None	1182
5	104741	Fulvestrant	0.944	0/1	0.000	-14.851	None	1136

Table 5: Admet Properties of finalized molecules

Name	M logP	S+logP	S+log D	M_NO	T_PSA	HBD_H
Kushenol N	2.235	3.894	3.871	7.000	116.450	4.000
Kushenol K	1.562	2.923	2.904	8.000	136.680	5.000
Flavobin	-0.261	1.789	1.653	10.000	155.140	5.000
CHEMBL66996	0.951	2.149	-0.747	8.000	144.520	5.000
Fulvestrant	6.073	7.196	7.196	4.000	57.530	2.000

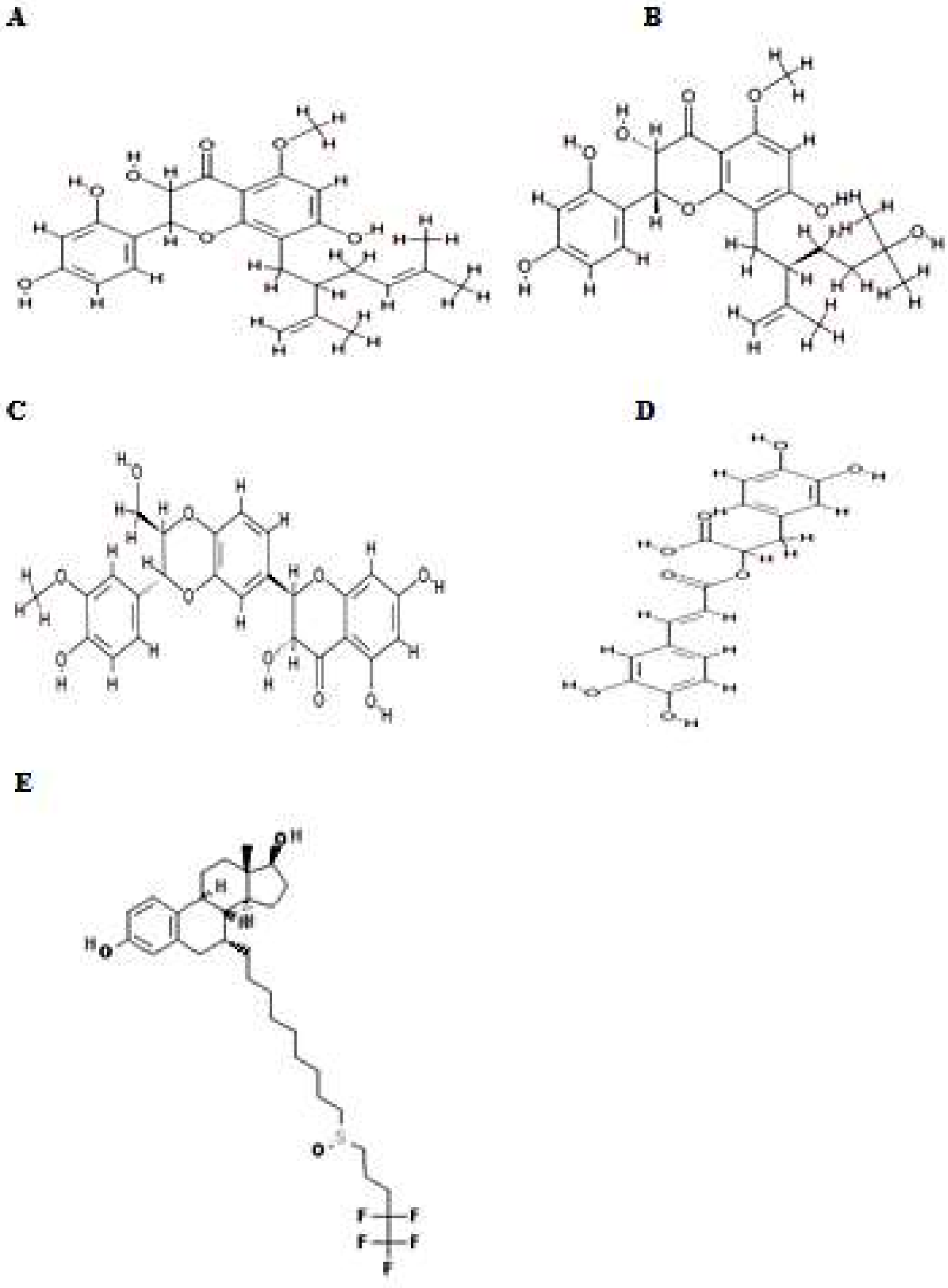

Fig. 1: Structures of four best selected ligand molecules, A) Kushenol N; B) Kushenol K; C) Flavobin; D) CHEMBL66996; and E) Fulvestrant

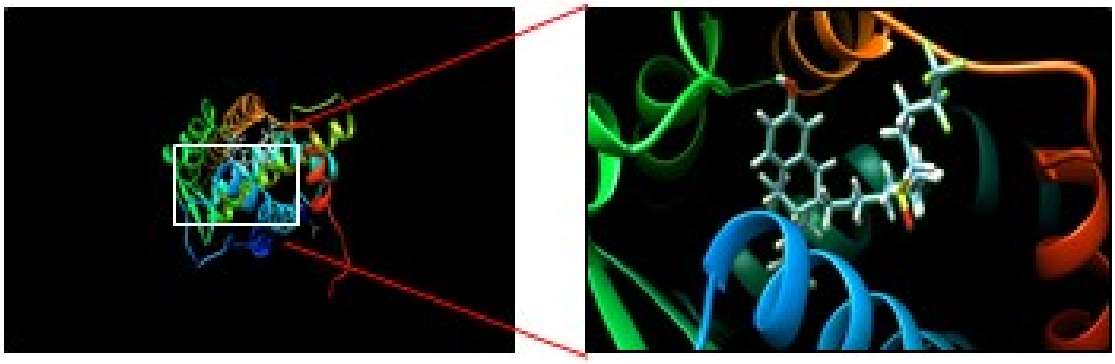

Fig. 2: Docked structure of reference drug with target enzyme ER-alpha

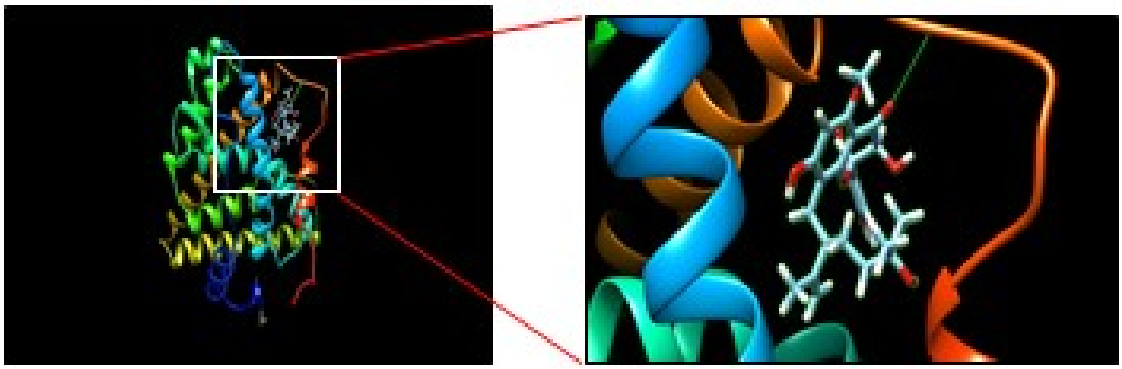

Fig. 3: Complete docked and zoomed docked structure of Kushenol K

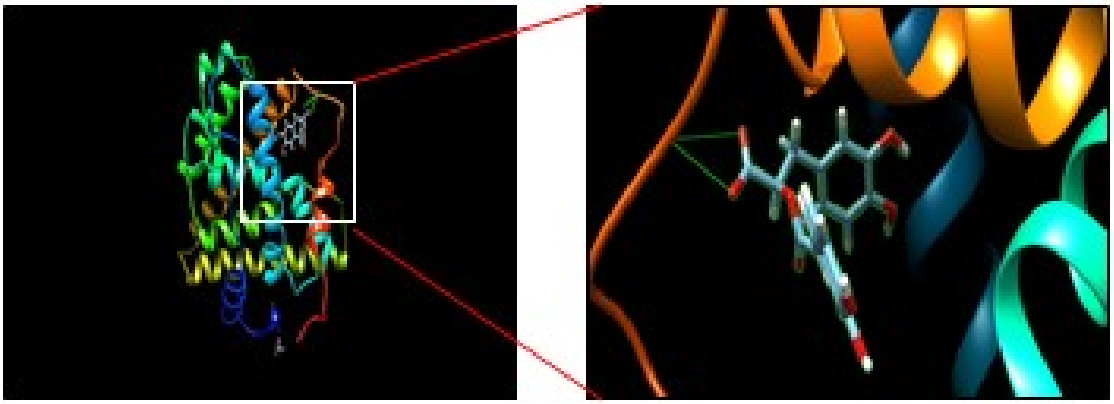

Fig. 4: Complete docked and zoomed docked structure of Kushenol N

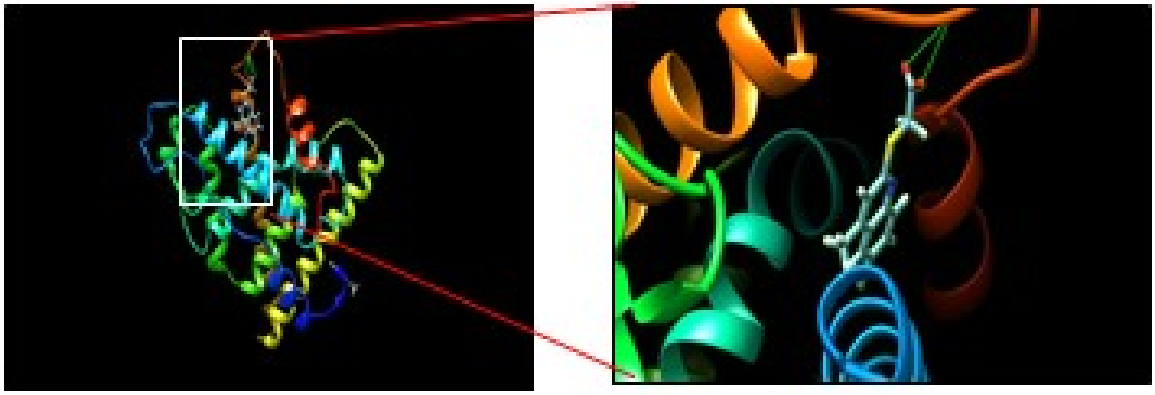

Fig. 5: Complete and zoomed docked structure of CHEMBL66966

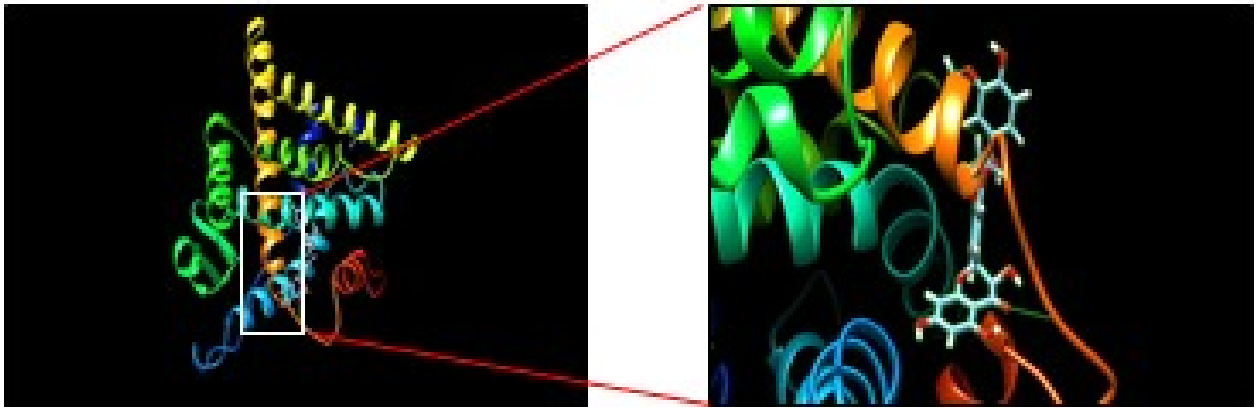

Fig. 6: Complete and zoomed docked structure of Flavobin

The docking was held by using swissdock, and we have found out the following parameters. Formation of Cluster's probability, full fitness in kcal/mol, simple fitness, full, inter, intra full fitness, estimated delta G in kcal/mol.

Drug scoring

For obtaining the drug score we have used DSX online: http://pc1664.pharmazie.uni-marburg.de/drugscore/. The information we obtained was related to tors-score, sas-score, atom-atom pairs and covalent bonds.

RESULTS

Target structure was taken by PDB in 3D format. The binding pockets were obtained from Dogsite scorer server and the properties of the selected active pocket of target ER- alpha based upon top scores are shown in table 1. Structure refinement, and other information related to target was analyzed by Chimera. A library of 5022 phytochemicals was studied; the phytochemicals with anticancer activity were screened out and docked against the target, which was estrogen receptor alpha. The information of chemical substances we used are given in table 2. By using swissdock, we may find the attachment potential of ligand molecule with target protein, whether it may attach to target or not? Docking results related to full fitness (kcal/mole), simple, inter and intra full fitness energy, delta G (kcal/mole) are given in table 3. Docked structures of top selected phytochemicals, used in the process of docking are given in (fig. 3-6) respectively, and the ADMET properties of top five are given in table 5. The binding pockets of our ER-alpha, which is our target, are given in table 1. We have find out the minimum similarity of Kushenol K with Target ER-alpha and it was 85%, by using VAMPIRE-LORD.

Fulvestrant was used as standard in this study. It is a drug that primarily blocks the estrogen receptor (Osborne *et al*., 2004). It acts against estrogen hormone throughout the body. It is used in treatment of metastatic breast cancer. It is approved by the FDA for advanced breast cancer for use in replacement of drugs tamoxifen or toremifene, if they do not give response. It often works in combination of luteinizing-hormone releasing hormone (LHRH) agonist to deactivate the ovaries. The properties of drug are given in table 2. Interaction of drug with target is shown in fig. 2. Surface full fitness, and binding energies (Delta G values in kcal/mol) of the selected ligands with the target protein are given in table 3.

Kushenols are members of the flavanone or flavonol classes of compounds, obtained from Sophora species, particularly from the roots of *Sophora flavences*. They show anti-estrogen activity. Kushenol N was forming two hydrogen bonds with target ptotein. Its delta G value (- 9.318) and its surface full fitness (205.588) was better than our control drug value, and its binding potential was also excellent. In addition, its drug-score (1396), far more than our control drugs score which was 1136.

Kushenol K was also forming hydrogen bond with target, its surface full fitness (203.251) and simple fitness (-1107.3) was also better than the control drugs surface full fitness (203.26) and simple fitness (-1103.8). Its drug scoring (1810) was much better than the reference drugs scoring (1136). Structural formulas of best selected molecules are shown in (fig. 1).

All the finalized molecules were assessed through drug scan tool which was DSX online. This tool gave us information about tars_score, the 'tors_score' is the sum of scores for each bond. The score for a single bond was the mean of its possible torsions, sas_score, the 'sas_score' was the solvent accessible surface score for solvation/desolvation contributions. The 'PCS' (per_contact_score) was the score divided by the number of atom-atom-interactions having any contribution to the total score. PCS drug score for a good drug must be higher than that of the standard. All information about drug scoring is given in table 4.

ADMET is abbreviated as absorption, distribution, metabolism, excretion and toxicity. The original ruleof5

has much importance in modern drug discovery, so according to that rule molecular weight should be less than 500 to avoid violation of rule, as our ligand molecules had. Secondly our M logP values were less than 4.15, HBD_H should be 5 or less than 5, M_N0 should be less than 10, our ligand molecules were full filling this rule, as showing in table 5.

DISCUSSION

In-silico drug designing is an advanced technique that has stopped wastage if money and time (Qadir *et al*., 2018). In this study, 5022 phytochemicals were searched from different plants, 31 phytochemicals out of 5022 showing anti-cancer activity were chosen and then docked against the target ER-alpha, and were further assessed by interaction analysis. Drug scoring was also obtained and then on that base, four were selected as top ligand molecules showing their potential to be a good future drug. These can be strong antagonist of ER-alpha and are showing more drug score than reference drug. They are showing the best potential to be used as anti-cancer drugs. Our ligand molecules are also following the Lipinki's 'Rule of 5' (Ro5). Log P values are obtaining by using two models M logP obtaining from Moriguchi and S+logP which is find out by using artificial neural network ensemble, while log D is founding out by using estimation of octanol-water distribution coefficient. The drug should not violate the following rules, MlogP which represents excessive lipophilicity, should not be more than 4.15.Moecular weight, hydrogen bond donors, hydrogen bond acceptor should not be more than 500, 5 and 10 respectively. Excessive lipophilicity (M logP) is dangerous because it may let the attachment of undesirable molecules to the target (Katzenellenbogen and Katzenellenbogen, 2000; Khan, 2010). Binding of the ligand molecules on to the surface of target protein are analyzed, confirming that these ligand molecules can enter the substrate-binding region of the protein active sites (Qadir *et al*., 2018). As a whole we have given total four substances which can be the best alternatives for the drug existing and may have more beneficial. They are showing the best potential to be used as anti-cancer drugs.

A recent similar study indicated Silybin as a good candidate (Ahmed *et al*., 2014). This study also supported our results and agreed that Kushenol N and Kushenol K may also be used against breast cancer but they compared the affinity with reference to tamoxifen, Whereas, we used Fulvestrant as standard, which even may be used for tamoxifen resistant patients. Moreover, they only studied binding potential and did not study the other properties like absorption, metabolism and excretion. But our study has confirmed that all the selected three compounds including Kushenol K also have good other biological properties and may be used effectively as antagonists of ER-alpha receptor and finally as potential candidates against breast cancer.

CONCLUSION

Four molecules showed more drug-scoring than the standard and these include Kushenol N, Kushenol K, Flavobin and CHEMBL66996. All these molecules were further assessed for their other biological activities and found better than the standard. Therefore, these compounds may be used effectively as antagonists of ER-alpha receptor and as potential candidates against breast cancer.

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