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Research Article

VIRTUAL SCREENING OF POTENTIAL INHIBITORS FROM HERBS FOR THE TREATMENT OF BREAST CANCER

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ABSTRACT

Objectives: Cancer is a disease which results in uncontrollable abnormal cells division and destruction of body tissues. Breast cancer occurs when malignant tumors develop in the breast. Breast cancer is the second leading cause of death among women. To study the role of herbs used in the treatment for breast cancer. To investigate the anti-breast cancer activity of compounds present on most common herbs and to analyse their interaction with amino acids in the active sites.

Methods: Complementary and alternative medicine is often used for curing cancer mainly the breast cancer. Also certain studies support the benefits of herbal medicines over others among Complementary and alternative medicine. Herbal treatments are more popular due to less complications and more safety. We selected a dataset of 38 compounds and performed virtual screening to identify the potential inhibitor against the known protein target BRCA1 involved in breast cancer using AutoDock4 as docking software. The binding site analyses were carried out using Discovery studio.

Results: From our study, we deduced that cimigenol (black cohosh) and glycyrrhetinic acid (licorice) were found to have the highest affinity with the target protein. The amino acid interactions with the top five compounds were also analysed.

Conclusion: During the course of our research we explored over common herbs used globally in treatment for breast cancer. Virtual screening was performed using AutoDock to search ligands to identify those structures which are most likely to bind to the protein. The high affinity compounds can bind more efficiently to the BRCA1 receptor and, hence, has potential to emerge as lead compound in the treatment of breast cancer.

Keywords: Protein, Ligands, AutoDock, Virtual screening, Visualization, BRCA1.

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INTRODUCTION

Cancer is a state when body cells begin to grow out of control. Cells in any part of the body can become cancerous and can spread to other areas. Germline mutations in a gene on chromosome 17q known as BRCA1 are responsible for a large proportion of inherited predispositions to breast and ovarian cancer [1]. Breast cancer is a fatal tumor; a cluster of cancerous cells that can enter adjoining tissues or can extent to far away areas of the body. These cells grow by disrupting away from the original tumor and then go into the blood vessels which branch into tissues within the body. The disease occurs majorly in women, but men can get it, too. Breast cancer generally begins in the inner lining of milk ducts that supply them with milk. A breast cancer of the lobules is known as lobular carcinoma while that of the ducts is called ductal carcinoma. It accounts for 16% of all female cancers and 22.9% of invasive cancers in women, also 18.2% of all cancer deaths worldwide, including both males and females, are from breast cancer [2].

Several techniques and clinical methods which include relaxation tactics such as - massage and herbal remedies are now recognized as essential part of alternative medicine. These practices suggest that the uses of alternative medicines are used by the section of population which have a particular education level and reached that level of income.

Most of the patients in the United Kingdom use various supplements to enhance their fitness or provide a good start to their immune system. Dietary therapies were often practiced which had special diet plans-low fat diet, eating only vegetarian food, freshly prepared meals, fruits, and whole grains. Herbal remedies include mixtures of herbs and plants, use of garlic oil for hypertension, *Echinacea* for proper functioning of the immune system. Many relaxation techniques that calm the body by lowering blood pressure and pulse rates are often used to heal the tension in the body. Meditation or spiritual healing which connects one to a higher power is also believed to calm the body tension.

Virtual screening is a method used in drug designing to generate or search large databases of small molecules (or ligands) to bind to a particular site in a protein or drug molecule [3]. It encompasses a variety of computational techniques, helping chemists to reduce a huge virtual library to a more manageable size [4]. In this technique, the crystal structure of the protein molecule was taken from the protein data bank [5]. All structures of the ligand molecules included in the database for testing whether the molecule binds or not were also taken from the protein data bank. These molecules were then docked using the AutoDock Software. Based on the binding energies and the three-dimensional (3D) structures generated the ligand molecules were sorted. These structures were then used to find out which of the structures that were docked in the given protein structure were stable (Based on hydrophilic and hydrophobic amino acids present in the structure and number of hydrogen bonds in the structure). Virtual screening, especially receptor-based virtual screening, has emerged as a reliable, inexpensive and accessible method for identifying leads [6].

In our research work, we installed the required Software's (AutoDock 4.2.3-i86 Linux 2.tar.gz, MGL Tools-1.5.6, and Tcsh) and then used python script. The protein and ligand were selected. Then series of steps were carried out to prepare a library containing ligand files and corresponding AutoGrid and AutoDock parameters files for library. After which AutoGrid was used to calculate maps also to launch AutoDock calculations for each ligand followed by two analyses step in which we extracted and evaluated results through which we got the sorted list of ligands according to the energy to bind with the protein [7]. Further analysis of result was carried out and the best conformation

of every ligand was selected. After which these ligands and the protein were visualized together as a complex in discovery studio to get their two-dimensional (2D) image.

METHODS

Protein structure

Mutations in BRCA1 and BRCA2 confer high risks of breast cancer [2]. From gene card database [8], proteins related to BRCA1 gene were obtained from which the protein with the best resolution (lowest) was selected. The X-ray crystal structure of the protein BRCA1 at 1.75°A resolution (PDB ID: 4IGK), were retrieved from protein data bank [9]. AutoDock expects that the input protein has polar hydrogen and that all the water molecules should be removed. Hence before protein preparation process, all the water molecules along with the heteromolecule attached with the structures were removed from the original crystal structure of 4IGK. Polar hydrogen atoms and Kollman charges were added to the protein structure.

Herbal compounds for breast cancer

The most common herbs (eleven) used in treatment for breast cancer were taken. Their composition was selected from "examine website database" (Table 1) [10]. Then the sdf structure of the components of these herbs was downloaded from Pubchem database [11]. These sdf files were converted to mol^2 format using Babel Software [12] and then we applied Lipinski rule of five to further filter the molecules as shown in Table 2.

Virtual screening using AutoDock

AutoDock is docking software structured in a way to predict the binding of a substrate to a receptor molecule and virtual screening is a technique used for identifying assuring compounds to bind to a target molecule with a known structure [13]. It automatically calculates the grid map and clusters the results in a way transparent to the user [14]. We have used the AutoDock version 4.2.3. This software has two main functions: First is AutoDock, which performs docking of a ligand molecule to a specific receptor site in the protein molecule [15]. Second is AutoGrid, for precalculating the grids or the specific locations where a particular ligand molecule bind in a protein molecule [16]. This software is often used for structure-based drug design as it helps in predicting atomic affinity grids in the molecule.

The ligand molecule is provided with a set of binding sites in the protein molecule which is found out by trying various conformations depending on the structure of the ligand and translations of the ligand molecule. The energy of the ligand-protein molecule is also calculated at different binding sites.

With the time, virtual screening has become an accepted tool in drug discovery. It has been successfully applied in a number of programs, in particular, at the lead discovery stage, where high-throughput molecular docking can play an important role [17]. Now, the virtual screening is carried out by creating a folder directory called "virtual screening" containing sub folders called "ligands," etc., in the "ligand" folder ligand files are set up. The ligand database consists of sdf files that are downloaded from the Protein Data Bank, which is then converted to mol^2 file format using babel software. Then, the preparing of ligand was done in which mol^2 files prepared in the previous step are then processed to ".pdbqt" file format using AutoDock software. These molecule files must be supported by AutoDock, following a given set of atom types. This set includes: United-atom aliphatic carbons, cycles having aromatic carbons, polar hydrogens, hydrogen-bonding nitrogens and directionally hydrogen-bonding oxygens among others, each with a partial charge. To determine the covering set of atom types a 3D lattice of equally spaced points placed over the ligand molecule and the location or active site in the protein molecule. AutoGrid software is used to calculate grid map for each constituent atom present in the ligand to be docked in the protein molecule. Now the receptor is prepared by saving the ligand files in pdbqt format. In pdbqt, "q" represents the charge and "t" is the type of AutoDock. Polar hydrogen atoms are added to the molecule and assigned a gasteiger partial charge. Further, the AutoGrid parameter files for the library is prepared. Dimensions are provided to grid box which completely surrounds the protein and ligand molecule. AutoGrid process the ligand molecule conformations that can be docked in the protein molecule on the 3D grid point in the grid box. All the energies of different sites of docking are noted by the software. Now to calculate atomic affinity maps for a ligand library, AutoGrid was used. The "ligand" folder contains the map files and ligand.pdbqt file formats. After AutoGrid takes place each of the ligand atoms will have a ".pdbqt" file and a docking parameter file with ".dpf" format. The docking directories and parameter files for each ligand in a library is prepared and AutoDock is run to find the docking energy and various docked conformations of the molecule. Finally, analysis is

Table 1: Herbal compounds with PubChem ID

S. N	Herb name	Constituent name	PubChem ID
1	Echinacea	Caffeic acid	689043
2	Echinacea	Chicoric acid	5281764
3	Echinacea	Echinacoside	5281771
4	Echinacea	Luteolin	5280637
5	Echinacea	Rutoside	5280805
6	Licorice	Formononetin	5280378
7	Licorice	Glabridin	124052
8	Licorice	Glycyrrhetinic acid	10114
9	Licorice	Glycyrrhizic acid	14982
10	Licorice	Liquiritigenin	114829
11	Cat's claw	Isopteropodine	9885603
12	Cat's claw	Lyaloside	11092621
13	Cat's claw	Mitraphylline	94160
14	Cat's claw	Tomentside B	21629530
15	Cat's claw	Uncaric acid	71448960
16	Cat's claw	Uncarinec	98363
17	Garlic	Diallyl Disulfide	16590
18	Garlic	Dithiins	12044565
19	Garlic	S-allyl cysteine	9793905
20	Flaxseed	Linamarin	11128
21	Flaxseed	Linustatin	119301
22	Flaxseed	Lotaustralin	441467
23	Flaxseed	Neolinustatin	119533
24	Flaxseed	Secoisolariciresinol Diglucoside	9917980
25	Turmeric	Bisdemethoxycurcumin	5315472
26	Turmeric	Calebin-A	637429
27	Turmeric	Curcumin	969516
28	Turmeric	Cyclocurcumin	69879809
29	Turmeric	Demethoxycurcumin	5469424
30	Burdock	Arctigenin	64981
31	Burdock	Chalcone	637760
32	Burdock	Flavanol	253959
33	Burdock	Flavanone	10251
34	Burdock	Flavone	10680
35	Burdock	Phlorotannin A	73162010
36	Burdock	Proanthocyanidin	108065
37	Carotenoids	Capsanthin	5281228
38	Carotenoids	Capsorubin	5281229
39	Green tea	Aminobutyric acid	119
40	Green tea	Catechins	9064
41	Green tea	Epicatechin	72276
42	Green tea	Epigallocatechin	72277
43	Green tea	Theanine	439378
44	Ginseng	Ginsenoside Rg3	9918693
45	Ginseng	Ginsenosides	3086007
46	Ginseng	Panaxtriol	73599
47	Ginseng	Ginsenoside Rb1	9898279
48	Ginseng	Ginsenoside RG1	441923
49	Black cohosh	Actein	21594792
50	Black cohosh	Cimicifugoside	441913
51	Black cohosh	Cimigenol	16020000
52	Black cohosh	Salsolinol	91588

Table 2: Binding energies for the docked herbal compounds

S. N	Herb name	Constituent name	PubChem ID	Molecular weight	ΔG values (kcal/mol)
1	Echinacea	Caffeic acid	689043	180.15742 g/mol	−4.7000
2	Echinacea	Chicoric acid	5281764	474.37112 g/mol	−4.2400
3	Echinacea	Luteolin	5280637	448.3769 g/mol	−6.6500
4	Licorice	Formononetin	5280378	268.26408 g/mol	−6.1100
5	Licorice	Glabridin	124052	324.3704 g/mol	−8.3100
6	Licorice	Glycyrrhetinic acid	10114	470.68384 g/mol	−8.4200
7	Licorice	Liquiritigenin	114829	256.25338 g/mol	−8.4200
8	Cat's claw	Isopteropodine	9885603	368.42626 g/mol	−6.5100
9	Cat's claw	Mitraphylline	94160	368.42626 g/mol	−6.5700
10	Cat's claw	Uncaric acid	71448960	354.39968 g/mol	−5.3300
11	Cat's claw	Uncarinec	98363	368.42626 g/mol	−6.2300
12	Garlic	Diallyl disulfide	16590	146.2736 g/mol	−2.9400
13	Garlic	Dithiins	12044565	116.20456 g/mol	−3.6000
14	Garlic	S-allylcysteine	9793905	161.22204 g/mol	−3.3900
15	Flaxseed	Linamarin	11128	247.24508 g/mol	−4.3400
16	Flaxseed	Linustatin	119301	409.38568 g/mol	−5.1700
17	Flaxseed	Lotaustralin	441467	261.27166 g/mol	−4.3500
18	Flaxseed	Neolinustatin	119533	423.41226 g/mol	−4.8600
19	Turmeric	Bisdemethoxycurcumin	5315472	308.32794 g/mol	−5.3500
20	Turmeric	Calebin-A	637429	384.3793 g/mol	−4.9100
21	Turmeric	Curcumin	969516	368.3799 g/mol	−5.7800
22	Turmeric	Cyclocurcumin	69879809	368.3799 g/mol	−7.4800
23	Turmeric	Demethoxycurcumin	5469424	338.35392 g/mol	−5.3300
24	Burdock	Arctigenin	64981	372.41166 g/mol	−5.4600
25	Burdock	Chalcone	637760	208.25518 g/mol	−5.2200
26	Burdock	Flavanol	253959	226.27046 g/mol	−5.7900
27	Burdock	Flavanone	10251	224.25458 g/mol	−5.9300
28	Burdock	Flavone	10680	222.2387 g/mol	−6.3600
29	Burdock	Phlorotannin A	73162010	388.40952 g/mol	−7.3100
30	Green tea	Aminobutyric acid	119	103.11976 g/mol	−3.2700
31	Green tea	Catechins	9064	290.26806 g/mol	−6.2400
32	Green tea	Epicatechin	72276	290.26806 g/mol	−5.9300
33	Green tea	Epigallocatechin	72277	306.26746 g/mol	−5.7900
34	Green tea	Theanine	439378	174.19766 g/mol	−3.3700
35	Ginseng	Ginsenosides	3086007	444.73268 g/mol	−8.0000
36	Ginseng	Panaxtriol	73599	476.73148 g/mol	−7.4600
37	Black cohosh	Cimigenol	16020000	488.69912 g/mol	−9.0100
38	Black cohosh	Salsolinol	91588	179.21572 g/mol	−5.1100

done using AutoDock tools. The results of docking are studied based on structural and conformational similarity and binding sites. Best docked conformation is found by a list provided with molecules sorted by lowest energy of docking [18].

Discovery studio

Discovery studio is a suite of software for simulating small molecule and macromolecule systems. It is developed and distributed by accelrys [19]. Discovery studio provides software applications covering the areas such as simulations, ligand design, and structure-based design. Discovery studio is used for the visualization of interaction between protein and ligand. To view the receptor ligand interaction the complex was opened and its 2D diagram was obtained.

RESULTS

After performing virtual screening using AutoDock, we obtained the sorted list of ligands based on their binding energy with the receptor (4IGK) which was further used to find the optimal conformation of the ligand to be used with the receptor [18].

Binding site analysis

1. Cimigenol compound

   The 2D crystal structure of complex was acquired from discovery studio after performing virtual screening using AutoDock. The residues bounded to the active site are TYR1845, GLN1846, CYS1847, ILE1760, LYS1759, ARG1758, SER1755, ARG1751, and GLU1754. The binding energy of this complex was found to be −9.0100 kcal/mol. Among the residues the one which is hydrophobic is CYS1847 while GLU1754, GLN1846, ARG1751, and ARG1758 are hydrophilic in nature. TYR1845 is aromatic in nature where as ILE1760 is nonpolar as well as aliphatic in nature. TYR1845, GLN1846, LYS1759, SER1755, and GLU1754 have van der Waals interactions with the Ligand (black cohosh cimigenol 2D) on the other hand ARG1751, ARG1758, ILE1760, and CYS1847 are singly bonded. Studies revealed that cimigenol and related compounds were screened as potential antitumor promoters by using the *in vitro* short-term 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced Epstein-Barr virus early antigen activation assay [20].

2. Glycyrrhetinic acid

   By the use of discovery studio, we acquired 2D crystal structure of protein-ligand complex after executing virtual screening using AutoDock. The binding energy of this complex was found to be −8.4200 kcal/mol. The residues bounding the active site are TYR1845, GLN1846, CYS1847, ILE1760, LYS1759, ARG1758, SER1755, ARG1751, and ARG1762. Among these residues, the one which is hydrophobic is CYS1847 while GLN1846, ARG1751, ARG1762, and ARG1758 are hydrophilic in nature. TYR1845 is aromatic in nature whereas ILE1760 is nonpolar as well as aliphatic in nature. TYR1845, GLN1846, and LYS1759 have van der Waals interactions with the Ligand (licorice glycyrrhetinic acid) on the other hand ARG1762, ARG1751, ILE1760, and CYS1847 are singly bonded. ARG1758 has unfavorable Donor-Donor bond and SER1755 has conventional hydrogen bond with the ligand. Recent study found that this compound impairs the p38 MAPK-AP1 signaling axis which accelerates the repression of breast cancer cell invasion [21].

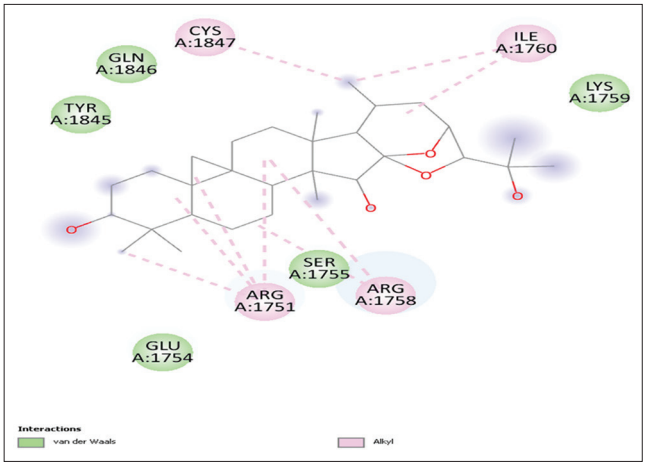

Fig. 1: Amino acid interaction of BRCA1 protein complex with cimigenol

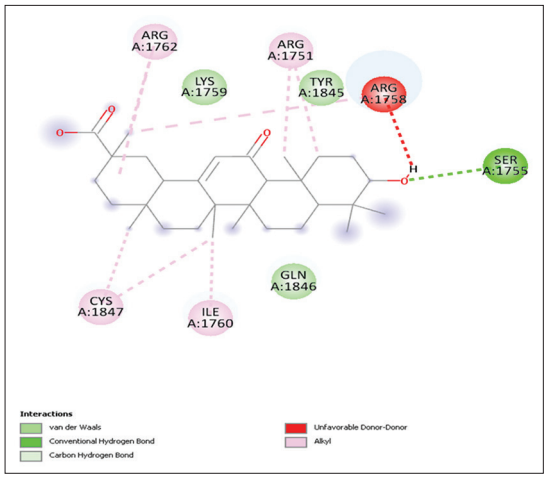

Fig. 2: Amino acid interaction of BRCA1 protein complex with glycyrrhetinic acid

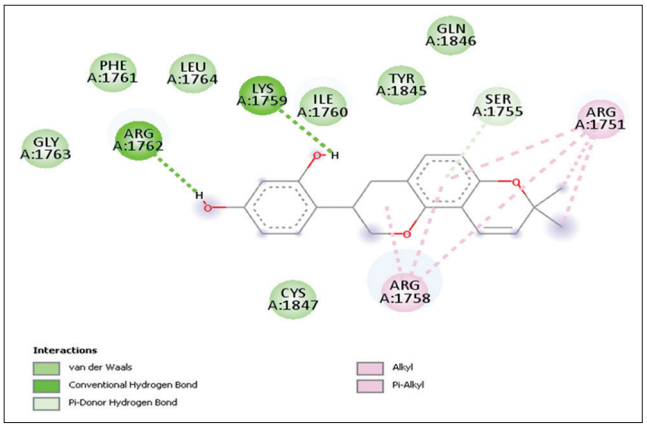

Fig. 3: Amino acid interaction of BRCA1 protein complex with glabridin

3. Glabridin compound

Discovery studio was used to obtain the 2D crystal structure of protein-ligand complex after executing virtual screening using AutoDock. The residues bounding the active site are TYR1845, GLN1846, CYS1847, ILE1760, LYS1759, ARG1758, SER1755, ARG1751, ARG1762, GLY1763, PHE1761, and LEU1764. The complex has a binding energy of −8.3100 kcal/mol. Amid

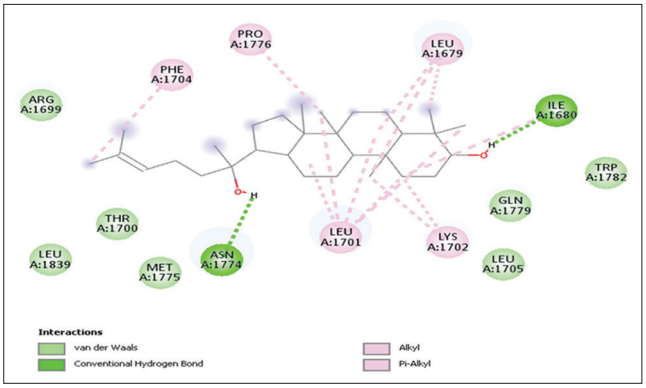

Fig. 4: Amino acid interaction of BRCA1 protein complex with ginsenosides

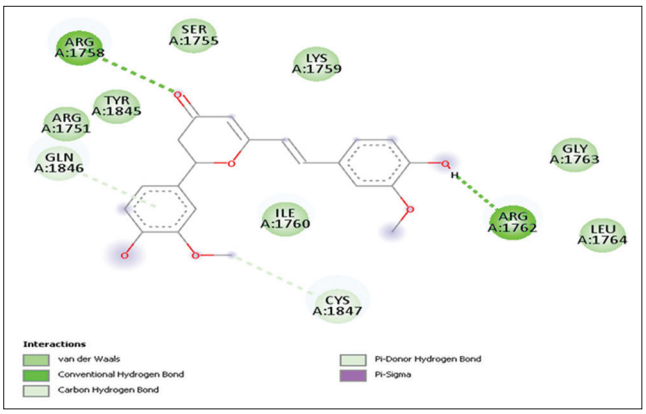

Fig. 5: Amino acid interaction of BRCA1 protein complex with cyclocurcumin

the residues the one which is hydrophobic is CYS1845 while GLN1846, ARG1751, ARG1762, and ARG1758 are hydrophilic in nature. TYR1845 and PHE1761 are aromatic in nature, whereas ILE1760, GLY1763, and LEU1764 are nonpolar as well as aliphatic in nature. TYR1845, GLN1846, CYS1847, ILE1760 LEU1764, PHE1761, and GLY1763 have van der Waals interactions with the ligand (licorice glabridin) on the other hand ARG1751 and ARG1758 are singly bonded and LYS1759 and ARG1762 has conventional hydrogen bond with the ligand. Furthermore, SER1755 has Pi-Donor hydrogen bond with the ligand. In mouse xenograft models, this glabridin compound was found to attenuated the tumor growth, mesenchymal characteristics, and cancer stem cells-like properties via demethylation-activated miR-148a [22].

4. Ginsenosides compound

By executing virtual screening using AutoDock in discovery studio, the 2D crystal structure of protein-ligand complex was obtained as shown in Fig. 4. The binding energy of this complex was found to be −8.0000 kcal/mol. The residues bounding the active site are ARG1699, PHE1704, PRO1776, LEU1679, ILE1680, TRP1782, GLN1779, LEU1705, LYS1702, LEU1701, ASN1774, MET1775, THR1700, and LEU1839. Among the residues the one which are hydrophobic are PHE1704, MET1775 and PRO1776 while GLN1779, ASN1774 and ARG1699 are hydrophilic in nature. PHE1704 and TRP1782 are aromatic in nature whereas ILE1680, and PRO1776 are aliphatic in nature. ARG1699, THR1700, LEU1839, MET1755, GLN1779, TRP1782, and LEU1705 have van der Waals interactions with the ligand (ginseng ginsenosides) on the other hand PHE1704, PRO1776, LEU1679, LEU1701, and LYS1702 are singly bonded and ASN1774 and ILE1680 has conventional hydrogen bond with the ligand. Ginseng root is one of the most commonly

used herbal medicines in the United States and East Asia for its multi-pharmaceutical functions [23,24]. Intake of ginseng root was reported to associate with reduced risk of various types of cancer in a case-control study conducted in Korea [25].

5. Cyclocurcumin

   Discovery studio helped in performing virtual screening using AutoDock through which the 2D crystal structure of complex was gained. The residues bounded to the active site are TYR1845, GLN1846 (Fig. 5), CYS1847, ILE1760, LYS1759, ARG1758, SER1755, ARG1751, LEU1764, ARG1762, and GLY1763. The binding energy of this complex was found to be −7.4800 kcal/mol. Among the residues, the one which is hydrophobic is CYS1847 while GLN1846, ARG1751, ARG1762, and ARG1758 are hydrophilic in nature. TYR1845 is aromatic in nature where as ILE1760, LEU1764, and GLY1763 are nonpolar as well as aliphatic in nature. TYR1845, ARG1751, ILE1760, LEU1764, LYS1759, SER1755, and GLY1763 have van der Waals interactions with the ligand (turmeric cyclocurcumin) on the other hand ARG1758 and ARG1762 have conventional hydrogen bonds with the ligand. Pi donor hydrogen bond is also present. CYS1847 and GLN1846 constitute this bond. Curcumin has been shown to exhibit antioxidant, anti-inflammatory, antimicrobial, and anticarcinogenic activities. Several studies suggested that cyclocurcumin compound were found to be inhibiting proliferation of breast cancer cells [26-28].

DISCUSSION

The herbal compounds from black cohosh and licorice were shown to be a better affinity towards breast cancer target protein. Black cohosh has a history of traditional use among Native Americans for the treatment of a variety of disorders, including various conditions unique to women such as amenorrhea and menopause. Contemporary uses of black cohosh are primarily geared toward the treatment of symptoms of menopause, such as hot flashes, and menopausal anxiety and depression [29]. Black cohosh is among the most frequently cited agent being used by breast cancer patients during their radiotherapy and chemotherapy. Black cohosh (Cimicifuga racemosa) is a shrub like plant commonly seen in the eastern forests of North America [30]. Black cohosh is being utilized by women who have been recommended to avoid HRT (Hormonal Replacement Therapy) by their doctors, who are at high risk for breast cancer or who have discontinued HRT after a diagnosis of breast cancer [30]. Glycyrrhetinic acid (GA), a major component of Radix Glycyrrhiza, is actually a significantly more potent agent to suppress invasion than cell survival[31].

Computational Biology and bioinformatics have the potential not only of speeding up the drug discovery process thus reducing the costs, but also of changing the way drugs are designed. Rational Drug Design (RDD) helps to facilitate and speedup the drug designing process, which involves variety of methods to identify novel compounds. One such method is the docking of the drug molecule with the receptor (target). The site of drug action, which is ultimately responsible for the pharmaceutical effect, is a receptor [32]. Docking is the process by which two molecules fit together in 3D space.

CONCLUSION

In our study, we have analyzed the interaction of different ligands with the receptor protein taken from the BRCA1 gene. We performed virtual screening using AutoDock to find the best conformation of each of these ligands and obtained the conformation having the best binding energy with receptor. This knowledge of the binding energy of different ligands with the receptor can help in the production of various complementary and alternative medicines for breast cancer which can help in curing it with less side effects and complications to the patient. From the research, we deduced that cimigenol; component of the herb black cohosh and glycyrrhetinic acid; component of the herb licorice have the highest affinity to bind with the protein. Thus these two herbs can prove to be most effective in curing breast cancer. Therefore, the future research can be extended to completely utilize the potential of this concept and bring about further novel applications which proved to be major scientific aspiration.

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