**GLOBAL ACADEMIC RESEARCH INSTITUTE**

COLOMBO, SRI LANKA


**GARI International Journal of Multidisciplinary Research**

ISSN 2659-2193

**Volume: 08 | Issue: 04**

On 31st December 2022

http://www.research.lk

Author: Humaid Luthufi, Heshani Mudalige, Ominda perera

School of Science, BMS, Sri Lanka

GARI Publisher | NCD | Volume: 08 | Issue: 04

Article ID: IN/ GARI/ICAS/2022/117 | Pages: 108-127 (19)

ISSN 2659-2193 | Edit: GARI Editorial Team

Received: 16.08.2022 | Publish: 31.12.2022

# PROTEIN-LIGAND DOCKING TO IDENTIFY POTENTIAL PHYTOCHEMICALS AGAINST BREAST CANCER PROTEIN RECEPTOR USING AUTODOCK

Humaid Luthufi, Heshani Mudalige, Ominda perera

*School of Science, BMS, Sri Lanka*

## *ABSTRACT*

Breast cancer is the most common cancer diagnosed among women worldwide with more than 2 million new cases estimated in 2021. Both hormone-dependent and hormone-independent breast cancers have a strong relationship with the estrogen receptor α (ERα). This study has been performed based on current therapy to target the hormone receptor Erα using phytochemicals. The 3D structure of Erα (PDB ID: 3ERT) was retrieved from Protein Data Bank and docked with 3D structures of phytochemicals retrieved from PubChem. Drug-likeness property was analyzed by applying the Lipinski's rule of five using ADMETlab 2.0. Receptor-Ligand site-specific docking was performed using Autodock 4.2.6. In site-specific docking, grid box parameters were set by targeting the common active amino acid residues in the binding pocket retrieved using CASTp (LEU346, ARG394, ALA350, LEU384, LEU387, PHE404, ILE424, HIS524) of the receptor with center grid box value; X= 31.371, Y= -1.046, Z=20.174 and spacing= 0.375Å. Docking poses and interactions were generated using UCSF Chimera and BIOVIA DS respectively. Then validation was carried out by performing redocking and obtained a value of -10.17 kcal/mol. This study based on binding energy, docking energy, drug likeness and other scores confirm that Daidzein has the best binding energy of -8.58kcal/mol and inhibition constant of 0.509uM out of the 20 phytochemicals used in this study. Other potential lead phytochemicals such as glycitein, genistein, curcumin, bergamottin, kaempferol and lignans (-8.55, -8.51, -8.48, -8.28, -8.04, -8.03 kcal/mol respectively) can be used to develop anti-therapeutic drugs for the treatment of breast cancer with lesser adverse effects.

Keywords: Erα, Site-specific, Lipinski's rule, Daidzein

## *INTRODUCTION*

More than one in ten new cancer diagnoses in women each year is due to breast cancer, which is the most prevalent cancer among women. It is the second-leading global cause of cancer death in women. Naturally, the breast possesses mammary glands in front of the chest wall. The ligaments that support and connect the chest to the chest wall are found on the pectoralis major muscle. The chest is made up of 15 to 20 lobes organized in a circular. The breast's size and morphology are defined by the fat that covers the lobes. Each lobe is made up of lobules that contain glands that produce milk when they are stimulated by hormones (Łukasiewicz et al.,2021). Breast cancer always develops insidiously. It is caused due to malignant proliferation of epithelial cells that line up the lobules and ducts of the breast. Regular screenings are how most patients learn about their condition. Perhaps others have an occasional swelling in the breast, a change in the breast's size or shape, the breast's skin is scaly or red, and fluid discharge from the nipples that is either clear or bloody when comes to symptoms (Feng et al., 2018).

However, mastalgia is not uncommon. Diagnosis of breast cancer requires an inspection of the body, imaging, particularly mammography, and tissue biopsy. With early diagnosis, survival rises. With a propensity for lymphatic and hematological dissemination, the tumour has a poor prognosis and may develop distant metastases (Moo et al., 2018).

Breast cancer incidence rises with age, reaching a peak of 421 cases per 100,000 in women aged 75 to 79. Women over the age of 40 account for 95% of newly diagnosed cases. Incidence rates in the 20 to 24 age group women is reported to be 1.5 cases per 100,000. 61 years is the average age of women at the time of breast cancer. Breast cancer accounts for 11.7% of all cancer cases worldwide as of 2021. In 2020, 25.7% of the total cancer cases diagnosed in Sri Lanka is also due to breast cancer (Sung et al., 2021).

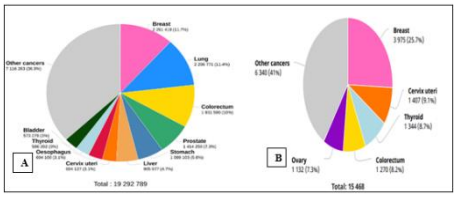


*Figure 1:A- Estimated number of cancer cases in 2020, worldwide. B-Number of breast cancer cases in 2020, females in Sri Lanka (Sung et al., 2021)*

Breast cancer occurs as a result of genetic mutations and DNA damage that may be affected by estrogen exposure. Inheritance is a possibility for some DNA defects or precancerous genes like BRCA1 and BRCA2. Therefore, having ovarian or breast cancer in the family increases the likelihood of getting breast cancer. In a healthy individual, cells with abnormal DNA or abnormal development are attacked by the immune system. In breast cancer patients, this fails, which promotes the growth and spread of the tumor (Shah, 2014).

A crucial tool in structural molecular biology and computer-aided drug creation is protein-ligand docking. This method aims to identify the predominant manner in which ligands will bind to three-dimensional proteins. The 2 docking techniques are blind and site-specific docking. Blind docking is done to identify an unknown binding site and site-specific docking is done by binding to the known binding site of the protein and site-specific docking targets the active amino acid residues (Hassan, 2017). Effective docking strategies efficiently search for high-dimensional spaces and using a scoring method that accurately rates potential dockings. Docking is a helpful tool for lead optimization since it can be used to virtually screen huge libraries of compounds, rank results, and develop structural ideas about how ligands inhibit a target (Gluterres and Im, 2020).

A wild type of protein receptor should be selected, not a mutated conformation. The resolution of the receptor should be less than 2Å. In presence of various conformation in a protein, the one with actives site is considered as a potential conformation and the active site amino acids in the protein receptor should be intact (Guedes., 2014).

Table 1: Receptor used in this study

| *Receptor* | PDB ID | Chains | Resolution | Method | Image |
|---|---|---|---|---|---|
| *Human Estrogen Receptor Alpha* | 3ERT | A | 1.90 Å | X-Ray diffraction |  |

Depending on the target, different ligands will be chosen for the docking procedure. It can be drawn using the Chemsketch tool or retrieved from a variety of databases, including ZINC or PubChem (Meng et al., 2011). It is often necessary to apply filters in order to reduce the number of docked molecules by analyzing the ADMET properties. Net

charge, molecular weight, polar surface area, solubility, commercial viability, similarity thresholds, pharmacophores, synthetic viability, absorption, distribution, metabolism, toxicological characteristics and excretion are a few examples (Guan et al., 2019).

Table 2: *Phytochemicals used in this study*

| Phytochemicals | Source | PubChem CID | Reference |
|---|---|---|---|
| **Xanthotoxol** | *Clausena lansium* | 65090 | (Acharya *et al*., 2019) |
| **Bergapten** | *Rhadinothamnus anceps* | 2355 | |
| **Psolaren** | *Citrus aurantiifolia* | 101626750 | |
| **Angelicin** | *Bituminaria bituminosa* | 10658 | |
| **Marmesin** | *Rhadinothamnus anceps* | 334704 | (Ahmed *et al*., 2020) |
| **Methoxsalen** | *Ammi majus* | 4114 | |
| **Bergamottin** | *Hansenia weberbaueriana* | 5471349 | |
| **Phellopterin** | *Zanthoxylum rhoifolium* | 98608 | (Han et al., 2018) |
| **Visnagin** | *Musineon divaricatum* | 6716 | (Jeengar *et al*., 2016) |
| **Catechins** | *Camellia sinensis* | 1203 | (Ahmed *et al*., 2020) |
| **Curcumin** | *Curcuma longa* | 969516 | (Jeengar *et al*., 2016) |
| **Daidzein** | *Glycine max* | 5281708 | (Sankaran and Mirunalini, 2022) |
| **Genistein** | *Glycine max* | 5280961 | (Pan *et al*., 2001) |
| **Glycitein** | *Glycine max* | 5317750 | (Pan *et al*., 2001) |
| **Glabridin** | *Glycyrrhiza glabra* | 124052 | (Wahab *et al*., 2021) |
| **Kaempferol** | *Spinacia oleracea* | 5280863 | (Chen *et al*., 2013) |
| **Lignans** | *Cucurbita moschata* | 443013 | (Mondaca *et al*., 2019) |
| **Quercetin** | *Malus pumila* | 5280343 | (Singh *et al*., 2010) |
| **Shogaol** | *Zingiber officinale* | 5281794 | (Bischoff and Furst, 2021) |
| **Trioxsalen** | *Psoralea corylifolia* | 5585 | (Husain *et al*., 2018) |

Table 3: *FDA approved drugs used in this study*

| FDA approved drugs | PubChem CID | Reference |
|---|---|---|
| **Tamoxifen** | 2733526 | (Acharya *et al*., 2019) |
| **Trastuzumab** | 146160902 | |
| **Lapatinib** | 208908 | (Schwartzberg *et al*., 2010) |
| **Letrozole** | 3902 | |
| **Gemcitabine** | 60750 | (Zhang *et al*., 2020) |

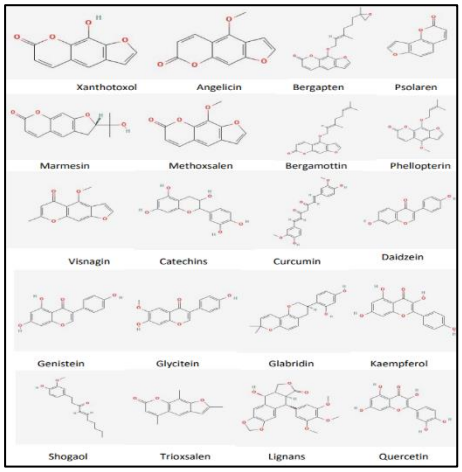


*Figure 2: 2D structures of phytochemicals used in this study*

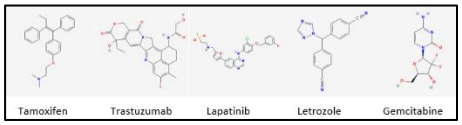


*Figure 3: 2D structures of FDA approved drugs used in this study*

Autodock suite 4.2.6 is an open-source computational software used to perform docking, analyze and to visualize results. PyMOL can be used as a molecular visualization software used to visualize 3D macromolecules (Rigsby et al., 2016). BIOVIA Discovery Studio 2021 is a free software used to view, analyze and visualize molecule data (Sharma, 2019). Ligplot is used to generate 2D schematic diagrams of protein ligand complexes (Caboche, 2013). ADMETlab 2.0 is a web tool used to analyze ADME parameters, identify drug nature and properties and to predict pharmacokinetic properties (Meng et al., 2011). UCSF Chimera is used to analyze and visualize molecular structural data and generate density maps (Huang et al., 2014).

Validation is performed to check the accuracy of docking. Validation can be performed using redocking or by generating Ramachandran plot. Ramachandran plot helps to generate a graph of amino residues present in the receptor before and after docking and thereby comparing the result helps in validating the result (Wlodawer, 2017). Redocking is performed by removing the ligand and docking it again to the receptor. It is done to ensure that the ligand is properly positioned and oriented to get a better result (Uchikoga et al., 2013)

**Significance**

Significance of breast cancer is that it is a disease without a natural cure. The treatments are based on removing, shrinking or inhibiting the growth of tumours. And the available treatments at present have various side effects such as hair loss, nausea, muscle pain, vaginal discharge, diarrhoea and nerve damage (Odle, 2014). Advancement in bioinformatics helps to do research on various phytochemicals and to discover a potential drug to treat breast cancer and other types of cancer with a better rate of recovery and less side effects (Hassan, 2017).

**Objectives**

Objective of this study is to identify best ligands and their binding sites against human ERα receptor for treatment of Breast Cancer disease. Some of the other specific objectives are to familiarize with software such as AutoDock Suite 4.2.6, UCSF Chimera, PyMOL, Open Babel GUI and BIOVIA DS, to identify best ligand binding site of FDA approved drugs for breast cancer using site-specific docking and to identify potent phytochemicals and FDA approved drugs and their binding site against human ERα receptor using site-specific docking.

**Materials**

The hardware consisted of a HP laptop Intel(R) Core (TM) i5-1035G1 CPU @ 1.00GHz 1.19 GHz, 64-bit operating system, x64-based processor, 8GB RAM, 10th generation and Windows 11. The software used were Autodock suite 4.2.6, BIOVIA Discovery Studio (DS), Open Babel GUI 2.4.1, PyMOL 2.5 and UCSF Chimera 1.1.6. Python 3.10.2 and Mgltools 1.5.7 were used as supporting softwares. Websites and webtools consisted of NCBI PubChem, CASTp 3.0, ADMET lab 2.0, Saves -PROcheck and Ramachandran plot analysis. Samples used were FDA approved drugs and phytochemicals.

## *METHODOLOGY*

**Protein receptor preparation**

The three-dimensional structures of the protein receptor involved in breast cancer, Erα was retrieved from the RCSB PDB with PDB IDs 3ERT. No chains were deleted during the modification of the receptor as there was only one chain. Modifications were done to the receptor by deletion of water molecules and selected atoms, addition of hydrogen and Kollman charges, assigning AD4 type to atoms and finally the file was saved as .pdbqt file to make it compatible with the ligand using Autodock suite 4.2.6.

**Ligand preparation**

The 3D structures of FDA approved drugs and phytochemicals were retrieved from NCBI PubChem in .sdf format. They were converted to .pdb using Open Babel GUI 2.4.1. Modifications were done to the ligand by setting torsions and the file was saved as pdbqt file to make it compatible to the receptor using Autodock 4.2.6.

**Site-specific docking using Autodock suite 4.2.6**

The receptor and ligands were modified to make them compatible to each other as mentioned in 3.1 and 3.2. To generate grid box, the macromolecule and ligand was opened, and the grid box parameter values were set as x, y, z dimensions 82, 50 and 90 respectively and x, y, z center values 31.371, -1.046 and 20.174 respectively (Figure 4) based on the active sites as mentioned in CASTp web tool. Finally, Autogrid and Autodock were run to obtain the docking log file which consists of the RMSD table along with the binding energies. Then the .dlg file was analyzed for best docking poses.

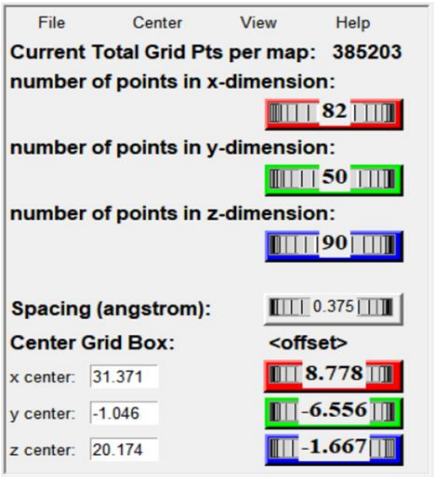


*Figure 4: Grid box parameter used in Site-specific docking*

**Analysis of docking parameters**

The DLG file and receptor file was opened using Autodock. Binding energy, inhibition constant and ligand efficiency were analyzed. The best phytochemical was determined based on the best binding energy.

**Visualization of poses and interactions**

Poses and interactions of docked protein-ligands, hydrogen bonds and hydrophobic bonds were visualized by opening the docked complex files and setting up parameters. 3D structures of docked complex files were also visualized to obtain the poses. Poses and interactions were obtained using following software such as Autodock suite 4.2.6, UCSF Chimera and BIOVIA Discovery Studio. The DLG file and receptor file was opened using Autodock suite 4.2.6. Binding information such as lowest binding energy, inhibition constant and ligand efficiency were analyzed. Finally, the complex file was saved as pdbqt file. UCSF Chimera was used to visualize and analyze the best docked poses. Background colour, quality and ribbons were adjusted to fit in. Then the file was saved as PDB. Dock complex.pdb file was opened in BIOVIA. Hydrogen bonds, hydrophobic interactions and other protein ligand interactions were obtained and analyzed using a 2D map. The 3D pose was also obtained by changing the background to white. PyMOL was used to generate poses and for superimposition.

**Validation**

Validation was done by generating a Ramachandran plot and by redocking. Redocking was performed by redocking its natural ligand OHT by the same method used in 3.1 and 3.2. Based on the results, stability of the protein-ligand complex was validated. Ramachandran plot was generated by uploading the PDB files for the receptor molecule before and after docking. Validation is done to make sure there is very less changes in the residues of the favoured regions of both complexes.

**Drug likeness analysis**

Drug likeness was identified using ADMETlab 2.0 web tool and potential phytochemicals were selected. Isomeric smiles were retrieved from PubChem and loaded into ADMETlab 2.0 to obtain the pharmacological properties of the ligands

## *RESULTS*

**Site-specific docking parameters**

Table 4: *Site-specific docking parameters retrieved using Autodock 4.2.6*

| Ligands | Binding Energy (kcal/mol) | Inhibition constant (μM) |
|---|---|---|
| **FDA Approved Drugs** | | |
| **Tamoxifen** | -9.80 | 0.0655 |
| **Trastuzumab** | -8.82 | 0.344 |
| **Lapatinib** | -8.42 | 0.669 |
| **Letrozole** | -8.25 | 0.891 |
| **Gemcitabine** | -4.82 | 0.291 |
| **Phytochemicals** | | |
| **Daidzein** | -8.58 | 0.509 |
| **Glycitein** | -8.55 | 0.540 |

| | | |
|---|---|---|
| **Genistein** | -8.51 | 0.577 |
| **Curcumin** | -8.48 | 0.610 |
| **Bergamottin** | -8.28 | 0.854 |
| **Kaempferol** | -8.04 | 1.27 |
| **Lignans** | -8.03 | 1.30 |
| **Glabridin** | -7.88 | 1.67 |
| **Catechins** | -7.75 | 2.80 |
| **Marmesin** | -7.72 | 2.20 |
| **Quercetin** | -7.70 | 2.25 |
| **Phellopterin** | -7.62 | 2.58 |
| **Trioxsalen** | -7.44 | 3.53 |
| **Visnagin** | -6.87 | 9.19 |
| **Psolaren** | -6.85 | 9.59 |
| **Shogaol** | -6.58 | 15.05 |
| **Xanthotoxol** | -6.55 | 15.78 |
| **Methoxsalen** | -6.53 | 16.39 |
| **Angelicin** | -6.41 | 20.02 |
| **Bergapten** | -6.29 | 24.50 |

Out of the FDA approved drugs, Tamoxifen had the best binding energy value of -9.80 kcal/mol and inhibition constant value of 0.0655 µM. Out of the phytochemicals, Daidzein obtained the best BE value of -8.58 kcal/mol and Ki value of 0.509 µM.

**Site-specific docking poses**

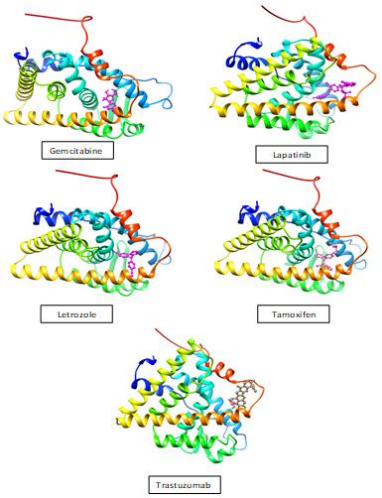


*Figure 5: Site-specific docking poses of FDA drugs retrieved using UCSF Chimera*

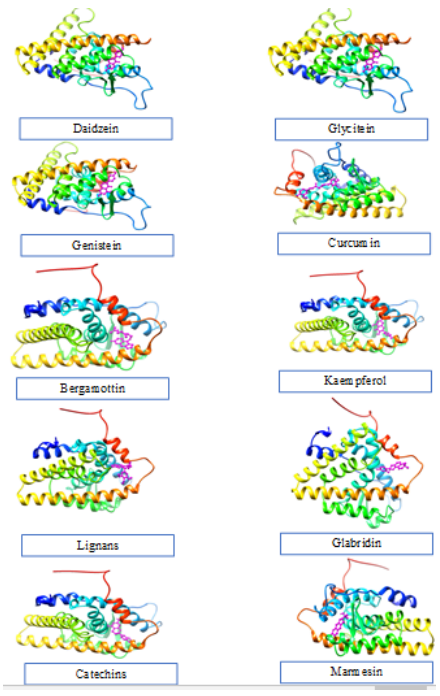

*Figure 6: Site-specific docking poses of phytochemicals retrieved using UCSF Chimera*

Site-specific docking interactions

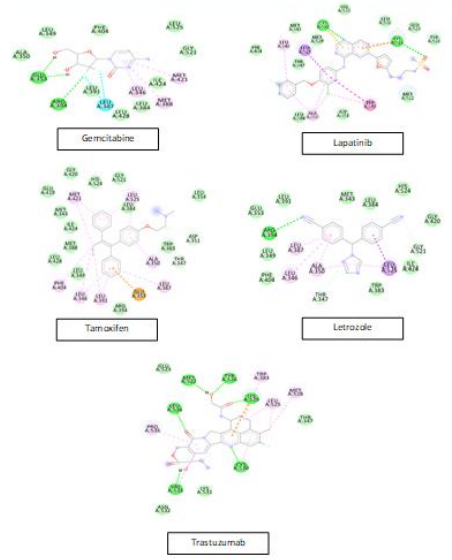


*Figure 7: Site-specific interactions of FDA drugs retrieved using BIOVIA DS*

*Figure 8: Site-specific interactions of phytochemicals retrieved using BIOVIA DS*

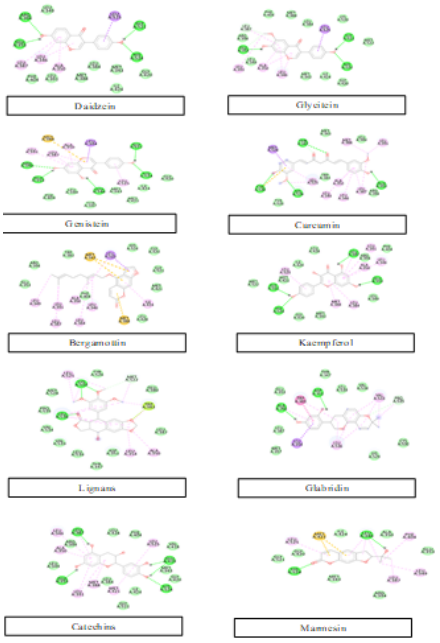


Table 5: *Site specific interactions retrieved using BIOVIA DS*

| Ligands | H bond-Amino acid residues | Hydrophobic bond-Amino acid residues |
|---|---|---|
| **FDA Approved Drugs** | | |
| **Tamoxifen** | | GLU419, GLY420, HIS524, GLY521, LEU384, LEU354, TRP383, ARG394, LEU349, LEU428, MET388, ILE424, MET343, MET421, LEU525, ALA350, LEU387, LEU391, LEU346, PHE404, GLU353 |
| **Trastuzumab** | LEU536, MET522, TYR526, CYS530, VAL533 | GLU523, THR347, LYS531, ASN532, TRP383, MET528, LEU525, PRO535 |
| **Lapatinib** | CYS530 | THR347, PHE404, MET343, MET528, VAL533, LEU536, GLU523, TYR526, MET522, ASP351, LEU384, LEU346, ALA350, TRP383, LEU525 |

| | | |
|---|---|---|
| **Letrozole** | ARG394 | GLU353, LEU391, MET343, LEU384, HIS524, GLY420, ILE424, TRP383, PHE404, LEU349, LEU387, ALA350, LEU346, LAU525 |
| **Gemcitabine** | ARG394, GLU353 | ALA350, LEU349, PHE404, LEU525, GLY521, ILE424, LEU384, LEU428, LEU391, MET421, LEU346, MET388, LEU387 |
| **Phytochemicals** | | |
| **Daidzein** | ARG394, GLU353, GLY521, HIS524 | LEU349, PHE404, LEU391, MET388, LEU384, MET343, ILE424, GLY420, LEU387, LEU346, ALA350, LEU525 |
| **Glycitein** | GLU353, GLY521, HIS524 | ARG394, PHE404, MET388, LEU384, LYS520, MET522, GLY420, ILE424, MET343, LEU349, LEU391, ALA350, LEU346, LEU525 |
| **Genistein** | ARG394, GLU353, LEU346, HIS524, GLY521 | PHE404, LEU349, THR347, MET343, MET421, ILE424, GLY420, LEU525, LEU391, LEU387, ALA350, LEU384 |
| **Curcumin** | CYS530, THR347, GLU353 | MET343, LEU384, ARG394, TRP383, TYR526, MET388, LEU391, LEU387, ALA350, LEU346, LEU349, LEU525, MET528 |
| **Bergamottin** | | GLU353, ARG394, TRP383, HIS524, GLY420, GLY521, MET421, LEU428, PHE404, LEU349, LEU391, LEU387, LEU384, ALA350, LEU346, ILE424, MET388, MET343, LEU525 |
| **Kaempferol** | LEU387, GLU353, HIS524, GLY521 | MET522, MET421, ILE424, LEU428, ARG394, PHE404, LEU349, MET343, GLY420, LEU525, LEU391, LEU346, ALA350, LEU384, MET388, LEU525 |
| **Lignans** | LEU536 | TYR526, GLU380, LEU387, ASP351, THR347, LEU539, VAL533, VAL534, PRO535, MET528, LEU525, ALA350, LEU354, TRP383 |
| **Glabridin** | ASP351, ALA350 | GLU353, THR347, LEU539, VAL534, CYS530, MET357, LEU387, VAL533, LEU536, LEU354, TRP383 |
| **Catechins** | LEU387, GLU419, HIS524, GLU353 | ARG394, LEU428, PHE404, VAL418, MET343, GLY420, ILE424, |

| | | |
|---|---|---|
| | | GLY521, LEU384, LEU349, LEU346, ALA350, LEU391, MET388, MET421, LEU525 |
| **Marmesin** | LEU346, HIS524 | GLY521, GLY420, ILE424, ALA350, GLU353, ARG394, MET343, LEU525, PHE404, LEU349, LEU387, MET421 |
| **Quercetin** | HIS524, GLU419, GLU353 | GLY521, LEU525, LEU384, LEU428, PHE404, ARG394, LEU349, VAL418, MET343, GLY420, MET388, LEU346, ALA350, LEU387, LEU391, MET421, ILE424 |
| **Phellopterin** | HIS524 | MET388, ILE424, ASP351, MET343, GLY521, GLY420, ALA350, TRP383, LEU525, LEU428, LEU391, PHE404, MET421, LEU346 |
| **Trioxsalen** | ARG394, LEU391 | MET343, GLU353, LEU384, ALA350, LEU349, LEU387, LEU346, MET388, LEU428, MET421, ILE424, PHE404 |
| **Visnagin** | | GLY390, PHE445, HIS356, PRO325, TRP393, ILE326, LEU387, MET357, PRO324, LEU327, GLU353, ARG394, LYS449 |
| **Psolaren** | HIS524, LEU525 | GLY420, GLU419, MET343, LEU346, PHE404, LEU391, LEU384, LEU428, MET388, ILE424 |
| **Shogaol** | LEU346 | HIS524, GLY521, MET343, LEU428, LEU402, PHE404, LEU387, LEU349, THR347, ARG394, MET388, ALA350, ILE424, lLEU391, MET421, LEU525, LEU384, GLU353 |
| **Xanthotoxol** | MET522 | TYR526, TRP383, LEU536, LEU525 |
| **Methoxsalen** | HIS524 | GLU419, GLY420, MET343, LEU346ala, PHE404, LEU428, LEU391, MET388, LEU384, LEU525, TRP383, ILE424, MET421, GLY521 |
| **Angelicin** | LEU327 | ARG394, PRO325, HIS356, ILE386, ILE326, PRO324, MET357, GLU353, LYS449 |
| **Bergapten** | HIS524, LEU525 | ILE424, GLY420, GLY521, MET343, PHE404 VAL418, LEU391, ALA350, LEU428, LEU346, MET421 |

ARG394, HIS524, LEU346, LEU387 and LEU525 were identified as common amino acid residues in both hydrogen and hydrophobic bonds. ALA350, MET4231, PHE404, ILE424 and LEU384 were identified as common amino acid residues in hydrophobic bonds of the binding pocket region.

ADMET analysis of phytochemicals

Table 6: ADMET analysis of phytochemicals using ADMETLAB 2.0

| Phytochemicals | BBB Penetration | HIA | Lipinski Rule | P-glycoprotein substrate | Cytochrome P (CYP2C9) inhibitory promiscuity |
|---|---|---|---|---|---|
| **Daidzein** | ✕ | ✕ | ✓ | ✓ | ✓ |
| **Glycitein** | ✕ | ✕ | ✓ | ✓ | ✓ |
| **Genistein** | ✕ | ✕ | ✓ | ✓ | ✓ |
| **Curcumin** | ✕ | ✕ | ✓ | ✕ | ✓ |
| **Bergamottin** | ✕ | ✕ | ✓ | ✕ | ✓ |
| **Kaempferol** | ✕ | ✕ | ✓ | ✕ | ✓ |
| **Lignans** | ✕ | ✕ | ✓ | ✕ | ✕ |
| **Glabridin** | ✕ | ✕ | ✓ | ✕ | ✓ |
| **Catechins** | ✕ | ✕ | ✓ | ✕ | ✕ |
| **Marmesin** | ✕ | ✕ | ✓ | ✕ | ✕ |
| **Quercetin** | ✕ | ✕ | ✓ | ✕ | ✓ |
| **Phellopterin** | ✕ | ✕ | ✓ | ✕ | ✓ |
| **Trioxsalen** | ✕ | ✕ | ✓ | ✓ | ✕ |
| **Visnagin** | ✕ | ✕ | ✓ | ✓ | ✓ |
| **Psolaren** | ✕ | ✕ | ✓ | ✕ | ✓ |
| **Shogaol** | ✕ | ✕ | ✓ | ✕ | ✓ |
| **Xanthotoxol** | ✕ | ✕ | ✓ | ✕ | ✕ |
| **Methoxsalen** | ✕ | ✕ | ✓ | ✕ | ✕ |
| **Angelicin** | ✕ | ✕ | ✓ | ✓ | ✓ |
| **Bergapten** | ✕ | ✕ | ✓ | ✓ | ✕ |

Based on the above 5 ADMET parameters, Lipinski's rule which is based on drug-ability was considered as the prime parameter. The rule of 5 include molecular weight >500 Da, H-bond donors <5, H-bond acceptors <10, CLog P value <5. Blood Brain Barrier (BBB) penetration and Human Intestinal Absorption were negative. Phytochemicals that didn't fulfil these parameters were ruled out. When two or more of these requirements were broken, a molecule is likely to be a non-orally available drug (Benet et al., 2016).

**Validation by redocking and Ramachandran plot**

Table 7: *Redocking values for site-specific docking*

| | **Binding Energy (kcal/ mol)** | **Inhibition constant/ Ki (µM)** |
|---|---|---|
| **Site-specific docking** | -10.17 | 0.0394 |

Site-specific redocking had the best redocking value of -10.17 kcal/ mol and 0.0394 µM.

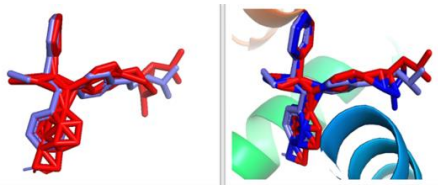

*Figure 9: Superimposed diagram of redocked receptor complex/ 3ERT and natural ligand OHT600 using PyMOL*

Superimposition was performed for the best redock value (-10.17 kcal/mol) which was obtained from the site-specific redocking. 3ERT receptor redocked to its natural ligand/ OHT600 had a good RMSD score of 1.695 Å. As the value is <2.0 Å, it can be concluded saying that this procedure is valid (Liu et al., 2009).

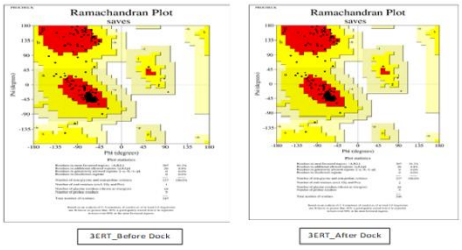


*Figure 10: Ramachandran plot validation of the receptor 3ERT*

As shown in figure 10, in both before and after docking the receptor, the residue in the most favourable region is 91.2%. Therefore, protein receptor structure is not affected by the docking procedure.

## *DISCUSSION*

Breast cancer is still a major public health concern and is given top priority in medical research despite advances in medical technology and significant study. This study primarily focuses on using various computational approaches to identify possible inhibitors of breast cancer. It was aimed to explore several phytochemicals against ER alpha proteins thought to be potential therapeutic targets and involved in the progression of breast cancer (Jha et al., 2022).

In Autodock suite 4.2.6, during the preparation of protein receptor 3ERT, the water molecule was deleted as they're not involved in binding, and they are eliminated to ease computations and get rid of any potential water molecules that would cause the pose search to be distorted in the binding pocket. No chains were deleted as the macromolecule had only one chain. Polar hydrogen addition makes it easier to discover hydrogen bond interactions and more favourable for anyone to discover ligand binding affinity against protein. Additionally, to stabilize the receptor by bridging the gaps. Kollman charges were added to the receptor to make sure it has a similar environment as in the body and to calculate the net atomic charge of the receptor molecule. Heteroatom (OHT600) was deleted to make room for the new ligand of interest as it can disrupt the binding of ligand with the receptor. Addition of missing atoms minimizes the effects on binding pocket and enhances protein preparation (Madhavi et al., 2013). Genetic algorithm was used to adjust one protein's surface in relation to the other and to determine the best complementary surface between the 2 molecules (Gardiner et al., 2001).

The ADMET characteristics of the filtered compounds were predicted using ADMETlab 2.0 web tool in order to determine the possible adverse effects of these phytochemicals in humans. ADMET parameters include the BBB, HIA, P-glycoprotein substrates and inhibitors, Lipinski's rule, renal organic cation transporter (ROCT), cytochrome P (CYP) inhibitory promiscuity, and toxicity risks. The phytochemicals that did not fulfill these parameters were not taken into consideration (Yousuf et al., 2017).

Stronger the ligand is bound to the receptor, the smaller the value of the inhibition constant indicates, and vice versa, higher the inhibition constant, the weaker the ligand is bound to the receptor. The decrease in the value of the inhibition constant is inversely proportional to the increase in the binding energy of the ligand. (Shivashankar and Sangeetha, 2022). Ramachandran plot analysis confirmed that the protein receptor structure was not affected by the docking procedure and redocking procedure obtained a value of 1.695 Å, which is less than <2 Å. Therefore, it can be confirmed that the docking procedure was accurate and valid (Liu et al., 2009). Phytochemicals docked using site specific docking using Autodock Suite 4.2.6 obtained higher binding energies and inhibition constants compared to the reference article as shown in Table 8. Other phytochemicals such as glycitein, curcumin, bergamottin and kaempferol also had binding energies above -7 kcal/mol and inhibition constant less than 1.30 µM.

Table 8: *Comparison of binding energy and inhibition constant from reference article and this study*

| **Ligand** | **BE from this study (kcal/mol)** | **BE from reference article (kcal/mol)** | **References** |
|---|---|---|---|
| Daidzein | -8.58 | -7.72 | Ferdous et al., (2013) |
| Genistein | -8.51 | -7.62 | |
| Quercetin | -7.70 | -7.13 | |
| Ligand | Ki from this study (µM) | Ki from reference article (µM) | References |
| Daidzein | 0.509 | 2.21 | Ferdous et al., (2013) |
| Genistein | 0.577 | 2.69 | |
| Quercetin | 2.25 | 5.55 | |

Daidzein had the best binding energy and inhibition constant values (-8.58 kcal/mol and 0.509 µM) for site-specific docking. Out of the FDA approved drugs, Tamoxifen had the best result for site-specific docking (BE= -9.80 kcal/mol and Ki= 0.0655 µM). Hence it can be proved that site-specific docking using Autodock suite 4.2.6 had the best results for the docking procedure.

In a study conducted by Kumar and Chauhan, Daidzein which obtained the highest value for site-specific docking in this study had a value of -9.30 kcal/mol for blind docking, reason for this can be due to the difference in software used for the docking procedure.

The experimental analysis in the research article shows that LEU346, ARG394 ALA350, LEU384, LEU387, PHE404, VAL418, MET421, ILE424, HIS524 and LEU525 act as catalytic site residues present in the 3D structure of Human estrogen receptor. And these residues were evaluated using QSiteFinder and CASTp.

Table 9: Common amino acid residues from reference article and this study

| **Common amino acid residues in this study** | **Common amino acids from article** | **References** |
|---|---|---|
| LEU346, ALA350, LEU384, LEU387, PHE404, MET421, ILE424, HIS524, LEU525, VAL418 | LEU346, ALA350, LEU384, LEU387, PHE404, MET421, ILE424, HIS524, LEU525, ARG394, TRP383 | Ferdous *et al*., 2013 |

Most common amino acids present in the binding pocket regions as per the article were 95% similar to the amino acids present in this study (Table 9). The ligands are stabilized at the target site by hydrogen bonding and hydrophobic interactions, which also contribute to change binding affinity and therapeutic efficacy (Patil et al., 2010).

In this study, LYS529 amino acid was identified as an 'Unfavourable bump', they are not classified as interactions present in hydrogen or hydrophobic bond region. Unfavourable bump can be formed due to steric interactions therefore, it can be tested by performing wet lab experiments like enzymatic assays (Fu et al., 2018). In this study, Daidzein which exhibited the best values in site-specific docking too showed the above interactions LEU346, ARG394 ALA350, LEU384, LEU387, PHE404, ILE424, HIS524 and LEU525. CASTp web tool was used to evaluate the residues in the binding pocket. HIS524 and ARG394 was obtained as the most repeated amino acid residues in Hydrogen bonds. Therefore, it can be proved that these amino acid interaction residues are important for estrogen receptor alpha targeted drug designing (Ferdous.S et al., 2013).

## *CONCLUSION*

In conclusion, the protein-ligand interaction plays a major role in structural based drug designing. The phytochemicals sorted based on ADMET analysis can be used as potential inhibitors for breast cancer disease with an efficient result. Best binding energies for both FDA and phytochemicals were obtained using Autodock suite 4.2.6. The most common acids identified in the binding pocket region (LEU346, ARG394 ALA350, LEU384, LEU387, PHE404, ILE424, HIS524 and LEU525) were crucial for the identification of binding pocket. In this study, phytochemicals such as daidzein, glycitein, genistein, curcumin, bergamottin, kaempferol, and lignans obtained very good results for binding energy and inhibition constant in site specific docking. Therefore, it can be

concluded that these phytochemicals can be used for further analysis to develop potent therapeutic target drugs for estrogen receptor alpha breast cancer.

As some of the phytochemicals had promising results against the estrogen receptor (3ERT), it can be analyzed and developed for the treatment of breast cancer. To obtain high throughput results for molecular docking simulations, computational tools such as DOCK, GOLD, FLEXX and ICM can be used (Dar and Mir, 2017). Schrodinger's GLIDE is a robust software which can be used for prediction of poses with an accuracy rate >90%. It is optimized for screening millions of compounds with full spectrum of speed (David, 2018). The Quantitative Structure-Activity Relationship (QSAR) is a mathematical tool that uses a compound's chemical structure to predict its physicochemical, biological, and environmental outcomes (Cherkasov, 2014). Traditional extraction methods have a significant drawback as they require a lot of time and energy. Therefore, techniques such as ultrasound-assisted extraction, microwave-assisted extraction or supercritical fluid extraction can be performed. Microwave-assisted extraction can be performed as it is possible to extract target molecules in 20 times less time than reflux extraction. Additionally, compared to the traditional two-step vacuum process, the microwave-vacuum method is ten times faster. Furthermore, it has been shown that the structure and composition of iso-flavonoids are unaffected by the extraction processes of ultrasonic disruption and microwave-vacuum drying. Extracted compounds can be isolated by High Pressure Liquid Chromatography (HPLC) as it accelerates the process of purification of the phytochemicals. UV-visible spectroscopy can be performed for qualitative analysis and purification. Finally, cytotoxicity assay can be carried out to determine the cytotoxicity of the phytochemical compound (Blicharski and Oniszczuk, 2017).

## *REFERENCES*


*Acharya, R., Chacko, S., Bose, P., Lapenna, A., & Pattanayak, S. P. (2019). 'Structure Based Multitargeted Molecular Docking Analysis of Selected Furanocoumarins against Breast Cancer', Scientific Reports, 9(1), 15743. Available at: https://doi.org/10.1038/s41598-019-52162-0 (Accessed: 18th March 2022).*

*Ahmed, S., Khan, H., Aschner, M., Mirzae, H., Küpeli Akkol, E., & Capasso, R. (2020). 'Anticancer Potential of Furanocoumarins: Mechanistic and Therapeutic Aspects', International Journal of Molecular Sciences, 21(16), 5622. Available at: https://doi.org/10.3390/ijms21165622 (Accessed: 27th March 2022).*

*Benet, L. Z., Hosey, C. M., Ursu, O., & Oprea, T. I. (2016). 'BDDCS, the Rule of 5 and drugability', Advanced Drug Delivery Reviews, 101, 89–98. Available at: https://doi.org/10.1016/j.addr.2016.05.007 (Accessed: 25th July 2022).*

*Bischoff-Kont, I., & Fürst, R. (2021). 'Benefits of Ginger and Its Constituent 6-Shogaol in Inhibiting Inflammatory Processes. Pharmaceuticals, 14(6), 571. Available at: https://doi.org/10.3390/ph14060571 (Accessed: 25th July 2022).*

*Blicharski, T., & Oniszczuk, A. (2017). 'Extraction Methods for the Isolation of Isoflavonoids from Plant Material', Open Chemistry, 15(1), 34–45. Available at: https://doi.org/10.1515/chem-2017-0005 (Accessed: 9th July 2022).*

*Caboche, S. (2013). 'LeView: automatic and interactive generation of 2D diagrams for biomacromolecule/ligand interactions', Journal of Cheminformatics, 5(1), 40. Available at: https://doi.org/10.1186/1758-2946-5-40 (Accessed: 18th March 2022).*

*Chen, A. Y., & Chen, Y. C. (2013). 'A review of the dietary flavonoid, kaempferol on human health and cancer chemoprevention', Food Chemistry, 138(4), 2099–2107. Available at: https://doi.org/10.1016/j.foodchem.2012.11.139 (Accessed: 27th July 2022).*

*Cherkasov, A., Muratov, E. N., Fourches, D., Varnek, A., Baskin, I. I., Cronin, M., Dearden, J., Gramatica, P., Martin, Y. C., Todeschini, R., Consonni, V., Kuz'min, V. E., Cramer, R., Benigni, R., Yang, C., Rathman, J., Terfloth, L., Gasteiger, J., Richard, A., & Tropsha, A. (2014). 'QSAR Modeling: Where Have You Been? Where Are You Going To?', Journal of Medicinal Chemistry, 57(12), 4977–5010. Available at: https://doi.org/10.1021/jm4004285 (Accessed: 29th July 2022).*

*Dar, A. M., & Mir, S. (2017). 'Molecular Docking: Approaches, Types, Applications and Basic Challenges', Journal of Analytical & Bioanalytical Techniques, 08(02). Available at: https://doi.org/10.4172/2155-9872.1000356 (Accessed: 29th July 2022).*

*David, T. I., Adelakun, N. S., Omotuyi, O. I., Metibemu, D. S., Ekun, O. E., Eniafe, G. O., Inyang, O. K., Adewumi, B., Enejoh, O. A., Owolabi, R. T., & Oribamise, E. I. (2018). 'Molecular docking analysis of phyto-constituents from Cannabis sativa with pfDHFR', Bioinformation, 14(9), 574–579. Available at: https://doi.org/10.6026/97320630014574 (Accessed: 29th July 2022).*

*Feng, Y., Spezia, M., Huang, S., Yuan, C., Zeng, Z., Zhang, L., Ji, X., Liu, W., Huang, B., Luo, W., Liu, B., Lei, Y., Du, S., Vuppalapati, A., Luu, H. H., Haydon, R. C., He, T.-C., & Ren, G. (2018). 'Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis', Genes & Diseases, 5(2), 77–106. Available at: https://doi.org/10.1016/j.gendis.2018.05.001 (Accessed: 17th March 2022).*

*Ferdous, S., Muhammad Usman Mirza, & Usman Saeed. (2013). 'Docking Studies reveal Phytochemicals as the long searched anticancer drugs for Breast Cancer', International Journal of Computer Applications, 67. Available at: https://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.403.6121&rep=rep1&type=pdf (Accessed: 18th March 2022).*

*Fu, Y., Zhao, J., & Chen, Z. (2018). 'Insights into the Molecular Mechanisms of Protein-Ligand Interactions by Molecular Docking and Molecular Dynamics Simulation: A Case of Oligopeptide Binding Protein', Computational and Mathematical Methods in Medicine, 1–12. Available at: https://doi.org/10.1155/2018/3502514 (Accessed: 29th July 2022).*

*Gardiner, E. J., Willett, P., & Artymiuk, P. J. (2001). 'Protein docking using a genetic algorithm', Proteins: Structure, Function, and Genetics, 44(1), 44–56. Available at: https://doi.org/10.1002/prot.1070 (Accessed: 29th July 2022).*

*Guan, L., Yang, H., Cai, Y., Sun, L., Di, P., Li, W., Liu, G., & Tang, Y. (2019). 'ADMET-score – a comprehensive scoring function for evaluation of chemical drug-likeness', MedChemComm, 10(1), 148–157. Available at: https://doi.org/10.1039/C8MD00472B (Accessed: 16th March 2022).*

*Guedes, I. A., de Magalhães, C. S., & Dardenne, L. E. (2014). 'Receptor–ligand molecular docking', Biophysical Reviews, 6(1), 75–87. Available at: https://doi.org/10.1007/s12551-013-0130-2 (Accessed: 16th March 2022).*

*Han, H. S., Jeon, H., & Kang, S. C. (2018). Phellopterin isolated from Angelica dahurica reduces blood glucose level in diabetic mice', Heliyon, 4(3), e00577. Available at: https://doi.org/10.1016/j.heliyon.20*

*18.e00577 (Accessed: 18th March 2022).*

*Hassan, N. M., Alhossary, A. A., Mu, Y., & Kwoh, C.-K. (2017). 'Protein-Ligand Blind Docking Using QuickVina-W With Inter-Process Spatio-Temporal Integration', Scientific Reports, 7(1), 15451. Available at: https://doi.org/10.1038/s41598-017-15571-7 (Accessed: 19th March 2022).*

*Huang, C. C., Meng, E. C., Morris, J. H., Pettersen, E. F., & Ferrin, T. E. (2014). 'Enhancing UCSF Chimera through web services', Nucleic Acids Research, 42(W1), W478–W484. https://doi.org/10.1093/nar/gku377 (Accessed: 17th March 2022).*

*Husain, F. M., Ahmad, I., Khan, F. I., Al-Shabib, N. A., Baig, M. H., Hussain, A., Rehman, M. T., Alajmi, M. F., & Lobb, K. A. (2018). 'Seed Extract of Psoralea corylifolia and Its Constituent Bakuchiol Impairs AHL-Based Quorum Sensing and Biofilm Formation in Food- and Human-Related Pathogens', Frontiers in Cellular and Infection Microbiology, 8. Available at: https://doi.org/10.3389/fcimb.2018.00351 (Accessed: 18th March 2022).*

*Jeengar, M. K., Rompicharla, S. V. K., Shrivastava, S., Chella, N., Shastri, N. R., Naidu, V. G. M., & Sistla, R. (2016). 'Emu oil-based nano-emulgel for topical delivery of curcumin', International Journal of Pharmaceutics, 506(1–2), 222–236. Available at: https://doi.org/10.1016/j.ijpharm.2016.04.052 (Accessed: 17th March 2022).*

*Jha, V., Devkar, S., Gharat, K., Kasbe, S., Matharoo, D. K., Pendse, S., Bhosale, A., & Bhargava, A. (2022). 'Screening of Phytochemicals as Potential Inhibitors of Breast Cancer using Structure Based Multitargeted Molecular Docking Analysis', Phytomedicine Plus, 2(2), 100227. Available at: https://doi.org/10.1016/j.phyplu.2022.100227 (Accessed: 16th March 2022).*

*Lemus-Mondaca, R., Marin, J., Rivas, J., Sanhueza, L., Soto, Y., Vera, N., & Puente-Díaz, L. (2019). 'Pumpkin seeds (Cucurbita maxima). A review of functional attributes and by-products', Revista Chilena de Nutrición, 46(6), 783–791. Available at: https://doi.org/10.4067/S0717-75182019000600783 (Accessed: 18th March 2022).*

*Liu, Y.-S., Fang, Y., & Ramani, K. (2009). 'Using least median of squares for structural superposition of flexible proteins', BMC Bioinformatics, 10(1), 29. Available at: https://doi.org/10.1186/1471-2105-10-29 (Accessed: 15th March 2022).*

*Łukasiewicz, S., Czeczelewski, M., Forma, A., Baj, J., Sitarz, R., & Stanisławek, A. (2021). 'Breast Cancer—Epidemiology, Risk Factors, Classification, Prognostic Markers, and Current Treatment Strategies—An Updated Review', Cancers, 13(17), 4287. Available at: https://doi.org/10.3390/cancers13174287 (Accessed: 15th March 2022).*

*Madhavi Sastry, G., Adzhigirey, M., Day, T., Annabhimoju, R., & Sherman, W. (2013). 'Protein and ligand preparation: parameters, protocols, and influence on virtual screening enrichments', Journal of Computer-Aided Molecular Design, 27(3), 221–234. Available at: https://doi.org/10.1007/s10822-013-9644-8 (Accessed: 18th March 2022).*

*Meng, X.-Y., Zhang, H.-X., Mezei, M., & Cui, M. (2011). 'Molecular Docking: A Powerful Approach for Structure-Based Drug Discovery', Current Computer Aided-Drug Design, 7(2), 146–157. Available at: https://doi.org/10.2174/157340911795677602 (Accessed: 18th March 2022).*

*Moo, T.-A., Sanford, R., Dang, C., & Morrow, M. (2018). 'Overview of Breast Cancer Therapy', PET Clinics, 13(3), 339–354. https://doi.org/10.1016/j.cpet.2018.02.006 (Accessed: 15th March 2022).*

*Odle, T. G. (2014). 'Adverse effects of breast cancer treatment', Radiologic Technology, 85(3), 297-319. Available at: https://pubmed.ncbi.nlm.nih.gov/24395910/ (Accessed: 17th March 2022).*

*Pan, W., Ikeda, K., Takebe, M., & Yamori, Y. (2001). 'Genistein, Daidzein and Glycitein Inhibit Growth and DNA Synthesis of Aortic Smooth Muscle Cells from Stroke-Prone Spontaneously Hypertensive Rats', The Journal of Nutrition, 131(4), 1154–1158. Available at: https://doi.org/10.1093/jn/131.4.1154 (Accessed: 14th March 2022).*

*Patil, R., Das, S., Stanley, A., Yadav, L., Sudhakar, A., & Varma, A. K. (2010). 'Optimized Hydrophobic Interactions and Hydrogen Bonding at the Target-Ligand Interface Leads the Pathways of Drug-Designing', PLoS ONE, 5(8), e12029. Available at: https://doi.org/10.1371/journal.pone.0012029 (Accessed: 29th July 2022).*

*Rigsby, R. E., & Parker, A. B. (2016). 'Using the PyMOL application to reinforce visual understanding of protein structure', Biochemistry and Molecular Biology Education, 44(5), 433–437. Available at: https://doi.org/10.1002/bmb.20966 (Accessed: 17th March 2022).*

*Sankaran and mirunalini. (2022). 'Structure-based molecular docking studies towards exploring phytoestrogen against breast cancer', Eurasian Journal of Medicine and Oncology. Available at: https://doi.org/10.14744/ejmo.2022.43656 (Accessed: 18th March 2022).*

*Schwartzberg, L. S., Franco, S. X., Florance, A., O'Rourke, L., Maltzman, J., & Johnston, S. (2010). 'Lapatinib plus Letrozole as First-Line Therapy for HER-2+ Hormone Receptor–Positive Metastatic Breast Cancer', The Oncologist, 15(2), 122–129. Available at: https://doi.org/10.1634/theoncologist.2009-0240 (Accessed: 18th March 2022).*

*Shah, R. (2014). 'Pathogenesis, prevention, diagnosis and treatment of breast cancer', World Journal of Clinical Oncology, 5(3), 283. Available at: https://doi.org/10.5306/wjco.v5.i3.283 (Accessed: 18th March 2022).*

*Sharma. S, (2019). 'Overview of BIOVIA Materials Studio, LAMMPS, and GROMACS. (2019). In Molecular Dynamics Simulation of Nanocomposites Using BIOVIA Materials Studio, Lammps and Gromacs', Elsevier (pp. 39–100). Available at: https://doi.org/10.1016/B978-0-12-816954-4.00002-4 (Accessed: 18th March 2022).*

*Shivashankar, S., & Sangeetha, M. K. (2022). 'The Natural Ligand for Metalloproteinase-A Multifaceted Drug Target', Applied Biochemistry and Biotechnology, 194(4), 1716–1739. Available at: https://doi.org/10.1007/s12010-021-03778-4 (Accessed: 9th July 2022).*

*Singh, B., Mense, S. M., Bhat, N. K., Putty, S., Guthiel, W. A., Remotti, F., & Bhat, H. K. (2010). 'Dietary quercetin exacerbates the development of estrogen-induced breast tumors in female ACI rats', Toxicology and Applied Pharmacology, 247(2), 83–90. Available at: https://doi.org/10.1016/j.taap.2010.06.011 (Accessed: 18th March 2022).*

*Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). 'Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries', Cancer Journal for Clinicians, 71(3), 209–249. Available at: https://doi.org/10.3322/caac.21660 (Accessed: 15th March 2022).*

*Uchikoga, N., Matsuzaki, Y., Ohue, M., Hirokawa, T., & Akiyama, Y. (2013). 'Re-Docking Scheme for Generating Near-Native Protein Complexes by Assembling Residue Interaction*

*Fingerprints', PLoS ONE. Available at: https://doi.org/10.1371/journal.pone.0069365 (Accessed: 18th March 2022).*

*Wahab, S., Annadurai, S., Abullais, S. S., Das, G., Ahmad, W., Ahmad, M. F., Kandasamy, G., Vasudevan, R., Ali, M. S., & Amir, M. (2021). 'Glycyrrhiza glabra (Licorice): A Comprehensive Review on Its Phytochemistry, Biological Activities, Clinical Evidence and Toxicology', Plants, 10(12), 2751. Available at: https://doi.org/10.3390/plants10122751 (Accessed: 18th March 2022).*

*Wlodawer, A. (2017). 'Stereochemistry and Validation of Macromolecular Structures' (pp. 595–610). Available at: https://doi.org/10.1007/978-1-4939-7000-1_24 (Accessed: 16th March 2022).*

*Yousuf, Z., Iman, K., Iftikhar, N., & Mirza, M. (2017). 'Structure-based virtual screening and molecular docking for the identification of potential multi-targeted inhibitors against breast cancer', Breast Cancer: Targets and Therapy, Volume 9, 447–459. Available at: https://doi.org/10.2147/BCTT.S132074 (Accessed: 09th July 2022).*

*Zhang, X., Zhang, J., Liu, W., Wang, Y., Wu, J., Zhao, S., Zhao, M., & Peng, S. (2020). 'Exploring the action of RGDV-gemcitabine on tumor metastasis, tumor growth and possible action pathway', Scientific Reports, 10(1), 15729. Available at: https://doi.org/10.1038/s41598-020-72824-8 (Accessed: 18th March 2022).*