https://doi.org/ 10.33472/AFJBS.6.Si2.2024.2674-2682

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Research Article

Computational Analysis of Some Phytoconstituents for Breast Cancer as Potential Anticancer Drugs

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ARTICLE INFO:

Volume 6,Issue Si2, 2024
Received:14 Apr 2024
Accepted : 03 May 2024
doi: 10.33472/AFJBS.6.Si2.2024.2674-2682

Abstract:

Recently, it has been suggested that adding natural substances, like phytochemicals, might help stop the growth of cancers. This study uses docking methods to look into what role these nutrients might play in cancers, especially prostate and breast cancer. Breast cancer is the most common type of cancer in women around the world. Breast cells, both healthy and cancerous, have receptors that connect to progesterone and estrogen, which causes the cells to grow. The important feature has been used to study how phytochemicals bind to these receptors and cause an opposite response that stops the growth of cancer cells that are out of control. Most of the drugs that are given to people with breast cancer stop estrogen from working on these cells. They used a docking method to join different groups of phytochemicals, such as quercetin, genistein, and daidin, to the active site of the human estrogen receptor (PDB ID: 2IOK). The molecular docking method used in this study was successful in finding possible plants that could be used as anti-cancer drugs to treat breast cancer. Researchers have found that Leu346, Leu384, Leu387, Phe404, and Leu525 are the most important residues that could be used as treatment targets. These results come from a lot of different numbers, such as binding energy, docking energy, drug similarity, and others. It's possible that Daidzein, Genistein, and Quercetin could be the first molecules to stop messages that are strong for human breast cancer. This study is the first step in the natural process of making new, strong, and specific human estrogen inhibitors for cancer treatment.

Keywords: Molecular Docking, docking score, phytoconstituent, drug design, CADD.

Introduction:

Breast cancer is the second most common type of cancer, with an 11% incidence rate. It is after lung cancer (Wadanambi, *et al.*, 2023). There are receptors on both normal breast cells and most breast cancer cells that can bind to progesterone and estrogen in the blood. A growth response happens when these hormones connect to the receptors and set off a chain of signals that encourages cells to divide and grow (Surana, *et al.,* 2021). Progesterone and estrogen have a big effect on cancer cells because they interact with genes that stop tumors from growing and genes that make cells grow out of control. When you look at breast cells that have estrogen and progesterone receptors versus cancer cells that don't have these receptors, the breast cells that do have these receptors have a better outlook and respond better to hormonal treatment (Surana, *et al.,* 2021). Tamoxifen is a pill that you take by mouth that stops estrogen from working properly. Many people who take tamoxifen end up with blood clots, strokes, womb cancer, and cataracts. These are all common side effects that can be life-threatening (Saleem, *et al.,* 2022). Blood clots can form very badly in the eyes, lungs, or legs when you take raloxifene. Leg pain, leg growth, breathing problems, chest pain, and changes in how you see things are some of the other effects that could happen (Pandya, *et al.,* 2020). Because these medicines have bad side effects, you should stop taking them and do more research to find a better option. The point of our study was to find natural drugs that bind strongly to breast cancer receptors so that we could find better ways to treat breast cancer (Pathan, *et al.,* 2023). Phytochemicals are very good at lowering the risk of getting cancer, according to scientific investigations. Because of this, our research shows that antioxidants have stopped breast cancer from spreading. There are different main groups of

phytochemicals, but isoflavones and flavonoids are the most well-known (Surana, *et al.,* 2021).There are many different kinds of chemicals found in plants that are called isoflavones. Many of them don't work like estrogen do. It's important to note that isoflavones and estrogen have molecules that are very similar to each other. The competitive binding against estrogen is thought to be because the structures are so close (Karthic, *et al.,* 2022). Isoflavones can change the way estrogen works, either making it stronger or weaker, based on the type of estrogen receptor that is found in the cell. Because estrogen and isoflavones compete for the same receptor sites, having too much estrogen might not be very bad for your health (Mondal, *et al.,* 2022). Isoflavones stop estrogen from working, which has been linked to a higher risk of breast cancer and other cancers that rely on hormones. Like the medicine tamoxifen, which is often prescribed to treat and avoid breast cancer, they work in a similar way. Genistein, quercetin, and daikon all had a strong connection with the estrogen receptor in this study (Kaloni, *et al.,* 2020). This could be because they have structures that are similar to estrogen and some phytochemicals. All of the polyphenols shown in Figure 1 are structurally related to estradiol, which is an animal sex hormone. Some parts of the structures are the same, like having a phenolic ring and two hydroxyl groups that are needed to connect to the different types of ER (Iman, *et al.,* 2016). The exact location of the hydroxyl group is very important for its ability to bind to the ER and start transcription. The most powerful effects happen when the hydroxyl group is in places four, six, or seven (Ahire, *et al.,* 2020 and Sharma, et al., 2018).

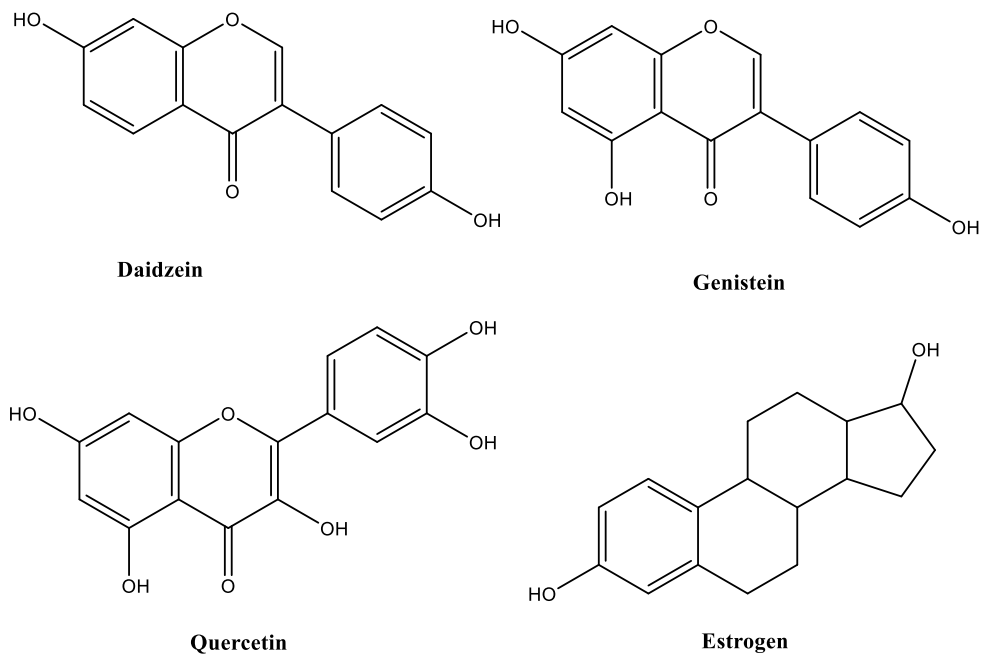

Figure 1: Structures of Phytoconstituents

Material and Method:

Selection of Protein and Ligand from database:

The 3D structure of the human estrogen receptor was found in the Protein Data Bank (PDB). It was the substance N-[(1R)-3-(4-hydroxyphenyl)-1-methylpropyl] that has a strong inhibitor. This building block's PDB ID is 2IOK (Keservani, *et al.,* 2019).The Pubchem molecule database was used to get the molecular structures of all the phytochemicals that were studied, such as 2-(2-phenyl-1h-indol-yl) acetamide (Mathivadani, *et al.,* 2020 and Parkhe, *et al.,* 2023).

Preparation of ProteinandLigandfor molecular docking:

For docking experiments, the raw protein from the protein data bank entitled Human Estrogen Receptor (PDB ID 2IOK) is further processed (Patel, *et al.*,2019). The protein receptor was first made by eliminating all heteroatoms and water molecules, then employing the program Chimera to perform successive energy minimization for 1000 steps at an RMS gradient of 0.02 with an update interval of 10, in order to eliminate any undesirable steric clashes. AMBER ff12SB was used as the force field. Open Babel was used to transform the 2D molecular structures into 3D ones, and the Hyperchem MM+ force field was used to minimize energy (Gaikwad, *et al.,* 2023).

Identification ofbindingsite:

Protein binding and active sites are frequently associated with structural gaps and pockets that can facilitate robust binding of a drug candidate. Information from the literature was utilized to determine the specific location where the human estrogen receptor facilitates the catalysis of the process (Keservani, *et al.,* 2020). The catalytic residue was further investigated using Q-SiteFinder and the CASTp service, which are tools for analyzing the surface topography of proteins. Q-SiteFinder utilizes the energy of interaction between a protein and a basic van der Waals probe to identify binding sites that possess favorable energy characteristics. There is concordance between the knowledge base data from the computer tool and the literature (Iman, *et al.*,2015). The CASTp server employs the alpha complex and weighted Delaunay triangulation algorithms to quantify geometric properties. It is capable of quantifying and detecting inaccessible internal compartments as well as surface pockets that are accessible for proteins and other molecules. Mathematics is employed to calculate the area and volume of each cavity and hollow, considering both the accessible surface for molecules and the surface accessible to solvents (Mondal, *et al.,* 2022).

Calculation of the docking score between phytochemicals and the human estrogen receptor

The computer docking studies were carried out with AUTODOCK 4.0. It had a 2.0 GHz Intel Core 2 Duo CPU, 4 GB of RAM, a 450 GB hard drive, and the Linux operating system. The software was put on a single computer. Following the instructions, automated dockings were done using the AutoDock4.0 tool to find the best ways for different inhibitors to bind to the human estrogen receptor within its binding pocket (Mohan, *et al.*,2013). We can say that the sensor proteins were connected to Kollman charges and polar hydrogen atoms. Ligands were linked to Gasteiger partial charges that were already set for hydrogen atoms that are not polar. During the docking process, the ligands were free to move around in three dimensions. The AutoGrid program was used to make the grid maps. The shape of the receptor being matched was shown at the center of each grid. In AutoDock, the translation, quaternion, and torsion steps were all left as they were (El-Shiekh, *et al.*,2023). For minimization, the Lamarckian genetic algorithm method was used with the usual settings. Standard practice for both rigid and flexible ligand docking was to do 50 iterations with a starting population of 150 randomly chosen people. The process involved evaluating 2.5 x 105 energies, going through a maximum of 27000 times, and a mutation rate (Surana and Mahajan, 2022).

It has a crossover rate of 0.80, an elitism value of 1, and a probability value of 0.02.With an RMS range of 1.0 angstrom, cluster analysis was done on the docked results. To find the binding energy of each cluster, take the average of all the conformations that cluster holds. The ligand's docked position is chosen based on the cluster that has the fewest binding energies and the most possible shapes (Ghasemzadeh, *et al.*,2014 and (Kamalidehghan, *et al.*,2018).

The binding site snugly fits into the active site cavity and closely interacts with numerous residues, including Leu346, Ala350, Leu384, Leu387, Phe404, Ile424, and Leu525. Table 2 provides supplementary details regarding the binding residues that are present in all of the plants (Ahmadipour, *et al.*,2015). The recent discoveries indicate that all of the examined phytochemicals share a common binding site with the human estrogen receptor (Maheswari and Sankar, 2024). The crucial residues for a potential therapeutic target include Leu346, Leu384, Leu387, Phe404, and Leu525. The free energy of binding (G) for the Human estrogen receptor target molecule was determined to be -8.82, -8.36, and -6.90 kcal/mol for Daidzein, Genistein, and Quercetin, respectively (Table 1). Daidzein, Genistein, and Quercetin exhibit a higher affinity for the human estrogen receptor compared to any other pharmacological molecule (Pakrashy, *et al.*, 2022).

Results and Discussion

Binding site analysis

These findings suggest that daidzein, genistein, and quercetin exhibit a higher number of intermolecular interactions in comparison to other pharmaceutical compounds. Quercetin, genistein, and daphzin efficiently bind to the human estrogen receptor. The value of inhibition is elevated due to the superior docking score and binding affinity. The experimental analysis suggests that the human estrogen receptor structure contains the catalytic site residues Leu346, Ala350, Leu384, Leu387, Phe404, Val418, Met421, Ile424, His524, and Leu525.

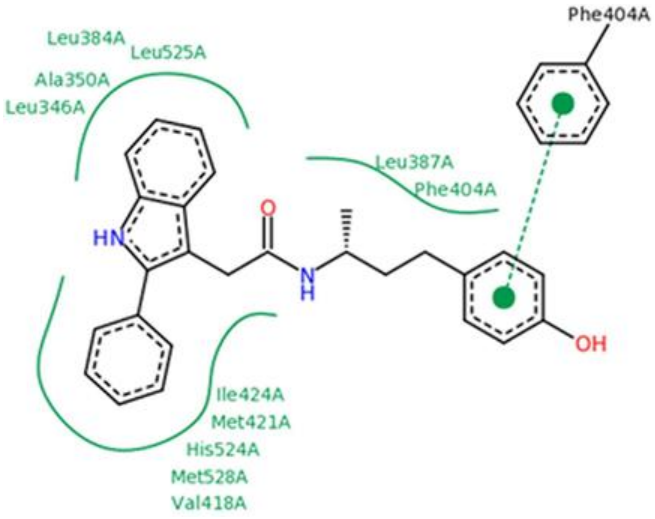

Figure 2: The binding site in the Human estrogen receptor consists of the amino acids

Q-SiteFinder and CASTp were utilized as computational techniques to analyze the catalytic residues. The first anticipated site of volume 603A^, as determined by Q-SiteFinder, contains catalytic site residues such as Leu346, Ala350, Leu384, Leu387, Phe404, Val418, Met421, and Ile424. There was a scarcity of data regarding quercetin, genistein, and daizenzin. This data unequivocally demonstrates that these three phytochemicals exhibit a greater propensity to bind to the human estrogen receptor, resulting in a decreased requirement for inhibition. The computational calculations and experimental results indicate that Leu346, Ala350, Leu384, Leu387, Phe404, Val418, Met421, Ile424, His524, and Leu525 serve as catalytic residues in the three-dimensional structure of the human estrogen receptor. The acquired results are consistent with the previous conclusions.

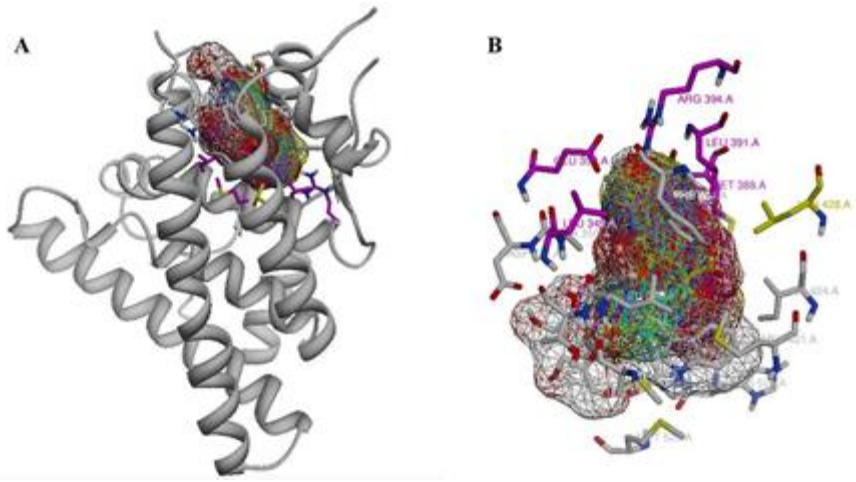

Figure3: Binding of Phytochemicals with Human estrogen receptor binding site

Molecular Docking studies of Human estrogen receptor with phytochemicals

The results of the current study demonstrated how the protein-ligand complex of the human estrogen receptor behaves when exposed to the phytochemicals listed in Table 1.

Table1: Phytochemicals free energies of binding (G) in the active site were computed by Autodock

Ligands	Binding energy G(kcal/mol)	Inter mol Energy (kcal/mol)	Docking Energy (kcal/mol)	Inhibition Constant(uM)
Daidzein	-8.82	-8.76	-8.96	2.23
Genistein	-8.36	-8.93	-9.75	2.71
Quercetin	-6.90	-8.56	-10.32	5.61

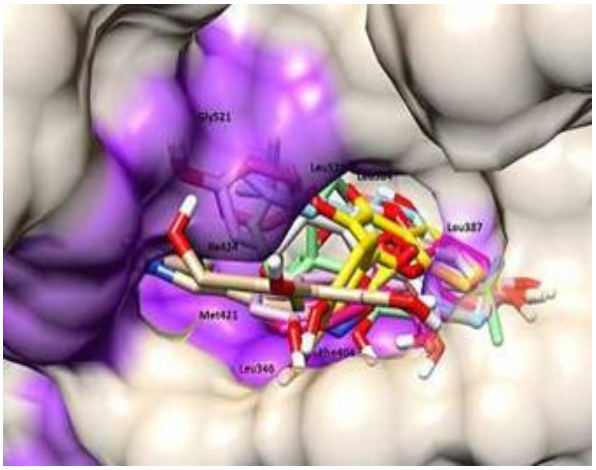

Figure 4: Plant compounds demonstrating their ability to connect to the estrogen receptor binding site

Conclusion

An essential element in the process of structurally based drug design is the interaction between the protein and the ligand. The present investigation involves the extraction of human estrogen receptors and the identification of potential pharmaceuticals for the treatment of breast cancer. Computational approaches have been employed to understand the mechanism of interactions and binding affinity between phytochemicals and the human estrogen receptor. The phytochemicals employed in this study exhibited binding energies ranging from -8.82 to -6.90 kcal/mol and docking energies ranging from -8.96 to -10.32

kcal/mol, which are within the optimal and customary limits for binding energy. The latest research findings indicate that the most crucial residues that could potentially serve as therapeutic targets are Leu346, Leu384, Leu387, Phe404, and Leu525. Daidzein, Genistein, and Quercetin are potential candidates for inhibiting the human estrogen receptor. Hence, these innate compounds can be utilized as a prototype for generating therapeutic precursor molecules, thereby reducing the duration required for the development of novel medicines. Conducting clinical studies and experimental validation of this research can help establish these phytochemicals as more potent drugs for treating different types of malignancies, including breast cancer. The discovery of these data will be essential in determining the key phytochemical for the subsequent stages of the drug-development process.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

All the authors approved the manuscript for publication.

Availability of data and material

All required data is available.

Competing interests

All authors declare no competing interests.

Funding

Not applicable.

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