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Virtual Screening of Apigenin and Luteolin as Natural Aromatase Inhibitors for the Treatment of Breast Cancer Patients

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Abstract

Aromatase is an enzyme that converts androgens (like testosterone) to estrogens (like 17- estradiol). It is also a highly successful therapeutic target for endocrine-responsive breast cancer. Aromatase inhibitors, which suppress estrogen synthesis in postmenopausal women, have been useful in the treatment of individuals with estrogen receptor-positive breast cancer. Anastrozole is an aromatase inhibitor medication that is used in the management and treatment of breast cancer. Flavonoids inhibit cancer cell proliferation by causing apoptosis, encouraging autophagy, and changing the cell cycle. Although several dietary flavonoids (like in parsley, celery and Broccoli) can inhibit aromatase, the tissue specificity and mechanism of binding are uncertain. According to several researches, flavonoids (apigenin and luteolin) dramatically suppress estrogen production. The study aims to examine binding of 3EQM (Aromatase) in the A chain with both of flavonoid (Apigenin and luteolin) and Anastrozole, using an *in silico* approach. PyRx default program was used to detect docking accuracy. Virtually, results showed that flavonoids have higher binding strength for apigenin and luteolin (which was -8.2 and -8.3) than Anastrozole (which was -7.6) with chain A of Aromatase. So, flavonoids can potentially be used as a natural medication to reduce breast cancer incidence. However, clinical trial studies are needed to investigate the role of apigenin and luteolin in the treatment of breast cancer.

1. Introduction

Estrogens are recognized to play a role in the development of breast cancer in both pre- and postmenopausal women. The majority of breast cancer patients are postmenopausal women, as the number of patients grows with age. Although estrogen is no longer produced in the ovaries after menopause, peripheral organs produce enough to stimulate tumor growth. Aromatase inhibitors are excellent targeted therapy for breast cancer because they catalyze the final and rate-limiting stage in the manufacture of estrogen [1].

Aromatase is a cytochrome P450 enzyme that is encoded by the gene CYP19A1. It is found in the placenta, ovary, testis, and other tissues and is required for the conversion of androgens to estrogens [2, 3]. Aromatase inhibitors are thus first-line therapy for oestrogen-dependent breast cancer. Aromatase transforms androstenedione, testosterone, and 16alpha-hydroxytestosterone to oestrone, 17beta-oestradiol, and 17beta, 16alpha-oestriol in three

steps, each requiring 1 mol of O2, 1 mol of NADPH, and coupling with its redox partner cytochrome P450 reductase. The first two processes are C19-methyl hydroxylation steps, while the third is Aromatase-specific aromatization of the steroid A-ring. Whereas most P450s are not very substrate selective, Aromatase is distinguished by its androgenic specificity [4, 5].

Aromatase inhibitors, which decrease estrogen synthesis in postmenopausal women, have shown beneficial in the treatment of patients with estrogen receptor positive breast cancer [6]. Aromatase inhibitors (AIs) prevent the enzyme aromatase from working. Aromatase (estrogen synthetase) is a monooxygenase that belongs to the cytochrome P450 superfamily and catalyzes the demethylation of androgen carbon 19 to produce phenolic 18-carbon estrogens [7].

The conversion of androgens to estrogens via this pathway in the adrenal glands, skin, muscle, and adipose tissue is the principal source of estrogen in postmenopausal women. Aromatase inhibitors disrupt this route, lowering estrogen levels in postmenopausal women. The breast cancer cells also exhibit aromatase activity, indicating a possible source of local estrogen for the tumor cells. Aromatase inhibition or inactivation reduces blood estrogen levels, which in turn reduces estrogen-mediated cancer cell proliferation in hormone receptor-positive breast cancer [8].

Aromatase inhibitors are drugs that aid in the reduction of estrogen levels. They are also referred to as hormone therapy or endocrine therapy. Aromatase inhibitors are a safe and efficient treatment for certain women with estrogen receptor-positive (ER+) breast cancer (the most frequent kind).

Anastrozole is a hormonal therapy. It works by reducing oestrogen hormone levels in the body. It is primarily administered to women who have gone through menopause and have hormone-dependent breast cancer [9]. Hot flashes, changed mood, joint discomfort, and nausea are common anastrozole adverse effects [10]. The risk of heart disease and osteoporosis is enhanced in severe cases.

Apigenin is a natural flavone found in many plant-based meals, including parsley and celery. Apigenin is used to treat hormone-receptor positive breast cancer patients by inhibiting aromatase, an enzyme that transforms androgens, including testosterone, into estrogen [11, 12].

Luteolin, a flavonoid found in a variety of fruits and vegetables, has been shown to be an anticancer agent by inducing apoptosis and cell cycle arrest, as well as inhibiting metastasis and angiogenesis in a variety of cancer cell lines including breast, colon, pancreatic, and lung. Luteolin has the potential to be a significant supplementary medicine for the prevention and treatment of various forms of cancer [13, 14].

The research aims to use flavonoids (Apigenin and Luteolin), which have a major role in inhibiting estrogen production, as well as due to their natural origin, safety, and low cost compared to synthetic cancer drugs and their side effects on the body.

2. Experimental Procedure

2.1. Protein Preparation and Ligands

Aromatase receptor in humans was acquired from the PDB database using the PDB ID: 3EQM (URL: https://www.rcsb.org). Furthermore, the target proteins are chosen for the docking experiment based on their X-ray diffraction. Proteins should be represented in PDB formats. To prepare the Aromatase receptor X-ray crystallographic structure for molecular docking, all heteroatoms, including (ions, water, and so on), were removed. Use the application Discovery Studio 2021 Client and the chimera tool. Some protein-binding sites in the chain are chosen while others are avoided.

2.2. Preparation of Ligands

The 3D chemical structures in SDF format were retrieved using Open Babel software and PubChem (https://pubchem.ncbi.nlm.nih.gov), and the compounds were converted to PDB format using PubChem (https://pubchem.ncbi.nlm.nih.gov). Apigenin and luteolin have been employed in all stages of docking research, including comparisons with the medication Anastrozole. These compounds were all retrieved via PubChem.

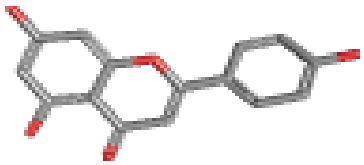
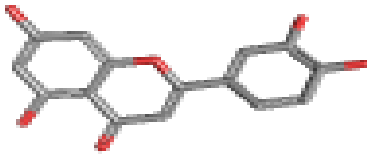
2.3. Molecular Docking

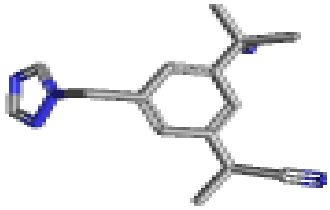
The structural interactions between the (target protein) 3EQM and the (ligand molecules) Apigenin, luteolin, and drug Anastrozole were investigated using an in silico method for analyzing ligand and receptor docking to show the conformation of this protein's target selectivity. PyRx, a virtual screening tool, was employed in this study to improve docking accuracy by using an algorithm as a score mechanism and merging Vina and Auto Dock [5, 15]. Aromatase cytochrome P450 is the sole enzyme known to catalyze the production of all oestrogens from androgens in vertebrates. The chemical composition of the macromolecule was determined using X-ray crystallography at a resolution of 3.20. The inquiry is continuing to prepare the target by eliminating the associated ligands and water molecules using the UCSF Chimera tool. Furthermore, we only keep Chain A because it only shows a chain containing a ligand-binding site. Then, recognizing that it was a macromolecule in the PyRx pipeline, we added it to the PyRx tool. Auto dock tools were utilized in this approach to ligand molecules and convert the protein to its correct readable file format (pdbqt). All docking investigations used blind docking, and the grid box was built to contain every potential ligand-receptor combination, and its dimensions were Apigenin, luteolin, and medication Anastrozole. [(X = 66.8225, Y = 72.0178, and Z = 56.7656), (X = 62.6751, Y = 72.2487, and Z = 53.8833), (X = 61.4343, Y = 74.7862, and Z = 56.8669)] respectively. All ligand binds were permitted to freely rotate, and all other software parameters were left alone while the receptor was still treated as rigid. The docked structure final portrayal was completed with Discovery Studio Visualizer 3.0. Prior to determining the efficacy of Urtica dioica compounds against 3EQM [5].

3. Results and Discussion

Aromatase is an enzyme that helps convert testosterone to estrogen. It is also a highly successful therapeutic target for endocrine-responsive breast cancer [2] . As shown in Table (1), molecular docking experiments were conducted between the Aromatase receptor, which has been a main target for Apigenin, luteolin, and Anastrozole. When energy levels are low, chemical compounds used as ligands with receptor binding affinity increase in affinity. The RMSD number, which is used to confirm docking tests, represents the average difference between the corresponding atoms of two proteins [16]. The RMSD upper bound, which ignores symmetry, matches each atom in one configuration with itself in the opposite conformation. Each atom in one conformation is compared to the closest atom of the same element type in the other conformation using the RMSD lower bound [16].

Table (1): Diagnosis of ligands using Aromatase receptor.

Docking term for ligands	Binding Affinity kcal/mol	RMSD/UB	RMSD/LB	substance name
3eqm _ 5280443_uff_E= 233.26	-8.2	0.0	0.0 	Apigenin 4′,5,7-trihydroxyflavone
3eqm _ 5280445_uff_E= 242.10	-8.3	0.0	0.0 	Luteolin 3′,4′,5,7-tetrahydroxyflavone

3eqm -2187_uff_E= 458.77	-7.6	0.0	0.0 	Anastrozole

3.1. Apigenin

Apigenin, which was obtained from PubChem. Figure (1) depicts the interaction state between Aromatase receptor and Apigenin. Table (2) depicts the interaction of the ligand with chain A of The Aromatase receptor with nineteen amino acids and the six different types of bonds formed between the ligand and receptor. Carbon hydrogen bond, Pi-alkyl, Pi-Donor Hydrogen Bond, Pi-Sulfur, and Pi-Pi T-shaped. The affinity between the two compounds was increased by the low binding energy (-8.2). The RMSD number is commonly used to validate docking experiments.

The RMSD between Apigenin's docked ligand and the experimental ligand was 0.0 angstrom. According to Table (2), the interaction of amino acids and the kind of bonds between ligands reflected the molecule Apigenin and chain A of the Aromatase receptor. The usage of the PyRx program to do virtual screening by docking in the active region of a target protein. The findings of this investigation also revealed that the linker had a very good interaction with the A chain of the Aromatase receptor, as illustrated in Figure (1).

Apigenin is a plant-derived molecule with promising anti-tumor actions, which may make it a desired adjuvant to minimize genomic instability and the risks of second malignancies in normal tissues. Furthermore, it has the potential to boost the efficacy of anticancer therapies [17].

Several studies have shown that apigenin's anticarcinogenic abilities are achieved via regulating the cellular response to oxidative stress and DNA damage, suppressing inflammation and angiogenesis, slowing cell proliferation, and inducing autophagy and apoptosis. Apigenin's capacity to trigger cell cycle arrest and apoptosis via the p53-related pathway is one of its well-known mechanisms. Apigenin also has a function in chemoprevention by inducing autophagy in various human cancer cell lines [18, 19]. This supports results obtained because it showed a strong affinity for Apigenin and this confirms the results of previous studies that Apigenin is an anticarcinogenic.

Table (2): Aromatase receptor & Apigenin with amino acids location within chain, quantity, and type of connections between bonds.

Amino acids	Location within chain	Type Bonds
MET	346	Van der waals
VAL	369	
MET	447	
MET	446	
MET	303	
SER	199	
PHE	203	
LEU	152	
GLY	439	
PRO	429	Carbon hydrogen bond
CYS	437	
SER	314	
THR	310	Pi-Donor Hydrogen Bond
MET	311	Pi-Sulfur

CYS	437	
PHE	340	Pi-Pi T-shaped
ALA	306	Pi- Alkyle
ALA	307	
ALA	443	
VAL	370	
CYS	437	

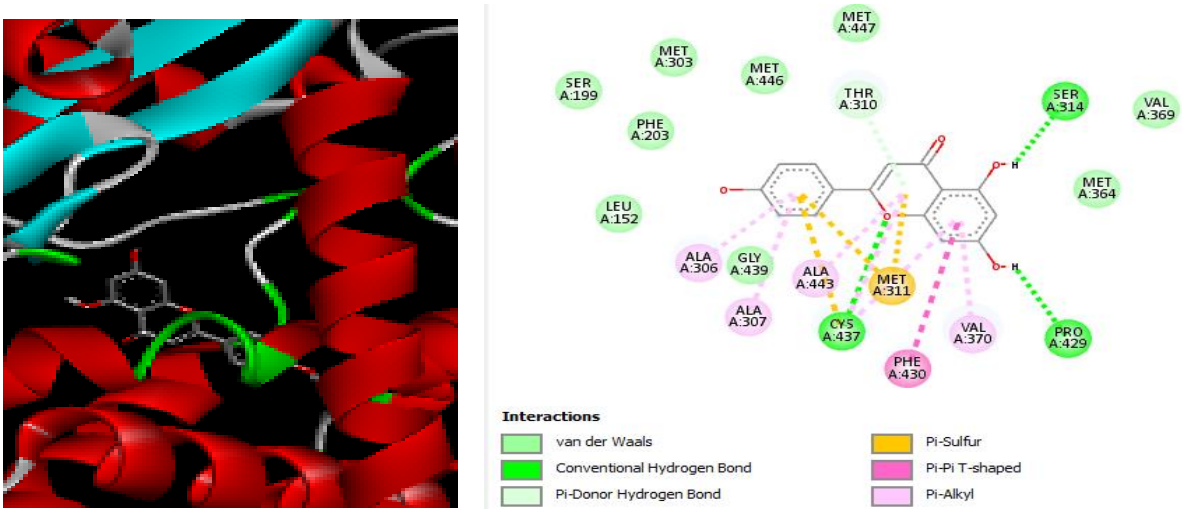


Figure (1): Relation between Aromatase receptor and Apigenin.

3.2. Luteolin

The docking analysis used luteolin, which was obtained from PubChem. Figure (2) depicts the interaction state between Aromatase receptor and luteolin. Table (3) depicts the interaction of the ligand with chain A of The Aromatase receptor with eighteen amino acids and the six different types of bonds formed between the ligand and receptor. Carbon hydrogen bond, Pi-alkyl, Pi-Donor Hydrogen Bond, Pi-Sulfur, and Pi-Pi T-shaped. The affinity between the two compounds was increased by the low binding energy (-8.3). The RMSD number is commonly used to validate docking experiments.

The docked ligand of luteolin and the experimental ligand exhibited an appropriate RMSD of 0.0 angstrom. According to Table (3), the interaction of amino acids and the kind of bonds between ligands reflected the compound luteolin and chain A of the Aromatase receptor. The usage of the PyRx program to do virtual screening by docking in the active region of a target protein. The results of this investigation also revealed that luteolin had a very good interaction with the linker with the A chain of the Aromatase receptor, as illustrated in Figure (2).

Luteolin is a flavonoid found in many plants, including fruits, vegetables, and medicinal herbs. Plants high in luteolin have been used to cure a variety of ailments in Chinese traditional medicine, including hypertension, inflammatory disorders, and cancer [20] .

Luteolin's anticancer activity has been extensively studied in a variety of cancer types, and it has been linked to its capacity to inhibit tumor growth by targeting cellular processes such as apoptosis, angiogenesis, migration, and cell cycle progression [20, 21]. This verifies the findings because it showed a strong affinity for luteolin, supporting previous findings that luteolin is anticarcinogenic.

Table (3): Aromatase receptor & Luteolin with amino acids location within chain, quantity, and type of connections between bonds.

Amino acids	Location within chain	Type Bonds
MET	346	Van der waals
ALA	438	
MET	447	
MET	446	
MET	303	
PHE	203	
LEU	152	
GLY	439	
PRO	429	Carbon hydrogen bond
CYS	437	
SER	314	
THR	310	Pi-Donor Hydrogen Bond
MET	311	Pi-Sulfur
CYS	437	
PHE	340	Pi-Pi T-shaped
ALA	306	Pi- Alkyle
ALA	307	
ALA	443	
VAL	370	
CYS	437	

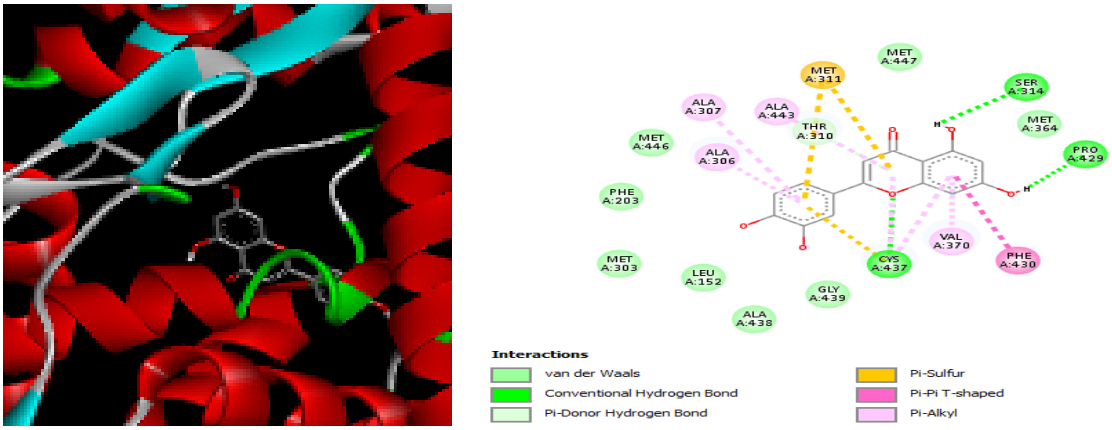


Figure (2): Relation between Aromatase receptor and luteolin.

3.3. Anastrozole

Anastrozole was used as the benchmark molecule for the docking analysis, which was obtained from PubChem. Figure (3) depicts the interaction state of the experiment's control ligand. Table (4) depicts the interaction of the ligand with chain A of The Aromatase receptor with sixteen amino acids and the six different types of bonds formed between the ligand and receptor. Carbon hydrogen bond, Pi-cation, Pi-sigma, Amide-Pi Stacked, and Pi-Alkyl The affinity between the two compounds was increased by the low binding energy (-7.6). The RMSD number is commonly used to validate docking experiments. The RMSD between Anastrozole's docked ligand and the experimental ligand was 0.0 angstrom. According to Table (4), the interaction of amino acids and the kind of bonds between ligands represented the molecule Anastrozole and chain A of the Aromatase receptor. The usage of the PyRx program to do virtual screening by docking in the active region of a target protein. The findings of

this investigation also revealed that Anastrozole had a good interaction with the linker with the A chain of the Aromatase receptor, as illustrated in Figure (3).

Anastrozole is a nonsteroidal drug that inhibits the aromatase enzyme selectively [22]. It is a chemical that reversibly inhibits the aromatase enzyme, causing estrogen levels to fall [23].Anastrozole reduces estrogen quickly, and certain side effects appear within 24 hours of starting Anastrozole. Hot flushes, nausea, vomiting, headache, and pain are some of the more severe side effects. Many of these will improve over the course of a few days or weeks.High blood pressure, high cholesterol, osteoporosis, and lymphedemas are long-term side effects [24]. The results showed that the affinity was (-7.6), indicating that the binding strength is good; however, when compared to apigenin and luteolin, their binding strength was higher; additionally, they Apigenin and luteolin Because of their natural origin, safety, and low cost in comparison to synthetic cancer drugs. More research on numerous pharmacokinetic properties, including human involvement, is required before apigenin and luteolin can become prescription medicines. The development of a standard dose may be undertaken through clinical studies.

Table (4): Aromatase receptor & Anastrozole with amino acids location within chain, quantity, and type of connections between bonds.

Amino acids	Location within chain	Type Bonds
TRP	224	Van der waals
ALA	306	
ITE	133	
PHE	134	
LEU	477	
LEU	372	
MET	374	
PRO	429	
ILE	398	
GLY	431	
THR	310	Carbon hydrogen bond
ARG	115	
ARG	115	Pi-Cation
PHE	430	Amide-Pi Stacked
VAL	373	Pi-Alkyl
CYS	437	
VAL	370	Pi-Sigma

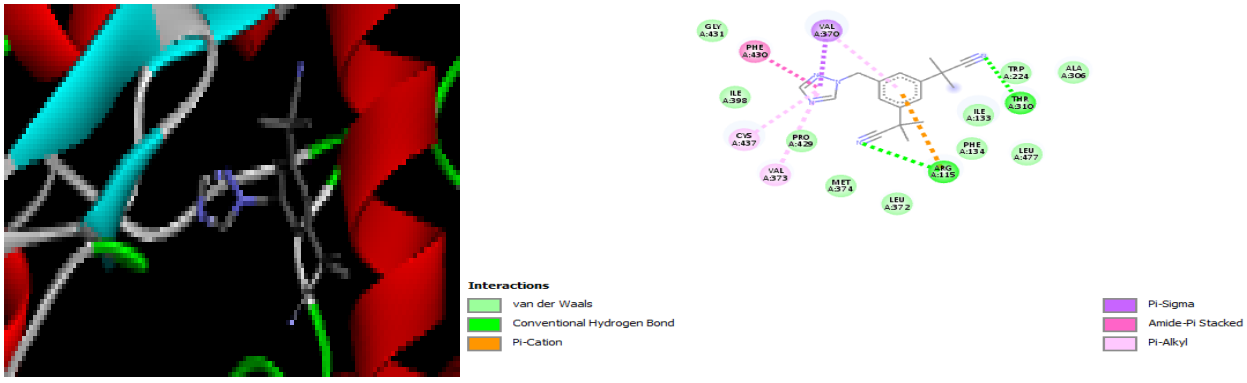


Figure (3): Relation between Aromatase receptor and Anastrozole.

4. Conclusions

Clinical trials are still ongoing to understand the role of apigenin and luteolin as preventive drugs against cancer. Cancer is the most common disease and the leading cause of death on a global scale. The current treatment strategy causes side effects such as anorexia, constipation, bleeding, diarrhea, edema, lethargy, and hair loss, in addition to killing good cells. Apigenin and luteolin serve as dynamic drug supply due to their low side effects, and they also appear to play a role in reducing cancer treatment resistance. The antioxidant properties of apigenin and luteolin suggest that they play an important role in reducing the generation of free radicals, controlling oxidative stress and inflammation, and finally, managing the genesis and progression of cancer. This flavonoid appears to have anticancer properties through altering several cellular signaling pathways such as angiogenesis, apoptosis, cell cycle, and several other carcinogenic pathways. Clinical trial studies are needed to investigate the role of apigenin and luteolin in cancer care and to explain potential mechanisms of action in this field.

Conflict of Interest: The authors declare that there are no conflicts of interest associated with this research project. We have no financial or personal relationships that could potentially bias our work or influence the interpretation of the results.

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