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In Silico Investigation of Novel Phytoactive Compounds as ER-α Inhibitors

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ABSTRACT

Breast cancer is one of the most common types of cancer worldwide, and it's a leading cause of cancer-related deaths, especially in women. It's classified into several subtypes, with hormone-dependent breast cancer being particularly tough to treat. ERα is a key player in the growth and survival of breast cancer cells, making it a prime target for treatment. Traditional therapies include selective estrogen receptor modulators (SERMs) and aromatase inhibitors, but these often lead to resistance and unwanted side effects. As a result, researchers are looking for natural, plant-based inhibitors as a promising alternative. This study explores the potential of plant-derived photoactive compounds, including Butein, Leonurine, Nobiletin, Genistein, and Luteolin, as ERα inhibitors through computational approaches. Molecular docking studies were performed using AutoDock Vina to predict binding affinities between ERα (3ERT) and selected bioactive compounds. Active sites of the ERα were determined by the CASTp analysis to obtain deep insights into ligand-protein interactions. To visualize the interaction of ligand-protein complexes Discovery Studio Visualizer was used. This docking studies showed strong binding interactions between ERα and the selected ligands. Butein and Genistein had the highest binding affinity, with -8.7 kcal/mol and -7.8 kcal/mol, respectively, with ERα. CASTp analysis confirmed that these ligands occupy key binding pockets of ERα, suggesting they may have an inhibitory role. Additionally, ADMET predictions indicated favorable pharmacokinetic properties, supporting their potential as drugs. The computational findings point to the potential of plant-derived photoactive compounds as ERα inhibitors, setting the stage for future in vitro and in vivo validation. These compounds show promising therapeutic potential against hormone-dependent breast cancer, making them worth exploring further for clinical applications.

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AB conducted the methodology and drafted the manuscript. AJ and FM contributed to figure preparation and manuscript revisions. EZ proposed the study, supervised the research work, and provided critical review. MK contributed to manuscript review and guidance.

Key words
Estrogen receptor α, Nuclear receptor, Breast cancer, Molecular docking, Genistein, Butein, Luteolin

INTRODUCTION

Breast cancer remains one of the most prevalent and significant health challenges worldwide, with an alarming rise in both incidence and mortality rates. As of 2020, there were an estimated 2.26 million new cases of breast cancer globally, with the disease becoming the leading cause of cancer-related deaths in women (Sung *et al*., 2020). This surge is strongly linked to human development, with regions undergoing economic transition experiencing a notable rise in cases. Breast cancer remains a leading cause of cancer-related mortality among women worldwide, with significantly poorer survival outcomes observed in low- and middle-income countries. These disparities are largely attributed to delayed diagnoses and limited access to effective treatments. Addressing this global challenge, the World Health Organization introduced the Global Breast Cancer Initiative (2022), aiming to improve survival through public health education, early detection, and integrated care strategies (Wilkinson *et al*., 2022).

Importantly, breast cancer is not a uniform disease; rather, it encompasses a spectrum of biologically distinct subtypes that differ in clinical presentation, molecular features, and genetic profiles (Sung *et al*., 2020). These subtypes heavily influence the selection of therapeutic interventions, which may include surgical procedures, radiation, chemotherapy, hormone therapy, or targeted biologics. For example, HER2-positive breast cancers are typically treated with chemotherapy and anti-HER2 agents, while hormone receptor-positive (ER+) cancers benefit from endocrine therapies. Despite these advances, the rising incidence particularly in younger women underscores the need for more precise diagnostic tools and innovative therapeutic strategies (Siegel *et al*., 2022). Estrogen is known to play a key role in the pathophysiology

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of hormone-responsive breast tumors, with estrogen receptor alpha (ERα) serving as a crucial driver of tumor proliferation by modulating gene expression linked to cell growth (Klinge, 2001). This makes ERα a prime target for therapeutic intervention in breast cancer treatment. This makes ERα a central target in current breast cancer treatments. Nonetheless, resistance to existing therapies such as selective estrogen receptor modulators (SERMs) and aromatase inhibitors remained a significant hurdle, often leading to treatment failure or recurrence (Russo, 2007).

In recent years, plant-derived bioactive compounds have garnered significant interest as potential inhibitors of estrogen receptor alpha (ERα) in breast cancer therapy. Several phytochemicals, including those derived from marine sources such as brown algae, exhibit promising anticancer effects, particularly by modulating estrogen receptor signaling pathways (Holdt and Kraan, 2011). Among these, photoactive compounds which become biologically active upon exposure to light are being explored for their ability to selectively disrupt estrogen signaling with enhanced specificity toward cancer cells (Nooreen *et al*., 2024). The investigation of such phytoactive compounds represents a novel and promising strategy in the treatment of ER-positive (ER+) breast cancer. By employing approaches such as molecular docking**,** virtual screening**,** and advanced drug delivery systems, researchers aim to identify new therapeutic candidates that can overcome limitations of current endocrine therapies. This study focuses on evaluating the inhibitory potential of selected natural compounds on Butein, Lenurine, Genistein, Nobiletin, and Luteolin against ERα through *in silico* docking techniques**,** thereby contributing to the development of more targeted and personalized breast cancer therapies.

MATERIALS AND METHODS

Protein preparation

The estrogen alpha receptor was dowloaded with the PDB ID 3ERT. AutoDock MGL tools were used to prepare the protein for docking studies. The protein preparation was performed using the standard protocol as discussed in our previous study (Hassan *et al*., 2024).

Molecular visualization of ERα complex

The Docked ERα complexes are visualized using Pymol, Biovia Discovery Studio Visualizer (DSV), and MGL tools. The 2D and 3D visualization of the structures were generated. The steps for visualization are as per the standard protocol given in our previous studies (Muhammad *et al*., 2021; Hassan *et al*., 2024).

Docking analysis

AutoDock vina was used for molecular docking analysis by keeping the receptor as a rigid molecule and ligands as flexible molecule for rotatable bonds. Grid box with the size of 40 (x, y, z) was used, the center of the grid box was set at coordinates x = 22.396, y = 5.644, and z = 21.988. After docking the nine poses for each ligand was generated. The ligand with the lowest binding energies was selected and were further subjected to visualization.

Active site prediction

The CASTp server was used to predict the effective sites of the studied protein. This software calculates the volume and surface area of the binding domains and its potential catalytic sites (Tian *et al*., 2018).

Physiochemical properties

The Lipinski rule of five and the ADMET analysis and drug toxicity was performed using the online available tools pkCSM and SwissADME. The specific canonical smiles for each ligand was retrieved from Pubchem and then was submitted to the ADMET tools used and the ADMET evaluation and toxicity results were obtained.

RESULTS

Molecular docking

In this study, molecular docking was performed to evaluate the interaction of plant-derived photoactive compounds with the ERα. The primary goal was to identify potential inhibitors that can modulate ERα activity, which is relevant to various hormone-dependent diseases, including breast cancer. We utilized offline tools that are PyMOL, Auto Dock Vina, and DSV to conduct and visualize the docking analysis. The docking results gave the binding affinities and interactions of docked compounds within the receptor's active site. The protein structure 3ERT was downloaded from the Protein Data Bank (PDB) in pdb format. Using Auto Dock Tools, the protein was prepared by removing water molecules, adding Kolman charges and only polar hydrogens, and converting it into .pdbqt format (Fig. 1). The ligand was downloaded from PubChem in SDF format. It was then converted to .pdb format using PyMOL and further processed in Auto Dock Tools and converted into .pdbqt format for docking studies. The resulting PDBQT files were then used as input for docking analysis in Auto Dock Vina 1.5.6.

Protein active sites prediction

Using the online available tool CASTp was used to predict the binding pockets of the respective protein 3ERT. A total of 33 binding sites were predicted with the effective

interactive amino acids including GLY521, GLY420, His 420, GLU353, LEU349, ILE326, ARG394, PRO324, MET522, LEU536, GLY 390, LYS 449 etc. Most of studied ligands are docked to the binding pocket 1 while some at binding pocket 3 as shown in Figure 2.

Fig. 1. 3ERT protein in PDBQT format.

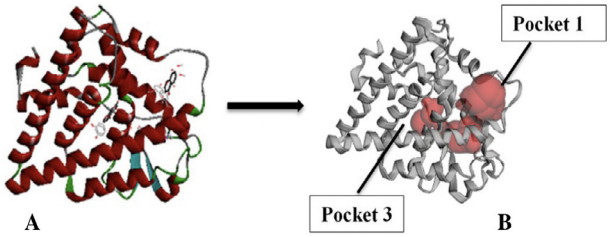

Fig. 2. (A) Image obtained via DSV indicates that all the studies are docked in binding pocket 1 and 3. (B) CASTp predicted a total of 33 binding pockets of the protein (3ERT) of which the two binding pockets (1 and 3) are shown highlighted in red color.

Molecular docking analysis

Out of the screened plant extracted compounds the selected compounds were subject to molecular docking analysis and visualization based on lowest binding energies that are Leonurine (Ligand 1), Butein (Ligand 2), Genistein (Ligand 3), Nobiletin (Ligand 4), Luteolin (Ligand 5) Table I. Ligand 1 and 2 exhibit the lowest binding energies and inhibition constant -6.2 kcal/mol, -7.8kcal/mol, and 2.731uM and 1.813uM, respectively. Ligand 1 showed hydrogen bonds with GLY521 and GLY420 amino acid residues while LEU525 and ALA350 showed hydrophobic interactions. However, Ligand 2 showed hydrophobic interactions with LEU387, LEU346, LEU525 and LEU349 (Fig. 3).

Ligand 4 and 5 showed the lowest binding energies and inhibition constant -6.6kcal/mol, -7.9kcal/mol, and 1.386uM and 1.531uM, respectively. Ligand 4 showed pi-sulfur bond interaction with MET522 amino acid residues and LEU536 with pi-alkyl interactions. However, ligand 5 showed hydrogen bond with GLY390 while electrostatic interactions was observed with ARG394, LYS449 AND GLU353 amino acid residues. Ligand 3 showed the highest binding affinity with lowest binding energy -8.7kcal/mol and inhibition constant 3.94uM. The docked complex exhibit hydrogen bond with GLY390 amino acid residue while ARG394, LYS449 and GLU353 showed electrostatic interactions (pi-cation and pi-ananion). However, PRO324 showed hydrophobic interactions (Fig. 4).

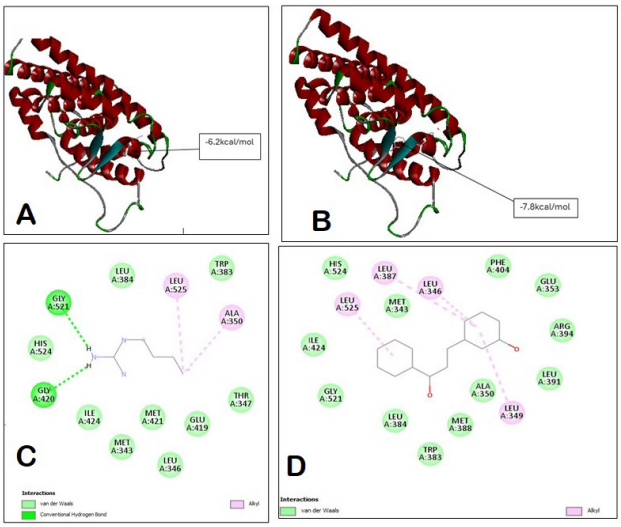

Fig. 3. Figure illustrating docked complexes. Ligand 1 docked complex and its visualization (A and C). Ligand 2 docked complex and its visualization (B and D).

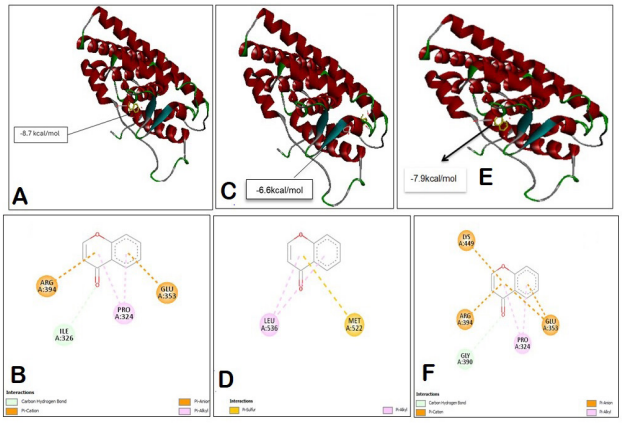

Fig. 4. Figure illustrating docked complexes. Ligand 3 docked complex and its visualization (A and B). Ligand 4 docked complex and its visualization (B and D). Ligand 5 docked complex and its visualization (E and F).

Physiochemical properties

Lipinski rule of five is the estimation of the compound's physiochemichal properties. Each ligand was subjected to SwissADME for Lipinski's rule of five.

Table I. Phytoactive compounds along with binding energies, amino acid residues and interaction types.

Ligand	CID	Protein	Binding energies (kcal/mol)	Amino acid residue	Interaction	Inhibition constant (uM)
Ligand 1	161464	3ERT	-6.2	GLY521 GLY420 LEU525 ALA350	Hydrogen Hydrogen Hydrophobic Hydrophobic	2.731
Ligand 2	5281222		-7.8	LEU387 LEU346 LEU525 LEU349	 Hydrophobic	1.813
Ligand 3	5280961		-8.7	ILE326 ARG394 GLU 353 PRO324	Hydrogen Electrostatic Electrostatic Hydrophobic	3.94
Ligand 4	72344		-6.6	MET522 LEU536	Pi-sulfur Hydrophobic	1.386
Ligand 5	5280445		-7.9	GLY390 ARG394 LYS449 GLU353 PRO324	Hydrogen Electrostatic Electrostatic Electrostatic Hydrophobic	1.531

All compounds meet the criteria, including molecular weight under 500 g/mol, fewer than 10 hydrogen bond acceptors, fewer than 5 hydrogen bond donors, and a LogP below 5, indicating good oral bioavailability. Leonurine shows favorable water solubility with a LogP of 0.62 and a polar surface area (PSA) of 129.39 Å². Butein and Genistein also fall within optimal ranges with moderate lipophilicity and PSA values supportive of membrane permeability. Nobiletin, despite having zero hydrogen bond donors, meets all the rules with a LogP of 3.51. Luteolin exhibits strong compliance, suggesting that all five compounds are suitable for further drug development studies targeting estrogen receptors (Table II).

Table II. Lipinski rule of five analysis of selected ligands.

Ligands	Moleular weight <500(g/mol)	H-Bond acceptor <10	H-bond donor <5	LogP <5	Polar surface area (Å²)
Ligand 1	311.33	6	3	0.62	129.39
Ligand 2	272.25	5	4	2.39	97.99
Ligand 3	270.24	5	3	2.58	90.90
Ligand 4	402.39	8	0	3.51	85.59
Ligand 5	286.24	6	4	2.28	111.13

Drug likeness and toxicity

The ADMET analysis of selected ligands; Leonurine, Butein, Genistein, Nobiletin, and Luteolin was conducted using the pkCSM online database, evaluating their absorption, distribution, metabolism, excretion, and toxicity profiles. All five compounds showed good intestinal absorption, with Genistein having the highest (87.13%) and Leonurine the lowest (70.33%). None of the ligands were identified as substrates or inhibitors of major cytochrome P450 enzymes (CYP2D6 and CYP3A4), indicating a low potential for drug–drug interactions. Water solubility values ranged from –2.317 to –3.251 LogS, suggesting moderate solubility, with Leonurine being the most soluble. Brain penetration, indicated by BBB permeability, was low in all compounds except Nobiletin, which showed a positive value (0.695), suggesting potential CNS activity. All ligands exhibited non-hepatotoxic and non-AMES toxic properties, except Nobiletin, which tested positive for AMES toxicity. Their total clearance values and non-inhibitory profiles for P-glycoprotein suggest good excretion characteristics and limited risk of efflux. Collectively, these findings support the drug-likeness and pharmacokinetic suitability of the selected ligands for further investigation in therapeutic development (Table III).

Table III. Drug likeness using pkCSM and SwissADME and ADMETlab 2.0 online databases server for the selected ligands.

ADMET		Ligand 1	Ligand 2	Ligand 3	Ligand 4	Ligand 5
Absorption	Water solubility (LogS) ml/L	-2.317	-3.177	-2.892	-2.892	-3.251
	Intestinal absorptipn (human) (% Absorption)	70.339	72.567	87.135	81.13	81.13
	P-Glycoprptein substrate	Yes	Yes	No	No	Yes
	P-Glycoprptein I inhibitors	No	No	No	No	No
	P-Glycoprptein II inhibitors	No	No	No	No	No
Distribution	VDss (human) (logL/kg)	0.442	0.741	0.011	0.011	1.153
	BBB permeability	-1.217	-0.895	-1.086	0.695	-0.907
	CNS permeability	-3.398	-2.395	-2.994	-1.317	-2.251
Metabolism	CYP2D6 substrate	No	No	No	No	No
	CYP3A4 substrate	No	No	No	No	No
	CYP2D6 inhibitor	No	No	No	No	No
	CYP 3A4 inhibitor	No	No	No	No	No
Excretion	Total clearance (Log ml/min / kg)	0.716	0.015	25.296	0.354	0.495
	Renal OCT 2 substrate	No	No	No	No	No
Toxicity	AMES toxicity categorical (Yes No/)	No	No	Yes	Yes	No
	Max. tolerable dose (log mg/kg/day)	0.486	0.117	0.438	0.438	0.499
	Hepatoxicity	No	No	No	No	No

DISCUSSION

Estrogen receptor alpha (ERα) plays a central role in the pathogenesis of hormone-dependent breast cancer and remains a prime target in endocrine therapy. However, resistance to existing agents like tamoxifen and aromatase inhibitors continues to limit their long-term efficacy (Manna and Holz, 2016). In this study, we employed molecular docking and ADMET prediction to evaluate five plant-derived photoactive compounds Leonurine, Butein, Genistein, Nobiletin, and Luteolin for their inhibitory interaction with ERα.

Among the tested ligands, Genistein exhibited the strongest binding affinity (-8.7 kcal/mol), followed by Luteolin and Butein, which also showed favorable docking scores and interaction profiles with key residues in the ERα binding pocket particularly GLU353, ARG394, and PRO324. These residues are critical for ligand binding and receptor activation. The CASTp analysis confirmed that these compounds occupied well-defined binding pockets, indicating potential allosteric or competitive inhibition (Tian *et al*., 2018).

Genistein, a soy isoflavone, has long been studied for its dual role as a phytoestrogen and anticancer agent. Recent work confirms that it not only binds ERα but also sensitizes cancer cells to hormonal therapies by modulating apoptotic and proliferative pathways (Mai *et al*., 2007). Similarly, Butein has been shown to interfere with Akt/mTOR signaling, reduce cell migration, and downregulate inflammatory cytokines, thereby enhancing its appeal as a multi-targeted anticancer compound (Golmei *et al*., 2024). Luteolin, widely distributed in many medicinal plants, inhibits breast cancer cell proliferation by modulating NF-κB and MAPK signaling cascades (Cook, 2018).

Our ADMET analysis supports the pharmacokinetic suitability of all five compounds. They exhibited high intestinal absorption, low hepatotoxicity, and no significant interaction with CYP450 enzymes essential features for oral bioavailability and low-risk drug interactions. Notably, Leonurine displayed the most favorable solubility and clearance profile, whereas Nobiletin, despite moderate docking performance, showed AMES toxicity, suggesting a need for further optimization or modification before therapeutic development. These findings are consistent with broader computational research efforts that explore dietary polyphenols and flavonoids as selective estrogen receptor modulators (SERMs). In a recent systematic review, flavonoids were found to engage ERα and HER2 targets with comparable or superior binding to synthetic ligands (Zand *et al*., 2002).

The potential photoactivity of some of these compounds opens up new possibilities for photoactivated

therapies that offer temporal and spatial control over drug action, especially relevant for localized tumor sites. This aligns with emerging interest in photodynamic estrogen receptor modulation, which could complement or replace conventional endocrine therapies with fewer side effects (Muniyandi *et al*., 2020).

Our study provides compelling computational evidence supporting the role of plant-derived compounds as ERα inhibitors. With favorable docking scores, strong interactions at biologically relevant binding sites, and drug-like ADMET profiles, the selected compounds especially Genistein, Luteolin, and Butein represent promising candidates for further *in-vitro*, *in-vivo*, and clinical exploration. These findings strengthen the case for integrating ethnopharmacology and molecular modeling in next-generation endocrine therapy development, particularly for resistant or relapsing breast cancer cases.

CONCLUSION

This study underscores the structural diversity of plant-derived compounds and their ability to bind effectively to estrogen receptor alpha (ERα), thereby contributing to their varied therapeutic potentials. Among the screened compounds, Genistein demonstrated the highest binding affinity, positioning it as the most promising candidate for ERα inhibition, followed by Luteolin and Butein. These findings support the potential of natural phytoactive compounds as safer and less toxic alternatives or adjuncts to conventional breast cancer therapies. The identification of these ERα-targeting compounds reinforces the growing interest in plant-based drug discovery, especially for the development of targeted therapies against hormone-dependent breast cancers.

DECLARATIONS

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Generative AI or AI-assisted technology statement

No generative AI or AI-assisted technologies were used in the preparation of this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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