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Prediction of Secondary Metabolite Compounds in Lotus (*Nelumbo nucifera* Gaertn.) Targeting Androgen-α Receptors for Breast Cancer Treatment Using Molecular Docking

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Abstract

Breast cancer (BC) is a malignant tumor that grows in the breast tissue and can spread to other organs. BC is often found at an advanced stage and therefore has a poor prognosis. With 68,858 cases, BC is the most common type of cancer in Indonesia. The development of breast carcinoma is strongly influenced by steroid hormones and their receptors, such as estrogen, progesterone, and androgen. Androgen receptors (AR) are found more (70-90%) compared to estrogen receptors (60-80%) and progesterone receptors (50-70%). Therefore, research on natural compounds that inhibit cancer cell proliferation influenced by AR needs to be increased. The lotus plant (*Nelumbo nucifera* Gaertn.) has been used as a traditional herbal medicine and food in Asia. Bioactive compounds in lotus have therapeutic potential against BC. In the pharmacophore screening results, five hit compounds were found: isorhamnetin, luteolin, catechin, kaempferol, and apigenin. The compound with the best pharmacophore fit score value is isorhamnetin with a value of 41.31, while the compound with the best binding affinity to AR is kaempferol with a binding affinity of -9.44 and an inhibitor constant of 121.12 nM.

Keywords: *breast-cancer, androgen-a receptor, lotus, molecular docking.*

INTRODUCTION

Breast cancer (BC) is one of the most common cancers globally and the leading cause of cancer deaths among women. In Indonesia, about 66,271 new cases and 22,598 deaths are expected in 2022 (WHO, 2022). BC treatment is challenging due to drug resistance and side effects from therapies like chemotherapy. Alternative targets, like the androgen receptor (AR), are being studied for their a roles in tumor growth, especially in subtypes such as Triple Negative Breast Cancer (TNBC). AR is present in 70–90% of BC cases and is often found with the Estrogen Receptor (ERα). Targeting AR could be a promising treatment, especially in cancers where other receptors are absent. This study uses computational techniques like molecular docking to explore lotus-derived compounds as potential AR inhibitors (Anestis, *et al*., 2020).

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Lotus (*Nelumbo nucifera* Gaertn.), known for its beauty, has been used in traditional medicine to treat inflammation, infections, and heart conditions. It contains various bioactive compounds, including alkaloids, flavonoids, and terpenoids, which are derived from various parts of the plant such as its seeds, flowers, leaves, and rhizomes (Bishayee, *et al*., 2022). Notably, compounds like liensinine and nuciferine from lotus have shown potential in inhibiting BC cell growth (Adrian, *et al*., 2024). This study investigates how lotus compounds interact with AR using *in silico* methods, without experimental validation. *In silico* methods, which use computer simulations to predict how compounds interact with receptors, are efficient for screening potential treatments (Elfita, *et al*., 2022; Kesuma, *et al*., 2018). This research aims to identify promising candidates for BC treatment by targeting AR, offering a cost-effective approach for future preclinical and clinical studies.

MATERIALS AND METHODS

Materials and Tools

The study utilized an ASUS VivoBook 15 laptop equipped with an 11th Gen Intel® Core™ i3-1115G4 processor, 4GB DDR4 RAM (expandable to 16GB), and operated on a 64-bit system. The software and databases used include the RCSB Protein Data Bank for receptor structures, PubChem for compound structures, ChemDraw Ultra 12.0 for drawing, Chem3D Pro 12.0 for energy minimization, DUDE for compounds and decoys, PreADMET for ADME/toxicity, BIOVIA Discovery Studio 2020 for preparation/visualization, and AutoDock Tools-1.5.6 for docking. Materials included PDB ID 2AM9 protein, androgen receptor, native ligands, and test compounds, which were crucial for computational analyses and simulations. The compounds used in this study were obtained from Lotus, specifically from its leaves, seeds, and flowers (Bishayee, *et al*., 2022).

Procedure

Lipinski's Rule of Five Prediction

Lipinski's rule of five was employed to predict physicochemical properties and assess the oral administration potential of active substances. The active compounds of Lotus (*Nelumbo nucifera* Gaertn.) were identified through a comprehensive literature review to gather compounds reported in previous studies. Relevant sources, including pharmacological studies and ethnobotanical surveys, were consulted to compile a list of bioactive constituents. The 2D structures of these compounds were drawn using ChemDraw and saved as SMILES. Physicochemical properties such as molecular weight, log *P*, hydrogen bond donors, and acceptors were predicted using the SwissADME software (Daina, *et al*., 2017).

Prediction of ADMET

ADMET parameters, including Human Intestinal Absorption (%HIA), Caco-2 permeability, Plasma Protein Binding (PPB), Blood-Brain Barrier (BBB) penetration, and toxicity (Ames Test and Rodent Carcinogenicity), were analyzed using the Pre-ADMET web service. Drug-likeness, ADME, and toxicity predictions were carried out by submitting the chemical structure from PubChem and saving the results in the appropriate format (Dulsat, *et al*., 2023).

Pharmacophore Screening

Active and decoy databases were downloaded at DUD-E (Database of Useful Decoys: Enhanced), prepared using LigandScout software, and saved in .ldb format. Ten pharmacophore models were created and validated to select the best one based on the ROC curve. The final model was then used to screen for compound hits (Wolber & Langer, 2005).

Molecular Docking

AR (PDB ID: 2AM9) was obtained from the Protein Data Bank (PDB) and prepared by

removing water molecules and native ligands using Biovia Discovery Studio. This preparation optimized interactions between the receptor and test compounds, which is essential for accurate docking and assessing therapeutic potential in breast cancer (Xiao, *et al*., 2018). The native ligand was isolated and refined by adding hydrogen atoms and Kollman charges. The ligand's structure was then optimized in 3D, with added torsion parameters and Gasteiger charges. Molecular docking was performed using AutoDockTools software, and the best binding poses were identified based on Gibbs free energy values, with lower values indicating more stable conformations (Shivanika, *et al.*, 2020).

RESULTS

Prediction of Lipinski Rule of Five (RO5)

Lipinski's rule of five states that a compound is considered to have drug-like properties if it meets the following criteria, including having a molecular weight (BM) of less than 500 Daltons, the number of hydrogen bond donors (HBD) is no more than 5, the log *P* partition coefficient value is no more than 5, and the number of hydrogen bond acceptors (HBA) is less than 10 (Lipinski, 2000). Test compounds that meet the requirements are predicted to be administered orally based on the results of the analysis of their physicochemical properties, as summarized in Table 1.

Table 1. Lipinski rule of five (RO5) prediction results.

No	Name of Compound	Molecular Weight (<500 Da)	Log*P* (<5)	Hydrogen Bond		Druglikeness
				Donor (<5)	Acceptor (<10)	
1	Neferine	624.77	5.47	1	8	Not Suitable
2	Gallic acid	170.12	0.50	4	5	Suitable
3	Liensinine	610.74	5.17	2	8	Not Suitable
4	Nuciferine	295.38	3.27	0	3	Suitable
5	Isorhamnetin	316.26	1.65	4	7	Suitable
6	Kaempferol	286.24	1.58	4	6	Suitable
7	Luteolin	286.24	1.73	4	6	Suitable
8	Syringetin	346.29	2.30	4	8	Suitable
9	Roemerine	279.33	3.16	0	3	Suitable
10	Myricetin	318.24	0.79	6	8	Suitable
11	Isoquercitrin	464.38	0.48	8	12	Not Suitable
12	Catechin	290.27	0.85	5	6	Suitable
13	Miquelianin	478.36	-0.55	8	13	Not Suitable
14	Rutin	610.52	-1.51	10	16	Not Suitable
15	Hyperoside	464.38	-0.38	8	12	Not Suitable
16	Apigenin	270.24	2.11	3	5	Suitable

Prediction of ADMET

The ADMET prediction evaluates the Absorption, Distribution, Metabolism, Excretion, and Toxicity of a compound to determine its drug-likeness. This method uses prediction scores to identify the most promising compounds for development (Guan, *et al*., 2018). Absorption refers to how a drug moves from its absorption site into circulation (Aslam, *et al*., 2003). Human Intestinal Absorption (HIA) data measure bioavailability, with compounds classified based on their absorption: poorly absorbed (0-20%), moderately absorbed (20-70%), and well absorbed (70-100%). Caco-2 permeability is another measure, with values categorized as low (<4 nm/sec), medium (4–70 nm/sec), or high (>70 nm/sec) (Amin, *et al*., 2021).

Drug distribution occurs after absorption when the drug enters systemic circulation (Aslam,

Table 2. ADME/Tox prediction results.

No	Compounds	Absorption		Distribution		Toxicity	
		HIA (%)	Caco-2 (nm/sec)	PPB (%)	BBB	Mutagen	Carcinogen
1	Gallic acid	53.69685	13.8492	65.384676	0.348084	+	M (-) / R (+)
2	Nuciferine	100.0000	57.5747	74.454071	1.65054	+	M (-) / R (-)
3	Myricetin	40.96404	0.991395	96.784810	0.110308	+	M (-) / R (+)
4	Isorhamnetin	78.34766	4.93924	83.545382	0.0580929	+	M (-) / R (+)
5	Kaempferol	79.43928	9.57744	89.608221	0.286076	+	M (-) / R (+)
6	Luteolin	79.42723	4.53973	99.717233	0.367582	+	M (-) / R (+)
7	Syringetin	18.30597	8.71698	43.221602	0.0308716	-	M (-) / R (-)
8	Roemerine	100.0000	56.7725	74.322985	1.80495	+	M (-) / R (-)
9	Catechin	66.70795	0.656962	100.00000	0.394913	+	M (-) / R (-)
10	Apigenin	88.122839	10.5468	97.253409	0.565113	+	M (-) / R (+)

M=Mouse; R=Rat

et al., 2003). Distribution is evaluated by PPB, with weaker binding (<90%) preferred for better target site delivery (Suherman, *et al*., 2020). The BBB prediction assesses brain-to-blood concentration, with high (>2.0), moderate (2.0~0.1), and low (<0.1) levels indicating the drug's ability to cross into the CNS (Soekardjo, 2008; Yang, *et al*., 2011). Toxicity predictions use tests like the Ames test to detect mutagenicity and carcinogenicity, essential for evaluating the safety of new drugs. For a detailed summary of the ADME/Tox prediction results, refer to Table 2.

ADMET tests on ten compounds (Table 2) revealed some key findings. Nuciferine, isorhamnetin, kaempferol, luteolin, roemerine, and apigenin showed good absorption based on HIA but did not have high permeability according to Caco-2 cell tests. In terms of distribution, myricetin, luteolin, catechin, and apigenin are strongly bound to plasma proteins, while others are not. None of the compounds crossed the BBB, suggesting a low risk of long-term brain effects. Syringetin was the only compound predicted to be non-mutagenic and non-carcinogenic in mice and rats (Been, *et al*., 2024).

Pharmacophore Modeling

Pharmacophore virtual screening helps identify active compounds by distinguishing them from decoys. In this study, active compounds were chosen based on their known biological activity, and decoys were selected from the DUD-E resource to ensure structural similarity but no reported activity against the target. The dataset consisted of 100 active compounds and 399 decoys to maintain a balanced screening process and validate the pharmacophore model's effectiveness. The screening process included lotus compounds post-ADMET and RO5 evaluations, alongside the active and decoy compounds. As shown in Figure 1, the pharmacophore model showed high sensitivity with an AUC-ROC value above 0.5 and a GH value above 0.7, indicating its ability to effectively distinguish active compounds from decoys (Suherman, *et al*., 2020; Meyer, *et al*., 1982).

The figure shows the pharmacophore model with aligned structures of isorhamnetin, luteolin, (-)-catechin, kaempferol, and apigenin. Colored spheres represent key pharmacophoric features: hydrophobic (yellow), hydrogen bond

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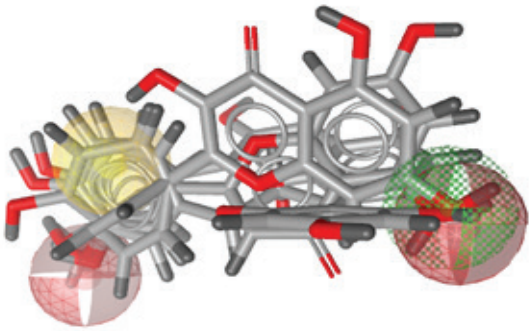

	Mark	Name	#	Matching Features	Pharmacophore-Fi...
1	☐	Isorhamnetin	2		41.31
2	☐	Luteolin	4		41.01
3	☐	(-)-Catechin	5		40.79
4	☐	Kaempferol	1		40.15
5	☐	Apigenin	3		40.11

Figure 1. Pharmacophore model and compound analysis.

donor (green), and hydrogen bond acceptor (red). The table summarizes matching features and pharmacophore-fit scores, with isorhamnetin achieving the highest fit score (41.31). These findings support their potential as androgen receptor inhibitors.

The figure illustrates the ROC curve of the 5th pharmacophore model, evaluating its ability to differentiate actives from decoys. From a dataset of 499 compounds (100 actives, 399 decoys), the model achieved an AUC of 1.00, identifying 148 hits with high sensitivity and specificity. This result confirms the model's reliability for virtual screening.

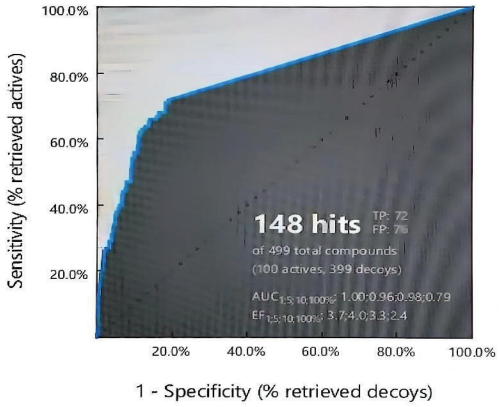

Figure 2. ROC curve of the 5th pharmacophore model.

The pharmacophore screening identified five potent compounds—isorhamnetin, luteolin, catechin, kaempferol, and apigenin—based on their interactions with the target site. A pharmacophore fitting approach is taken before molecular docking to ensure the matching of important chemical features such as hydrogen donors/acceptors, hydrophobic interactions, and aromatic rings required for interaction with the target. Molecular docking involves detailed simulation of interactions at the active site of the protein, including ligand and protein flexibility, resulting in more computational time. Isorhamnetin achieved the highest Pharmacophore Fit Score of 41.31, indicating optimal alignment with the model and superior interactions at the target site (Figure 2), demonstrating its predictive capability

and confirming the reliability of the model for identifying key compounds such as isorhamnetin. This positions isorhamnetin as a top candidate for further drug discovery compared to luteolin, catechin, kaempferol, and apigenin, highlighting its potential efficacy and therapeutic significance. While other compounds also showed promise, their slightly lower fit scores suggest varying interaction strengths. These results underscore isorhamnetin's potential and advocate for its exploration in future research efforts.

Molecular Docking

Protein and Ligand Structure Preparation and Optimization

Five secondary metabolite compounds—isorhamnetin, luteolin, catechin, kaempferol, and apigenin—were selected based on their prediction via RO5, ADMET properties, and pharmacophore screening for docking with the AR active site. The focus on AR as a target stems from its varied expression across BC subtypes and its interaction with key ligands like testosterone and 5α-dihydrotestosterone, which are more abundant in men than women. Other androgens, such as androstenedione and dehydroepiandrosterone, also bind to AR but with lower potency (Kolyvas, *et al*., 2022). This study used molecular docking of these compounds to explore their potential binding interactions with AR for breast cancer treatment.

Preparation and Optimization of Native Ligand Structure

Docking of the lotus-derived compounds resulted in varying binding affinities with the AR. The native ligand (testosterone) exhibited a binding energy of -11.99 kcal/mol, with an root mean square deviation (RMSD) of 0.78, confirming the stability and reliability of the docking method.

Grid Box Determination

Determination of the size of the grid box on the ligand needs to be done before docking the ligand to the androgen-α receptor. The docking parameters were set using the "Genetic Algorithm", the number of GA runs was made as many as 100 times the ligand and receptor interaction and the distance obtained was 0.375 Å. The size of the grid box used in AR was specified by the dimensions in x, y, and z coordinates with values 26.907, 2.557, and 5.181, respectively. These values represent the spatial boundaries of the docking region, ensuring that the receptor's active site is properly targeted for ligand interactions. The grid box size is shown in Table 3.

Table 3. Androgen receptor (AR) grid box size data.

Protein	PDB Code	Native Ligand	Grid box size					
			Centre X	Centre Y	Centre Z	Size		
						X	Y	Z
Androgen-α	2AM9	TES	26.907	2.557	5.181	24	32	16

Validation of Molecular Docking Method

Method validation was performed by re-docking the native ligand of the androgen-*α* receptor (PDB code: 2AM9). The native ligand, testosterone (TES), is a steroid hormone known to regulate AR activity tightly. The choice of testosterone aligns with the receptor's natural ligand-binding conditions, though its suitability for modeling conditions in women, where androgens play a different a roles, warrants careful consideration. Figure 3. shows the interaction between TES and AR, highlighting key residues that confirm docking accuracy.

The figure shows TES binding to the AR. The left panel highlights the 3D binding pose with key residues, while the right panel illustrates a 2D

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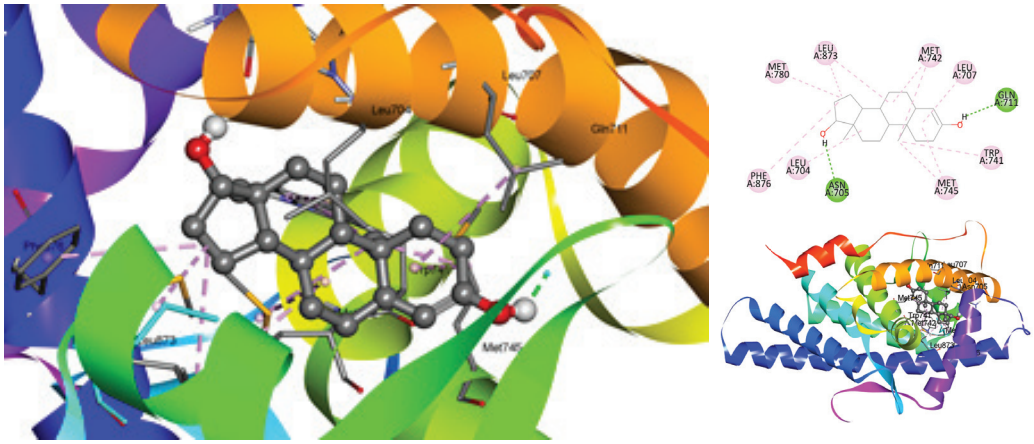

Figure 3. Interaction between TES and AR.

interaction map of hydrogen bonds and hydrophobic contacts. The bottom-right view shows TES's location in the AR pocket. Molecular docking confirmed TES's strong interaction with AR.

It's important to note that while testosterone is a primary androgen in males, it also plays a roles in females, albeit at lower concentrations. In breast tissue, testosterone can be converted to dihydrotestosterone (DHT) by the enzyme 5α-reductase. Both testosterone and DHT are distinct compounds; however, they can bind to the AR and elicit biological responses (Anestis, *et al*., 2020). The re-docking results for the native ligand (testosterone) in this study are presented in Table 4, which summarizes the binding affinities and key interaction parameters obtained using AutoDock Tools. These results demonstrate that the re-docking process achieved a RMSD value below 1 Å, confirming the reliability and precision of the molecular docking method (Eliaa, *et al*., 2020). Future studies should consider alternative ligands or models to better capture sex-specific physiological and pathological conditions.

The outcome of the docking process is the interaction activity of the ligand with its protein in the form of bond energy value between the ligand and receptor. Gibb's theory states that the smaller the energy generated from the bond of a ligand with the receptor, the more stable the bond between the ligand and the receptor will be (Earlia, *et al*., 2019). The docking method's validity was assessed using a re-docking approach, as presented in Table 5.

Redocking is performed as a form of validation for the method used. The validity can

Table 4. Native ligand docking results using autoDock tools.

Protein	Native Ligand Code	Binding Energy (kcal/mol)	Ki (μM)	Interaction with Amino Acids			
				Hydrogen Bond	Van der Waals Bond	Interaction	RMSD
Androgen-α	TES	-11.99	1.64	ASN A: 705 GLN A: 711	-	MET A: 780 LEU A: 873 MET A: 742 LEU A: 707 TRP A: 741 MET A: 745 LEU A: 704 PHE A: 876	0.78

Table 5. Method validation (redocking).

Rank	Subrank	Run	Binding Energy (kcal/mol)	Cluster RMSD	Reference RMSD
1	1	90	-11.99	0.00	0.78
1	2	57	-11.99	0.07	0.78
1	3	60	-11.99	0.13	0.78
1	4	23	-11.99	0.12	0.78
1	5	81	-11.99	0.14	0.78
1	6	24	-11.99	0.02	0.78
1	7	27	-11.99	0.01	0.78
1	8	53	-11.99	0.10	0.78
1	9	52	-11.99	0.02	0.78
1	10	12	-11.99	0.10	0.78

be monitored in RMSD parameters. According to the results presented in Table 6, the RMSD values for the redocked native ligand were consistently below the threshold of 2Å. A smaller RMSD score signifies that the conformations are more similar, thereby confirming the reliability of the docking method used.

Based on the molecular docking results, three key parameters were evaluated: binding energy, inhibition constant (Ki), and the similarity in amino acid interactions between natural ligands and test compounds. Kaempferol exhibited the highest binding affinity (-9.44 kcal/mol) among the tested compounds, indicating its strong interaction

Table 6. Output of molecular docking simulation.

No	Compounds	Cluster	Binding Energy (kcal/mol)	Ki (μM)	Interaction with Amino Acids		
					Hydrogen Bond	Van der Waals Bond	Interaction
1	Isorhamnetin	1	-8.29	836.63	ASN A: 705 THR A: 877 MET A: 780 GLN A: 711	-	PHE A: 764 MET A: 749 MET A: 745 LEU A: 707 LEU A: 873 MET A: 742 MET A: 787
2	Luteolin	1	-8.90	297.00	ASN A:705 LEU A:873 MET A:745 ARG A:752 GLN A:711 THR A: 877	-	LEU A: 704 LEU A:707 PHE A:764
3	Catechin	1	-9.25	166.59	ASN A: 705 LEU A: 704 MET A: 745 GLN A: 711 ARG A: 752 MET A: 780	-	LEU A: 707 LEU A: 873 PHE A: 764
4	Kaempferol	1	-9.44	121.12	ARG A: 752 GLN A: 711 LEU A: 704 ASN A: 705 THR A: 877 LEU A: 873	-	LEU A: 707 MET A: 745
5	Apigenin	1	-9.46	115.75	GLN A: 711 ARG A: 752 LEU: 873 ASN A: 705 LEU A: 707	-	LEU A: 707 MET A: 745 PHE A: 764

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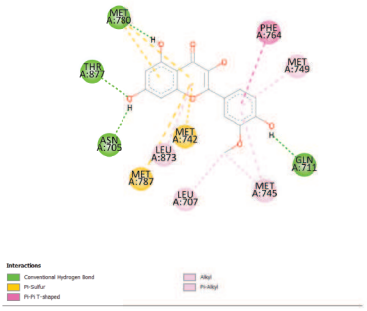
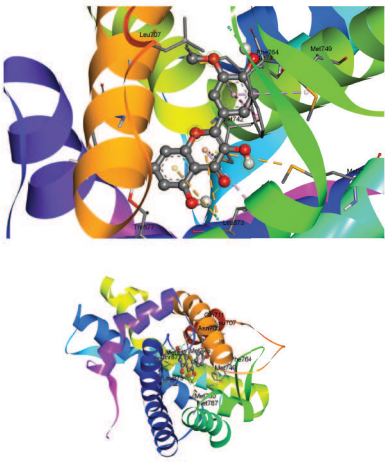
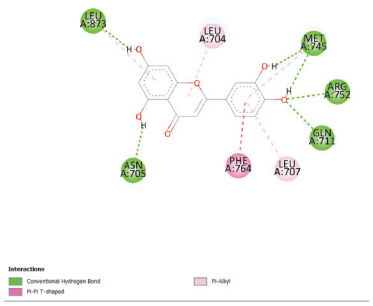
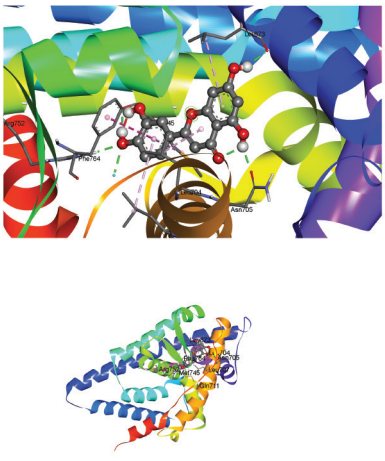
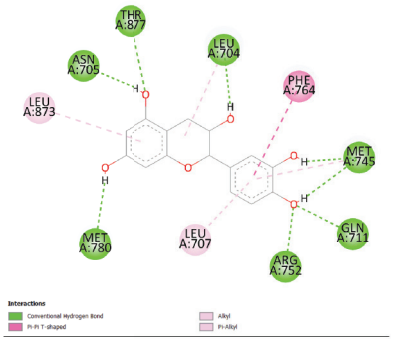
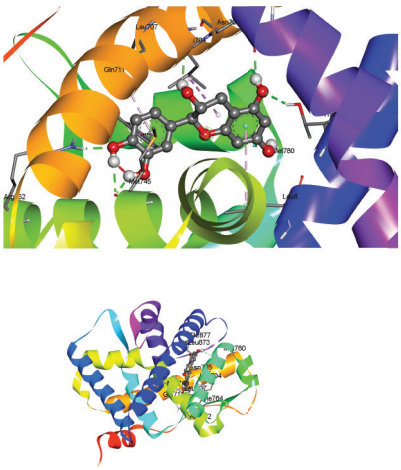
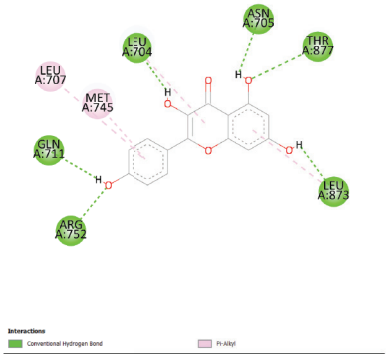
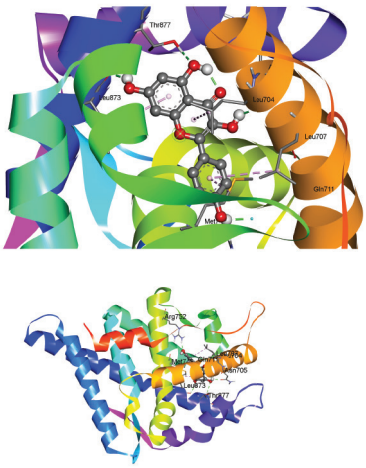
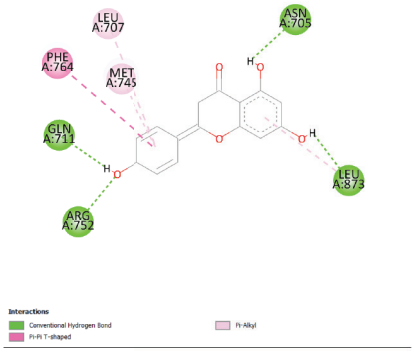
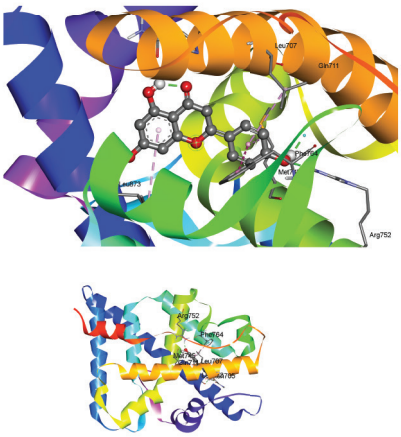
with AR. This is supported by its low inhibition constant (121.12 µM), highlighting its potency as an AR inhibitor. The pharmacophore fit score further corroborates these findings, with isorhamnetin achieving the highest fit score (41.31), suggesting a complementary structural alignment with AR's active site. These results emphasize the critical relationship between structural compatibility and binding efficacy, underscoring the therapeutic potential of kaempferol and isorhamnetin. (Gaspersz & Sohilait, 2019). Table 7. shows the visualization of the interaction between the ligand (natural ligand and test compound) and the receptor (2AM9) in two-dimensional and three-dimensional.

DISCUSSION

Lipinski's Rule of Five helps predict if a compound can be administered orally by assessing its physicochemical properties (Susanti, *et al.*, 2021). Compounds with a molecular weight over 500 Da may struggle to cross cell membranes, and higher log *P* values can indicate increased hydrophobicity, which can lead to toxicity as the

Table 7. Ligand-protein complex visualization.

Test Compound-Receptor	Two-Dimensional Visualisation	Three-Dimensional Visualisation
Isorhamnetin-2AM9		
Luteolin-2AM9		

Test Compound-Receptor	Two-Dimensional Visualisation	Three-Dimensional Visualisation
Catechin-2AM9		
Kaempferol-2AM9		
Apigenin-2AM9		

molecule accumulates in the body (Syahputra, *et al*., 2014). While receptor-ligand interactions are important for drug viability, evaluating Absorption, Distribution, Metabolism, Excretion (ADME), and toxicity properties is also crucial for determining a compound's drug potential (Hermanto, 2021).

Molecular docking is a key computational tool that predicts interactions between receptors and ligands, aiding in drug discovery by simulating chemical interactions (Pinzi & Rastelli, 2019). The AR has gained attention as a target in BC therapy due to its expression in various BC subtypes (Kono, *et al*., 2017). AR is found in 30-80% of BC cases and often coexists with estrogen receptors, which makes it a useful prognostic marker (Kensler, *et al.*, 2019; Majumder, *et al*., 2017).

AR's potential in BC treatment is enhanced by its interaction with bioactive compounds from lotus. These include alkaloids like nuciferine and flavonoids like quercetin and kaempferol, which have shown anti-cancer properties. *In vitro* studies indicate that these compounds can inhibit BC cell proliferation, induce apoptosis, and affect key oncogenic pathways such as PI3K/AKT and mTOR (Bishayee, *et al*., 2022; Kolyvas, *et al*., 2022). *In vivo* studies have also shown that nuciferine can reduce tumor growth and improve survival by modulating the tumor microenvironment (Bishayee, *et al*., 2022). These findings suggest that lotus-derived compounds could serve as valuable agents for targeting AR in BC.

Molecular docking studies further suggest that these compounds have strong binding affinities to AR, potentially influencing AR pathways in BC cells (Wahl & Smieško, 2018). The biological outcomes of such binding—whether inhibitory, stimulatory, or neutral—depend on the conformational changes and AR-co-regulator interactions (Sakkiah, *et al*., 2018; Wahl & Smieško, 2018). Future research should focus on identifying the specific active compounds in lotus and exploring their a roles as AR agonists or antagonists. Additionally, examining their effects on other cancers and in combination with other therapies could further develop targeted treatments.

CONCLUSION

This study highlights the potential of lotus-derived compounds as AR inhibitors in breast cancer treatment. Computational approaches, including Lipinski's Rule of Five, ADMET analysis, pharmacophore screening, and molecular docking, identified promising candidates such as kaempferol and isorhamnetin. These compounds showed strong binding affinities to AR, supporting their potential therapeutic value. Future experimental and clinical studies are essential to validate these findings and advance the development of lotus-derived compounds as effective anti-cancer agents.

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