

Pharmacophore Modeling and Molecular Docking of Flavonoid Derivatives in *Abelmoschus manihot* Against Human Estrogen Receptor Alpha of Breast Cancer

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Abstract: Tamoxifen is the most commonly used anti-estrogen adjuvant therapy for estrogen receptor-positive breast cancer. However, it is associated with an increased risk of some serious side effects, such as uterine cancer, stroke, and pulmonary embolism. The flavonoid compounds in the leaves of *A. manihot* inhibited the growth of 4T1 breast cancer cells at a CTC50 concentration of 185.06 µg/ml. Therefore, this study aims to examine the molecular interactions and pharmacophore modeling based on the interaction of 4-OHT with human ER, followed by the molecular docking of the flavonoid derivatives with human ERα. The molecular docking simulations and 3D structure-based pharmacophore models were used to identify the molecular interactions of flavonoid derivatives in *A. manihot* on estrogen receptors (ERα) (PDB ID: 3ERT). The results showed that the binding energies of the flavonoid derivatives in isorhamnetin and isoquercitrin were -8.68 kcal/mol and -8.75 kcal/mol, respectively. This compound also interacted with Arg394 and Glu353 important amino acid residues in the ERα-binding pocket. Meanwhile, the pharmacophore fit scores of isorhamnetin and isoquercitrin were 82.36% and 84.91%, respectively. The flavonoid derivatives in *A. manihot* had pharmacophore fit resulting from the 4-OHT complex with ER, and therefore they had potential as ERα antagonists. Out of the 10 flavonoid derivatives, isorhamnetin and isoquercitrin showed the best docking scores and could be used as candidates for new anti-breast cancer drugs with antagonistic activity against ERα.

Keywords: *Abelmoschus Manihot*; Estrogen Receptor Alpha; Molecular Docking; Pharmacophore.

1. Introduction

Cancer is currently the leading cause of death worldwide. In 2020, almost 10 million deaths were registered as a result of cancer, and according to report, breast cancer ranks first with 2.26 million cases and 685,000 deaths (1). Estrogen receptors are the human prognostic marker used to identify tumors in breast tissue (2), and they consist of two subtypes, ERα and ERβ, which have different affinities for estrogen. ERα is a transcription regulator-activated ligand as a major regulator of breast differentiation and proliferation (3). Furthermore, it plays an important role in the development and progression of hormone-dependent breast cancer (4). Tamoxifen, as an antiestrogen can block estrogen signaling through a mechanism of competition with endogenous estrogens to bind to the estrogen receptor and alter its activity as a regulator of gene transcription. Additionally, it has antagonistic activity in the breast but is an agonist in the uterus and bone (5). Tamoxifen and its active metabolite 4-hydroxytamoxifen (4-OHT) have cytotoxic activity against MCF-7

breast cancer cells with an IC₅₀ of 5 μ M and 1 μ M (6). However, the effectiveness of tamoxifen is limited by the presence of intrinsic resistance. Amplification and overexpression of COPS5 (complex subunit COP9) was a major cause of tamoxifen resistance in 86.7% of patients with ER α -positive breast cancer. Overexpression of COPS5 through isopeptidase activity leads to proteasome-mediated NCoR degradation, which is a major repressor of ERCC, and therefore, alternative medicine is needed (7).

Flavonoids are one of the promising natural remedies for breast cancer (8). One of the plants that contain active flavonoid compounds is *Abelmoschus manihot* L. Medik. Most people in Palu and Manado cities, Indonesia use the leaves as food, which were reported to have total flavonoids of 61.763% (9). It was reported that the flavonoid compounds in the leaves of *A. manihot* inhibited the growth of 4T1 breast cancer cells at a concentration of 50% inhibitory cell growth (CTC₅₀) of 185.06 μ g/ml (10). Furthermore, flavonoid compounds can act as selective estrogen receptor modulators (SERMs) as they are a group of compounds with a C6-C3-C6 chemical structure. SERMs can enter the cell and then bind to the ER, forming a complex bond. This complex binding binds to the Estrogen Response Element (ERE), which is located near the gene whose transcription is controlled. Additionally, the complex will activate nuclear receptor co-repressor (NCoR) protein and suppress cancer cell replication, therefore its proliferation can be controlled (11,12). Currently, studies on pharmacophore and molecular docking of flavonoid derivatives in *A. manihot* to ER α are very limited. Therefore, it is necessary to conduct pharmacophore modeling tests based on the interaction of 4-OHT with human ER α followed by molecular docking of flavonoid derivatives of *A. manihot* with human ER α .

2. Experimental Section

2.1 Hardware and Software

The hardware used is a computer with Windows 10 64-bit OS, AMD A6-7310 APU Processor with AMD Radeon R4 Graphic 2.00 GHz and 6 GB RAM. The software is ChemDraw professional 15.0 (Academic License), LigandScout 4.4.5 Advanced (Universitas Padjadjaran License), AutoDock 4.2.6, AutoDockTools 1.5.6 (The Scripps Research Institute), and BIOVIA Discovery Studio 2021 (Academic License).

2.2 Preparation of Ligands and Molecular Docking

3D X-ray crystallographic structure of ER α in complex with 4-OHT (PDB ID: 3ERT) crystallized and stored by Shiau et al., (1998) 1.90 Å resolution downloaded from the Protein Data Bank online (<https://www.rcsb.org/structure/3ERT>) (13). The ligands were separated from the receptor structure using the BIOVIA Discovery Studio 2021 R2 client. The 3D-mangostin structure and its derivatives as ligands were optimized by ChemOffice 2010 and ChemDraw Ultra 12.0 (PerkinElmer Inc.) and LigandScout 4.4.5 Advanced (Inte: Ligand GmbH). Molecular docking simulations were carried out according to previous validation studies (14). ER α receptors and ligands were prepared for docking simulation using AutoDockTools 1.5.6. Furthermore, receptors as macromolecules are added with Kollman charges, while ligands are added with Gasteiger charges (15). The grid parameter file corresponds to a grid consisting of 40×40×40 points spaced 0.375Å and centered on the active edge ER α (x = 30,010, y = -1,913, and z = 24,207). AutoDock 4.2.6 (The Scripps Research Institute) was used to perform molecular docking simulations. The docking parameter file corresponds to the Lamarckian Genetic Algorithm (LGA) with 100 run counts, 150 population sizes, 2,500,000 energy evaluations, 0.02 gene mutation rate, and 0.8 crossover rate (16). The conformational results from the simulation were grouped using the root mean square deviation (RMSD) tolerance of 1.0. The ligand conformation with the lowest free bond energy (Δ G) was selected from the best cluster, and the best ligand conformation was used for the next analysis stage. The receptor-ligand complex from the docking simulation was visualized using the BIOVIA Discovery Studio Visualizer 2021. The determination of ligand interaction features for each pose in the receptor-binding pocket was analyzed with LigandScout 4.4.5 Advanced Inte: Ligand GmbH. Wina, Austria (8).

2.3 3D Structure-based Pharmacophore Modeling

Structure-based 3D pharmacophore modeling was derived from an ER α X-ray structure complexed with 4-OHT (PDB ID: 3ERT) using Ligandscout 4.4.5 Advanced (17). Feature validation was performed by filtering 626 active sets and 20,773 bait sets obtained from the Database of Useful Decoys (18). The flavonoid derivatives in *A. manihot* were screened

virtually using a 3D structure-based pharmacophore model validated with the LigandScout 4.4.5 advanced algorithm. The result of this process is the adjustment value of the pharmacophore. The pharmacophore-fit score was used to measure the feature and geometry similarity of each achieved compound based on a 3D structure with a feature pharmacophore model with 4 feature counts removed for the combined pharmacophore, 10.0% optional threshold partial adaptation feature, and 1.0 feature tolerance scale factor.

3. Result and Discussion

Two hydrogen bonds were observed in the interaction between 4-OHT and human ER α . Hydrogen bonding was observed in the interaction between 4-OHT and human ER α bound to Glu353 as a hydrogen bond donor (HBD) and Arg394 as a hydrogen bond acceptor (HBA), as shown in Figure 1.

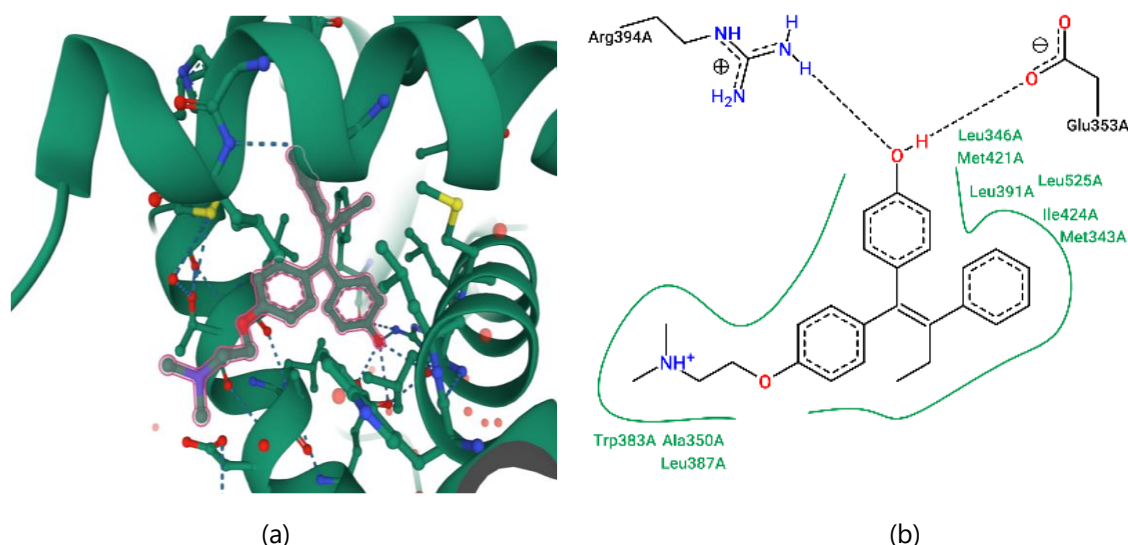


Figure 1. Interaction of hydrogen bonds between 3D (a) and 2D (b) structure 4-OHT with Arg394 and Glu353 in human ER α (Black dotted lines indicate hydrogen bonds, salt bridges, and metal interactions. Solid green lines indicate hydrophobic interactions).

The hydrophobicity of 4-OHT mostly interacts with the aromatic ring and the butenyl group also forms positive ionized interactions with the secondary amine nitrogen. Hydrogen bond interactions are formed with hydroxyl oxygen and phenoxy as shown in Figure 2.

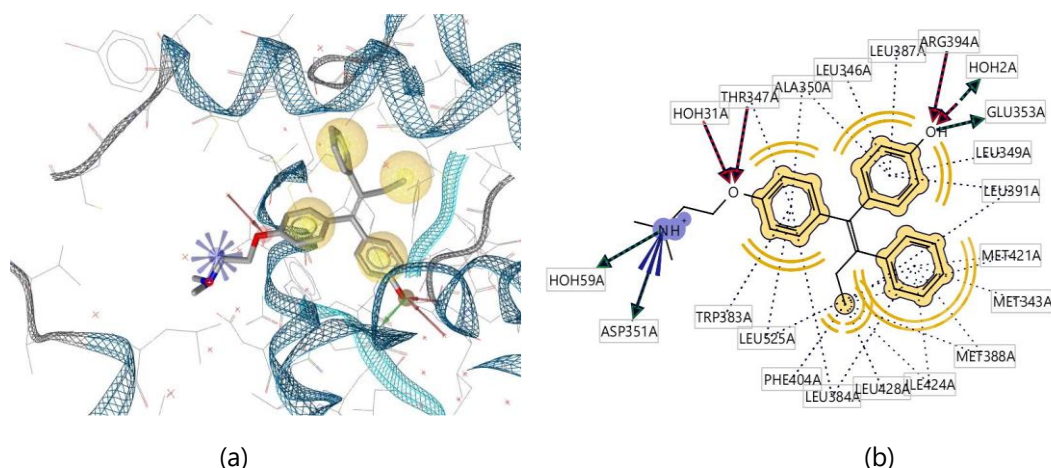


Figure 2. (a) 3D structure-based pharmacophore modeling of 4-OHT with ER α (PDB ID: 3ERT) (The interactions of positively ionizable hydrophobic hydrogen bond donors and acceptors are represented as blue stars, yellow balls, green and red arrows). (b) 2D structure-based 3ERT shows the hydrophobic interactions with the binding pocket residues.

The docking results in Figure 4 show that the dimethylaminoethoxy group of 4-OHT is more elongated than the methoxy and hydroxy groups of isorhamnetin and isoquercitrin. This difference can be explained by the lower binding free energy (ΔG) of 4-OHT (-11.93 kcal/mol; inhibitory constant 1.79 nM) compared to G isorhamnetin (-8.68 kcal/mol; inhibitory constant 0.43 μ M) and isoquercitrin (-8.75 kcal/mol; inhibitory constant 0.38 μ M) (Table 2). The structural design of the flavonoid derivatives focused on the modifications of the methoxy group and the dihydroxy-substituted aromatic ring and was based on the key interactions between 4-OHT and ER α , as well as according to Lipinski's Five rules as shown in Table 1.

Table 1. The properties of flavonoid derivatives calculated in *A. manihot* based on Lipinski's Rule of Five.

No	Molecular Names	Molecular Weight	Log P	Number of Hydrogen Bond Donors	Number of Hydrogen Acceptors
1	Cannabiscitrin	480.38	2.49	10	12
2	Hibifolin	494.37	4.58	9	14
3	Hyperoside	464.38	2.66	9	11
4	Isoquercitrin	464.38	2.66	9	11
5	Quercetin	302.24	4.22	6	6
6	Isorhamnetin	316.27	4.16	5	6
7	Myricetin	318.24	4.05	7	7
8	Quercimeritrin	464.38	2.66	9	11
9	Quercitrin	448.38	3.45	8	10
10	Rutin	610.53	2.23	11	15

Results in Table. 1 shows Lipinski's rule of five for orally administered drugs and this rule specifies four physicochemical parameters, namely molecular weight ≤ 500 , $\log P \leq 5$, donor hydrogen bond ≤ 5 , and acceptor hydrogen bond ≤ 10 . These physicochemical parameters are related to the solubility and acceptable intestinal tract permeability and are part of the initial stage to determine the bioavailability of orally administered drugs (19). The protein structure of hER α has hydrogen bonds in its constituent amino acid residues, namely between Glu419 and His524 and Glu419 with Lys531 (hydrogen bonding network). A disruption of this hydrogen-bonding network can be suggested by fluctuations in ligands that are potential antagonists of the hER α receptor (14). The ligands isorhamnetin and isoquercitrin disrupt hydrogen bonds with fluctuations with residues Ser432 and Ser521, therefore it can be explained that isorhamnetin and isoquercitrin may have antagonistic activity against hER α .

Table 2. The Docking Simulation Results of Flavonoid Derivatives in *A. manihot* in Ligand Binding Domain of ER α .

No	Molecule Name	ΔG (Kkal/mol)	Ki (μ M)	HB	HB Interaction with Arg394 or Glu353
1	Cannabiscitrin	-7.37	3.96	5	2
2	Hibifolin	-6.25	26.28	2	0
3	Hyperoside	-8.28	0.85	4	2
4	Isoquercitrin	-8.75	0.38	7	3
5	Quercetin	-7.81	1.89	4	2
6	Isorhamnetin	-8.68	0.43	5	3
7	Myricetin	-7.34	4.18	3	1
8	Quercimeritrin	-5.62	75.77	7	1
9	Quercitrin	-8.54	0.54	3	1
10	Rutin	-4.63	402.49	3	1

The validation of the pharmacophore model and the interaction properties based on 3D structures was performed by filtering 626 active sets and 20,773 bait sets obtained from the Database of Useful Decoys (DUDe) (18). The results showed that the 100% enrichment factor (EF100%) was 32.4 with a 100% AUC of 1.00 as shown in the Receiver Operating

Characteristic (ROC) curve (Figure 5). These results indicate that the 3D pharmacophore model can distinguish the active molecule from the feed molecule.

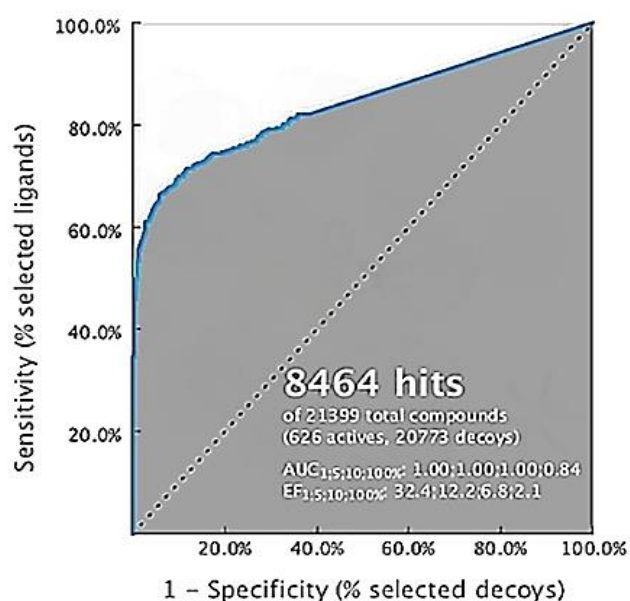


Figure 5. Receiver Operating Characteristic (ROC) Curve Validation of the 3D structure-based Pharmacophore Model Using a set of 626 Estrogen Receptor Alpha Active and 20,773 Decoy Molecules.

The pharmacophore fit score of the flavonoid derivatives in *A. manihot* is presented in Table 3. The pharmacophore fit score is a measure of the geometric similarity of the molecular features to the 3D pharmacophore model. The results indicate that isorhamnetin and isoquercitrin have high pharmacophore compatibility (82.36 and 84.91, respectively), meaning that the chemical properties of isorhamnetin and isoquercitrin are geometrically aligned with the chemical properties of 4-OHT.

Table 3. The Pharmacophore Fit-score of Flavonoid Derivatives in *A. manihot*.

No	Molecular Names	Docking Score (kcal/mol)	Pharmacophore Fit-score
1	Cannabiscitrin	-7.37	83.12
2	Hibifolin	-6.25	84.44
3	Hyperoside	-8.28	84.75
4	Isoquercitrin	-8.75	84.91
5	Quercetin	-7.81	84.16
6	Isorhamnetin	-8.68	82.36
7	Myricetin	-7.34	84.84
8	Quercimeritrin	-5.62	83.13
9	Quercitrin	-8.54	84.86
10	Rutin	-4.63	83.87

Pharmacophore modeling can be used to determine the suitability score of pharmacophore against flavonoid derivatives in *A. manihot*. The pharmacophore fit score is the percentage measure of the geometric similarity of chemical features compared to the active 3D model of the pharmacophore ligand, namely tamoxifen (14). The results show that isorhamnetin and isoquercitrin have high pharmacophore compatibility values ($\geq 50\%$) of 82.36% and 84.91%, respectively, which means that there are similarities in the chemical properties of isorhamnetin and isoquercitrin with the chemical properties of 4-OHT. Therefore, it was stated that isorhamnetin and isoquercitrin have a good affinity for hER α .

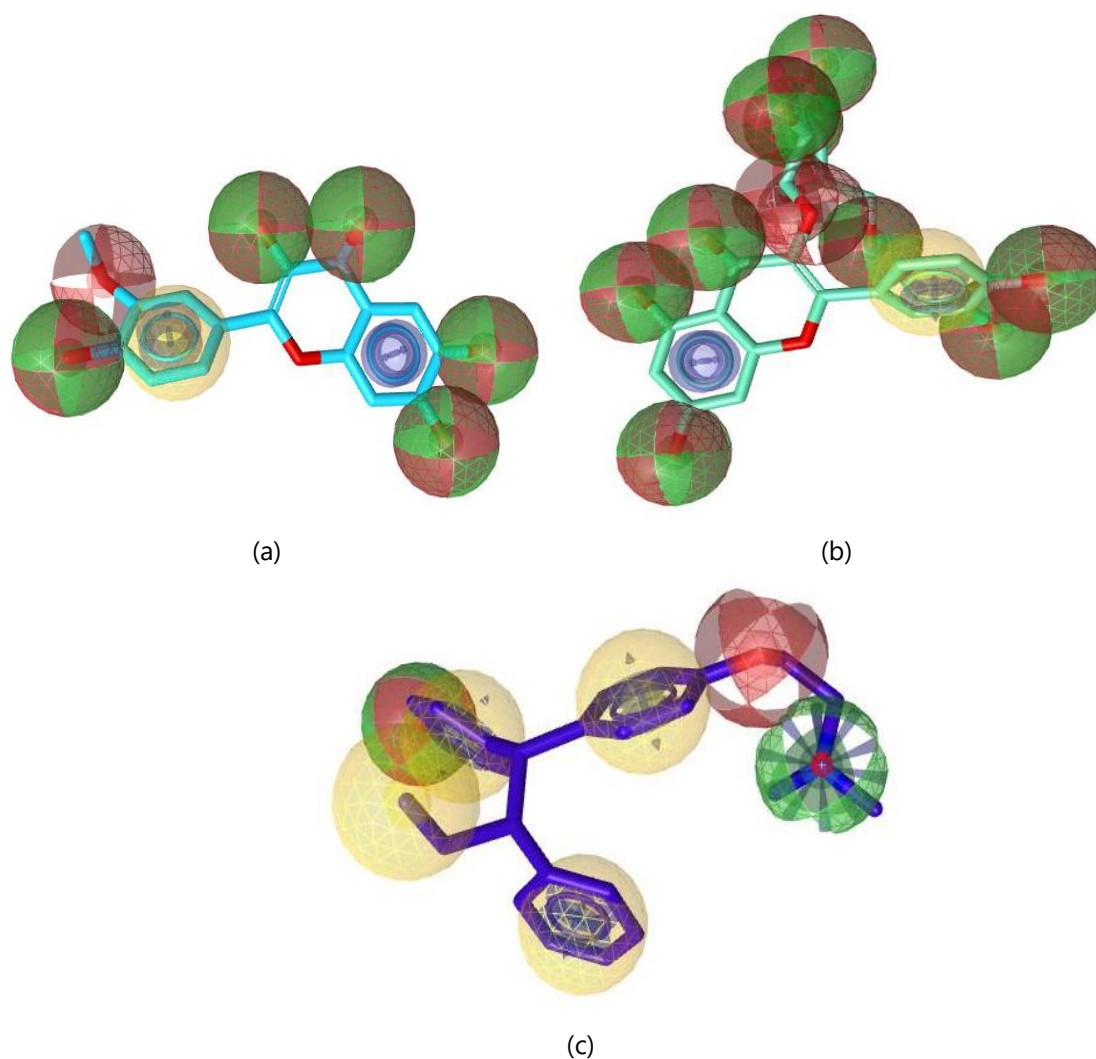


Figure 6. Conformity of isorhamnetin (a) and isoquercitrin (b) with a 4-OHT-derived 3D structure-based pharmacophore model (c) with ER α (PDB ID: 3ERT) (3D pharmacophore model produced using LigandScout 4.4.5 Advanced. The interactions of positively ionizable and hydrogen bond donors and acceptors are represented as blue stars, green arrows, yellow and red balls, respectively).

4. Conclusion

The flavonoid derivatives of *A. manihot* have similar pharmacophore properties resulting from the complexation of 4-OHT with ER, and therefore they have potential as ER α antagonists. Out of the 10 flavonoid derivatives in *A. manihot*, isorhamnetin and isoquercitrin showed the best docking scores and can be used as candidates for new anti-breast cancer agents with antagonistic activity against hER α .

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Conflict of Interest

The author declares no conflict of interests.

Authors contribution

Conceptualization : Recky Patala

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