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In Silico Study of Anticancer Activity of *Acalypha indica* Bioactive Compounds Against the ERα Receptor

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In Silico Study of Anticancer Activity of *Acalypha indica* Bioactive Compounds Against the ERα Receptor

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Abstract. Cancer is caused by the growth of abnormal cells in body tissues and is the second leading cause of death in the world, especially in women. The search for anticancer drug compounds continues in order to reduce the toxic effects of chemotherapy, making alternative treatments necessary. This study aims to evaluate the anticancer activity of sixteen bioactive compounds from *Acalypha indica* plants through the identification of target receptors and interaction studies using molecular docking methods against ERα (Estrogen receptor alpha) receptors. The results of molecular docking showed that, after optimization, the best anticancer candidates bioactive compounds tested from *A. indica* were myristyl sulfate, di-n-amyl phthalate, and catechins. The myristyl sulfate compound, with a ΔGbind value of -36.97 kcal/mol, forms hydrogen bond interactions with amino acids Gly216 and His219; the di-n-amyl phthalate compound, with a ΔGbind value of -32.81 kcal/mol, forms hydrogen bond interactions with the amino acid Thr42; and catechin, with a ΔGbind value of -25.5 kcal/mol, forms hydrogen bond interactions with amino acids Glu48, Met38, and Thr42, which are similar to those of the comparative drug doxorubicin. Therefore, the docking results for the bioactive compounds myristyl sulfate, di-n-amyl phthalate, and catechin with the ERα receptor suggest that they have the potential to serve as alternative anticancer drug candidates.

Keywords: Acalypa indica, Estrogen receptor alpha, Molecular docking

1. Introduction

Cancer is one of the major health problems and the second leading cause of death worldwide, resulting from the abnormal growth of body tissue cells. Cancer cells develop rapidly, uncontrollably, and continue dividing indefinitely [1]. It has become a critical issue in the global health sector and remains the second leading cause of death globally. According to WHO data from 2020, Indonesia recorded 348,809 cancer cases, with 207,210 deaths. Breast cancer has the

highest incidence rate in Indonesia at 16.7%, with a mortality rate of 22.9%, and 11% of total cancer cases lead to death [2].

Cancer treatment, especially before it reaches a more dangerous stage is crucial to inhibit the division of abnormal cells. Generally, cancer treatment involves chemotherapy using drugs [3], one of which is doxorubicin. Doxorubicin is an anthracycline-class antibiotic widely used to treat various types of cancer, including breast cancer. However, some cancer cells have developed resistance to doxorubicin [4].

The use of chemical drugs can cause side effects in the body, which highlights the need for alternative drugs for anticancer treatment. This is because no anticancer drug is universally effective against all types of cancer cells; most tend to target specific cancer types. Furthermore, natural compounds are needed to prevent, slow down, suppress, or reverse the carcinogenic process as potential chemotherapeutic agents [5]. Many plants are used as raw materials for herbal medicines, one of which is *Acalypha indica*.

A. indica which is abundant in tropical areas, has long been used in traditional medicine to treat various diseases, such as diarrhea, rheumatism, and diabetes [6]. This plant also shows various pharmacological benefits, including as an antibacterial, antidiabetic, anti-inflammatory, and anticancer [7][8]. Research shows that Acalypha indica extract is effective against several types of cancer cells, including breast cancer [9].

Breast cancer cells used in this study are MCF-7. In silico analysis requires careful receptor selection, as the protein must be overexpressed in MCF-7 cancer cells. Protein receptors commonly used in breast cancer studies include Estrogen Receptor α (ERα) [10]. However, there has been no analysis of active compounds in *A. indica* extract targeting ER-positive breast cancer. There are two types of estrogen receptors: Estrogen Receptor α (ERα) and Estrogen Receptor β (ERβ). Among these, ERα is the main receptor targeted in breast cancer therapy because it is upregulated in most breast cancers and plays a significant role in hormone-dependent tumor growth [11].

In this study, sixteen active compounds of *A. indica*, the results of our research using LC-HRMS [12]mmmwere tested for anticancer activity using the in silico method, which is more efficient in terms of time and cost. This method is used to predict molecular interactions and biochemical evaluations, supporting laboratory research. Molecular docking plays an important role in the discovery of micro-compound-based drugs that can inhibit or trigger the activity of target proteins, such as MCF-7 [13]. This study aims to determine the potential of *A. indica* based on the binding energy and interactions between its active compounds and MCF-7 breast cancer receptors.

2. Materials and Method

2.1 Material

Acalypha indica contains bioactives that have the potential to be candidates for breast cancer agents. Based on LC-HRMS analysis of *Acalypha indica* [12], the active compounds used as test compounds include oleic acid alkyne, 16-hydroxyhexadecanoic acid, artemotil, 13 (S) -HpOTrE, Acetyphenylalanine, 9-HpODE, 13-Oxo-ODE, DL-Malic acid, misoprostol, 9 (Z), 11 (E) -conjugated linoleic acid, myristyl sulfate, di-n-amyl phthalate, salicylic acid, catechin, traumatic acid and hernano which will be tested against MCF-7 type breast cancer (pdb code 3ERT).

The hardware used in this study was a laptop with an AMD Ryzen 3 5300U processor running at 2.60 GHz with Radeon Graphics, 8 GB DDR2 800 MHz memory, and a 250 GB hard disk, which were sufficient to run molecular docking simulations and perform visualization analysis of ligand-

receptor interactions. The software used included Discovery Studio 2021, AutoDockTools version 1.5.7, PyMOL, Chimera 1.16, SwissADME, Open Babel, and YASARA, which played roles in docking analysis, visualization, and the prediction of pharmacokinetic parameters. Data sources were obtained from the PubChem and Protein Data Bank (PDB) databases.

2.2 Preparation of breast cancer structure (MCF-7) and ligands

Ligand preparation begins by downloading the 3D structure of the ligand from PubChem in **.sdf** format, which is then converted to .pdb using Open Babel. The ligand is further prepared using AutoDockTools (ADT), including the addition of charges, hydrogens, and torsion settings. Free torsion is set on the part of the ligand with free movement space, referring to the geometry of the human estrogen receptor alpha and the 4-hydroxytamoxifen receptor complex, and the ligand is saved in .pdbqt format.

Receptor preparation is carried out by downloading the 3D structure of the protein from the Protein Data Bank (PDB) in .pdb format. The receptor structure is cleaned of other molecules using Chimera 1.16 and saved in .pdb format. Further stages involve using Chimera for visualization, the selection and removal of water molecules, and re-saving the structure as a .pdb file.

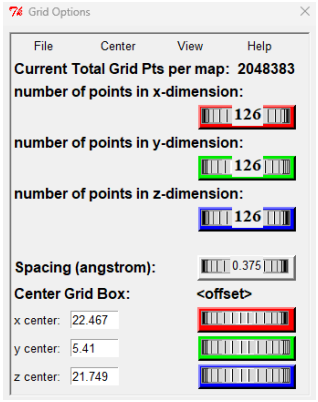


Figure 1. Docking grid setup

2.3 Validation of the docking method

Validation of the docking method should include steps to demonstrate the method's validity. The validation process involves re-docking the pure ligand to the isolated receptor. The docking method is considered adequate if it yields a Root Mean Square Deviation (RMSD) value of 2 Å or less.

2.4 Molecular docking

The docking of ligand molecules to target receptors using AutoDock 1.5.7 begins with the addition of ligands and macromolecules, followed by the determination of the grid box for the protein's active site. After setting the size and coordinates of the grid box, a GPF (Grid Parameter File) is created to define the active site area of MCF-7 and the grid map parameters.

The GLG (Log Grid) file is generated after running Autogrid4, which produces a summary of the grid map creation process. The DPF (Docking Parameter File) is created by setting the docking parameters using the genetic algorithm and the bioactive compound of the plant as a ligand.

Finally, the DLG (Docking Log File) is generated to run the docking between the receptor and the active compound. Binding energy scoring is performed, and a low affinity value (≤ -8.0 kcal/mol) indicates the feasibility of visualizing the docking results. Redocking with a control ligand is performed first for validation [14].

2.5 Visualization of Docking result

Cancer is one of the major health problems and the second leading cause of death worldwide, resulting from the abnormal growth of body tissue cells. Cancer cells develop rapidly, uncontrollably, and continue dividing indefinitely [1]. It has become a critical issue in the global health sector and remains the second leading cause of death globally. According to WHO data from 2020, Indonesia recorded 348,809 cancer cases, with 207,210 deaths. Breast cancer has the highest incidence rate in Indonesia at 16.7%, with a mortality rate of 22.9%, and 11% of total cancer cases lead to death [2].

2.6 Optimization of Docking Result molecules

Optimization was performed on the receptor-ligand docking complex from the two lowest-energy conformations (clusters 1 and 2) obtained from the bioactive docking of the anting-anting plant compound, using YASARA. This process generates a YASARA scene file and calculates energy using the NOVA force field.

First, the initial energy (single point) of the system is calculated before minimization. This is done by opening YASARA and selecting Analyze → Energy → Potential → Object, then choosing the 3ERT data object from the original PDB (before minimization). After clicking OK, select all energy components to calculate the initial potential energy.

Next, the energy minimization process is carried out by selecting Option → Choose Experiment → Minimization. YASARA runs the energy minimization process until it is complete. Finally, the single point energy after minimization is recalculated using the same steps as before, selecting Analyze → Energy → Potential → Object and choosing the object. The result is the optimized energy of the minimized structure.

2.7 Data analysis

Interaction analysis is carried out by reviewing the value of the Gibbs free energy of ligand binding (ΔG), the amino acid residues involved, the types of interactions formed, and the ligand pose on the active site of the protein. The data from the interaction analysis will then be used to determine the potential of the test ligand as an MCF-7 inhibitor. This analysis process is conducted individually for each ligand that meets the criteria as a drug according to Lipinski's rules.

3. Result and Discussion

3.1 Redocking

Molecular docking simulations of oleic acid alkyne, 16-hydroxyhexadecanoic acid, artemotil, 13(S)-HpOTrE, acetylphenylalanine, 9-HpODE, 13-oxo-ODE, DL-malic acid, misoprostol, 9(Z),11(E)-conjugated linoleic acid, myristyl sulfate, di-n-amyl phthalate, salicylic acid, catechin, traumatic acid, and hernanol were performed to identify their potential binding modes and investigate their similarity to the binding modes of standard ligands. The test compounds were docked to the human estrogen receptor alpha (ERα) (PDB ID: 3ERT), while 4-hydroxytamoxifen (4OHT), the native ligand of 3ERT, and the test drugs doxorubicin and genistein were also docked as a comparison and to validate the proposed docking method. The overlay of the native ligand with the docking pose of the 4OHT compound in the active site showed the same binding pose as

the crystallized ERα receptor, with a root mean square deviation (RMSD) of 0.96. Thus, the proposed docking protocol can be used for further steps.

3.2 Validation of docking method

Redocking was performed to validate the docking method by docking the human estrogen receptor alpha with its native ligand, 4-hydroxytamoxifen, at the enzyme's binding site. The RMSD value obtained from redocking was used to evaluate the accuracy of the binding mode prediction, with RMSD ≤ 2 Å considered good validation. The redocking results showed a binding energy of -11.79 kcal/mol and an RMSD of 0.96 Å (Table 1), indicating the success of the docking method used. The interactions formed include Van der Waals forces and hydrogen bonds with amino acid residues in the enzyme's active site (Figure 2)

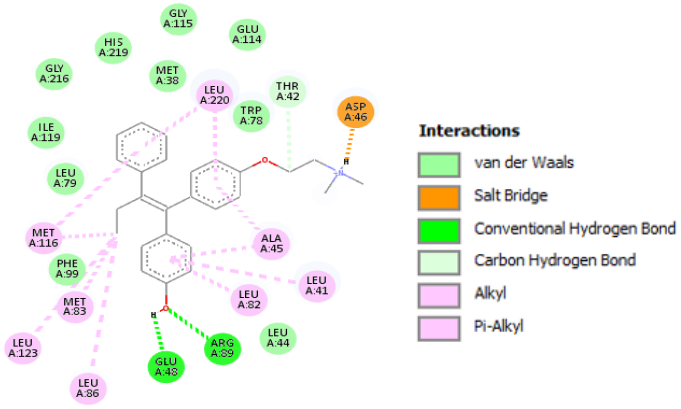
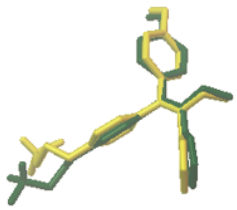


Figure 2. Visualization of terminal A redocking interaction

Table 1. Redocking of 4-hydroxytamoxifen terminal A.

Terminal	Conformation	Binding Energy	Reference RMSD
A		-11.79	0.96 Å

*green color is the conformation of the native ligand bound to the receptor and yellow color is the conformation resulting from redocking

3.3 Interaction Analysis

Molecular docking was performed on 16 compounds from *Acalypha indica* against the human estrogen receptor alpha to analyze their interactions at the enzyme's active site. The docking aims to provide insights into ligand-receptor interactions relevant to drug design. The docking data were analyzed from output files in .dlg format, focusing on the spatial fit, position, and orientation

of the ligands, along with the enzyme's macromolecular residues interacting with them. These interactions include hydrogen bonding, van der Waals forces, electrostatic interactions, and hydrophobic effects. The docking results identified key residues in the enzyme's active site that play a role in forming interactions with the ligands. This active site is where the enzyme binds to the ligand, influencing the enzyme's function or conformation. Detailed data on the amino acid residues interacting with the ligands are provided in the Appendix.

The optimization stage of the docking results was performed using the YASARA application with the NOVA force field to achieve the lowest energy and a more stable conformation. Optimization was applied to docking results selected based on the similarity of amino acid residue interactions and hydrogen bonds with the reference ligand. The ligand-enzyme interaction energy was calculated before and after minimization, with the initial docking results obtained from AutoDock and the optimization results from YASARA. The AMBER force field was used to minimize the protein and ligand structures, yielding more accurate and stable interaction energies. This optimization aims to validate the ligand's interaction pose with the target enzyme.

Table 2. Data on hydrogen bond residues of the test ligands at terminal A which have similar interactions to the redocking ligands from docking results and after optimization.

Compound Name	Hydrogen Bonds Resulting from Docking	Hydrogen Bonds After Optimization
Redocking 4-hydroxytamoxifen	Arg89, Glu48	Glu48
Comparative drug doxorubicin	Arg89. Glu48, Glu114, Thr42	Glu48, Glu114, His219, Met38, Thr42
Genistein comparative drug	Arg89, Glu48, Gly216, His219, Leu41	Glu48, Gly216
Oleic acid alkyne	Gln48, Leu22	Glu48, Leu22
16-Hydroxyhexadecanoic acid	Arg89, Glu48	Arg89, Glu48
Artemotil	-	-
13(S)-HpOTrE	Glu114, His219, Leu41	Glu114, His219, Leu41
Acetyphenylalanine	Glu48, Lys144	Arg89, Glu48, Lys144
9-HpODE	Gly85, Lys144	Arg89, Gly85, Lys144
13-Oxo-ODE	Lys144	Lys144
DL-Malic acid	Lys224, Tyr221	Lys224, Tyr221
Misoprostol	Glu75, Val229	Glu75, Val229
9(Z),11(E)-Conjugated linoleic acid	Arg89, Gly85	-

Myristyl sulfate	Gly216, His219	Gly216, His219
Di-n-Amyl phthalate	-	Thr42
Salicylic acid	Ala2, Asp64, Leu1	Ala2, Asp64, Leu1
Catechine	Arg89, Glu48, Met38, Thr42	Arg89, Glu48, Met38, Thr42
Traumatic acid	Glu18, Lys144	Glu18, Lys144
Hernanol	Arg89, Glu48, Trp88	Arg89, Glu48

*Similar residues are marked with an underline on the residue

Molecular docking results of 16 test ligands at Terminal A showed that eight of them had similar amino acid residue interaction patterns with the reference ligands 4-hydroxytamoxifen, doxorubicin, and genistein. Some compounds, such as oleic acid alkyne and 16-hydroxyhexadecanoic acid, formed hydrogen bonds similar to the reference ligands at residues such as Gln48, Arg89, and Glu48. In addition, other compounds such as 13(S)-HpOTrE and acetyphenylalanine also showed similar interactions with hydrogen bonds at residues such as Glu114 and His219. This analysis suggests that the similarity of amino acid residues between the test ligand and the reference ligand potentially indicates that the test ligand can inhibit the target protein, which increases the possibility of similar biological activity to the reference ligand.

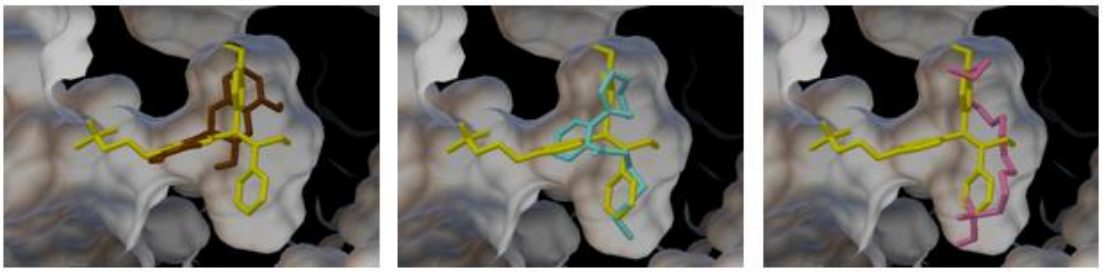


Figure 3. Visualization of the position and spatial suitability of the test ligand at the A terminal of the docking results with (a) Comparison of the position of Catechin with 4-hydroxytamoxifen redocking (yellow) (b) Comparison of the position of myristyl sulfate with 4-hydroxytamoxifen redocking (yellow) (c)

Furthermore, the position and orientation of the docked ligands in the receptor binding site were analyzed using AutoDock 4.2. 3D visualization shows that ligands such as di-n-amyl phthalate, myristyl sulfate, and catechins occupy the active site of the human estrogen receptor alpha enzyme effectively, indicating an optimal shape match between the ligands and the receptor. Figure 2 confirms that the positions of the test ligands do not differ significantly from the redocking positions of 4-hydroxytamoxifen, with all test ligands interacting within the enzyme's active site. The similarity in shape and volume between the ligands and the enzyme enhances these interactions, aiding in identifying the best ligand position during docking and assessing its potential as an enzyme inhibitor [15].

Table 3. Binding affinity of docking results and optimization results of test compounds at terminal A

Compound Name	Docking Result Energy (Kcal/mol)	Interaction Energy (E_{R-L}) Optimization Result (Kcal/mol)
Redocking 4-hydroxytamoxifen	-11.79	-40.56
Comparative drug doxorubicin	-9.70	-55.49
Comparative drug genistein	-8.42	-25.52
Myristyl sulfate	-5.27	-36.97
Di-n-Amyl phthalate	-5.95	-32.81
Catechin	-7.56	-25.5

3.4 Visualization analysis of Docking results

The next step in the docking analysis is to observe the position, spatial fit, and orientation of the ligand anchored in the receptor binding site using AutoDock 4.2. The three-dimensional shape of each ligand is analyzed to assess its spatial fit and interactions with the protein macromolecule. Test compounds with similar amino acid interactions—namely, oleic acid alkyne, 16-hydroxyhexadecanoic acid, 13(S)-HpOTrE, acetylphenylalanine, di-n-amyl phthalate, myristyl sulfate, catechin, and hernanol—are compared with the redocking results of 4-hydroxytamoxifen. The visualization in Figure 3 shows that the three test ligands—di-n-amyl phthalate, myristyl sulfate, and catechin—align their surfaces well with the human estrogen receptor alpha enzyme, effectively filling the active site.

The shape fit analysis in Figure 3 indicates that the positions of the test ligands in each selected compound are not significantly different from the redocked 4-hydroxytamoxifen, with all ligands interacting within the enzyme's active site. The match between the solvent-accessible receptor surface and the interacting ligand surface in terms of shape and volume helps determine the optimal position for the ligand to interact with the target enzyme [15].

3.5 Energy Analysis

The final analysis of the docking results focuses on the binding affinity between the ligand and the receptor. The interaction between the protein receptor and the drug molecule ligand will produce a*binding* energy (*binding affinity*) and activity of the drug molecule, with energy conditions tending to be the lowest which will produce the most stable molecular conditions. The lower the value of*binding* energy (*binding affinity*), the more stable it is.

The lowest energy analysis is performed in the final step, as the binding affinity alone is not meaningful if the test ligand does not exhibit similar biological activity to the reference ligand. The goal is for the test ligand to interact with the target enzyme in a manner similar to the reference ligand. Based on the visualization of the ligand's position and amino acid similarity, three selected compounds will be analyzed for their binding affinity, as shown in Table 2 below.

The energy value of the molecular docking results is an important parameter in assessing the conformational stability of the ligand when binding to the receptor. This stability is indicated by a low ΔGbind value, which reflects the energy required for the ligand to interact with the enzyme

macromolecule at the binding site. A lower ΔGbind value corresponds to a more stable ligand-receptor interaction. Data on docking energy and energy optimization for the selected test compounds are presented in Table 3.

The binding affinity results from Terminal A show that the three test compounds have ΔGbind values ranging from -5.97 to -7.56 kcal/mol. These lower binding affinity values are comparable to or close to those of the reference ligands, such as 4-hydroxytamoxifen (4OHT), doxorubicin, and genistein, indicating strong potential for interaction with the human estrogen receptor alpha (ER-α). A ΔGbind value below 0 for the three test compounds indicates strong affinity for the receptor's active site, supporting their potential as inhibitors of the target enzyme.

Energy optimization using the YASARA tool aims to minimize the ΔGbind value, enhancing the stability of the ligand-receptor interaction. This optimization employs the AMBER force field, which includes parameters tailored for organic molecules such as proteins and nucleic acids. The molecular optimization results, listed in Table 3, show ΔGbind values ranging from -36.97 to -25.5 kcal/mol. Myristyl sulfate and di-n-amyl phthalate exhibit the lowest ΔGbind values, at -36.97 kcal/mol and -32.81 kcal/mol, respectively, followed by catechin at -25.5 kcal/mol. A more negative ΔGbind value after optimization indicates improved stability of the ligand-receptor interaction compared to the initial docking results obtained using AutoDock4.

However, low binding affinity values do not always guarantee a compound's potential as an enzyme inhibitor. Therefore, in addition to the energy value, the similarity of amino acid residue interactions with native ligands and reference drugs must also be considered. In this study, myristyl sulfate, di-n-amyl phthalate, and catechin exhibit significant residue interaction.

4. Conclusions

The molecular docking results of 16 compounds from Acalypha indica at Terminal A identified potential inhibitors of the ERα receptor, specifically myristyl sulfate, di-n-amyl phthalate, and catechins. Myristyl sulfate has a ΔGbind binding energy of -36.97 kcal/mol and interacts with the amino acids Gly216 and His219 through hydrogen bonds. Di-n-amyl phthalate has a ΔGbind of -32.81 kcal/mol and interacts with Thr42. Meanwhile, catechin, with a ΔGbind of -25.5 kcal/mol, forms hydrogen bonds with Glu48, Met38, and Thr42, similar to the anticancer drug doxorubicin. In the future, we intend to test other receptors to gain a more thorough understanding of the bioactive potential of A. indica through in silico studies.

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