RESEARCH ARTICLE | OCTOBER 17 2018

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Aida Fitriah ✉; Kholifah Holil; Umaiyatus Syarifah; Fitriyah; Didik Huswo Utomo

AIP Conf. Proc. 2021, 070003 (2018)
https://doi.org/10.1063/1.5062801

In silico Approach for Revealing the Anti-Breast Cancer and Estrogen Receptor Alpha Inhibitory Activity of *Artocarpus altilis*

Aida Fitriah[1,3, a)], Kholifah Holil[1], Umaiyatus Syarifah[1], Fitriyah[1], Didik Huswo Utomo[2,3]

[1]*Biology Department, Faculty of Science and Technology, Maulana Malik Ibrahim State Islamic University, Malang, Indonesia*
[2]*Biology Department, Faculty of Sciences, Brawijaya University, Malang, Indonesia*
[3]*Research and Education Center for Bioinformatics, Nusantara Institute of Science and Technology, Malang, Indonesia*

[a)] Corresponding author: aidafitriah97@gmail.com

Abstract. Estrogen receptor alpha (ERα) is a common target for breast cancer treatment and is mainly involved in cell proliferation. Breadfruit (*Artocarpus altilis*) is a well-known traditional herb that has anticancer potential, specifically for inhibiting breast cancer proliferation. This study sought to identify the active compound from breadfruit as a candidate ERα inhibitor. This study used flavonoid compounds as anticancer candidates. Eleven flavonoids compounds were considered, including: cycloaltilisin 7, isocyclomorusin, cyclomorusin, cycloaltilisin, cyclomulberrin, isocyclomulberrin, quercetin, cyclocommunal, artocarpin, artonin E, and morusin. Research was conducted using the exploratory descriptive method. The first step was ligand and receptor preparation before the docking process using VegaZZ. The molecular docking process was calculated using AutoDock Vina in PyRx v.0.8. The docking site was predicted in the inhibitory site of ERα based on the control drug (tamoxifen). Screening for the best docking complex was considered according to highest binding affinity and accurate binding site. The selected compounds were predicted for pharmacokinetics to evaluate the possibility of absorption in the human intestinal tract. The potential of the active drug to pass the membrane was assessed based on the Lipinski rule of five. Finally, the biological process of the best compound was identified using PASS SERVER to explain the accuracy before conducting the experiment in the laboratory. The results suggest that isocyclomorusin had the lowest binding affinity (8.4 kcal/mol) and this compound could act as an anti-neoplastic (Pa > 0.7) towards breast cancer. Isocyclomorusin could be easily absorbed in the human intestine due to an HIA+ score above 0.9 and could pass the bilayer membrane in breast cancer cells because it meets the Lipinski rule of five standard. Based on the results of this study, it appears that isocyclomorusin is a potential candidate for anti- breast cancer treatment derived from *Artocarpus altilis* that could act as an ERα inhibitor.

Keyword: Breadfruit, breast cancer, estrogen receptor alpha, molecular docking

INTRODUCTION

Breast cancer is one of the most common diseases causing death in the worldwide.[1,2] Breast cancer is caused by abnormal cells growing in breast tissue. These cancer cells continue to proliferate due to hormonal imbalances.[3] Estrogen hormone production in postmenopausal women is no longer synthesized in large quantities in the ovaries, but estrogen will be more highly synthesized in adipose tissue, so women who are obese are more susceptible to breast cancer.[4,5]

High estrogen production affects the activation of estrogen receptors in breast tissues. The estrogen receptor alpha (ERα) is a dominant estrogen receptor found in breast tissues. ERα has a stronger affinity in

The 8th Annual Basic Science International Conference
AIP Conf. Proc. 2021, 070003-1–070003-6; https://doi.org/10.1063/1.5062801
Published by AIP Publishing. 978-0-7354-1739-7/$30.00

estrogen binding than ERβ.[6,7,8] The interaction could activate the co-activator protein SRC-3, which triggers replication of cancer cells and becomes immortal.[9,10,11]

Cancer cells could be treated with active compounds from herbs. One of the plants that contain active flavonoid compounds known to have anticancer activities is breadfruit (*Artocarpus altilis*).[12] Arung et al.,[13] states that flavonoids compounds from breadfruit have been shown to potentially reduce the viability of breast cancer cells T47D. However, this plant has many active compounds and the molecular mechanisms are not clear. This study will reveal the molecular mechanisms of breadfruit and the potential active compounds computationally.

Herbal remedies are a well-known alternative solution for conventional medication. Herbs can act systemically and have less adverse effects. This study also compares the existing drug that binds to ERα, namely, tamoxifen, which has proved able to prevent cancer cell proliferation by binding to ERα.[14] The use of tamoxifen over the long term has a harmful effect on the body, minimizing its usefulness. This study therefore computationally evaluates the active compounds derived from breadfruit flavonoids for inhibiting ERα and reveals their therapeutic potential.

METHOD

Samples Preparation

Flavonoid derivatives of breadfruit include isocyclomorusin, quercetin, isocyclomulberrin, cycloaltilisin 7, morusin, artocarpin, cyclocommunal, cyclomorusin, artonin E, cyclomulberrin, and cycloaltilisin, which act as ligands, were retrieved in 3D format via PubChem (http: //PubChem.ncbi.nlm.nih.gov).[15] Preparation was done by minimizing all structures of active compounds using the steepest decent minimization in PyRx 0.8. The 3D structure of ERα)was downloaded from the Protein Data Bank (RCSB) PDB ID: 3ERT (http://www.rscb.org).[15] Water molecules and drugs attached to the protein structure were eliminated, then the hydrogen atom was added to the structure of the protein since as a result of X-ray diffraction most the hydrogen atoms were incomplete.

Molecular Docking Analysis

Molecular docking was performed to obtain the best inhibitor from the active compound mimicking tamoxifen. The docking process was initiated by selecting the ligand and the prepared receptor. Thus, the docking process was started by setting the gird box on the active site receptor. The docking results were saved in PDB format and the value of the binding affinity was saved in Microsoft Excel format.[15] The docking result visualization was performed using LigPlot v.1.4.5 software, while the interaction visualization in 3D form was done using Discovery Studio software.

Pharmacokinetics Analysis

The human intestine absorption (HIA) test was used to determine which active compounds of the herb could be well absorbed in the human digestive system. The HIA test was performed using preADMET online software (http://preadmet.bmdrc.org/). Upon visiting the preADMET software site, the ligand structure data is input in molfile format (*mol), and the data submitted.[16] The Lipinski rule of five test was performed to understand the pharmacokinetics of the active compounds, including whether any of the active compounds would be able to enter the cell and interact with the target protein. The analysis was conducted in SCFBIO Server (http://www.scfbio- ittd.res.in/software/drugdesign/lipinski.jsp).[17]

PASS (Prediction of Activity Spectra for Substances) Test

The biological activity test was important to confirm before lab tests were conducted. The results would be shown by the probability activity score, which predicts the chance of success if tests were performed in the lab. The PASS test was performed using the PASS Online software (http://www.pharmaexpert.ru/passonline). First SMILES was searched for breadfruit derivative compounds using PubChem

(http://pubchem.ncbi.nlm.nih.gov), then the ligand compounds were input into the PASS software and the activity prediction is performed (Get Prediction).

RESULT AND DISCUSSION

Evaluation of Estrogen Receptor Alpha Inhibitory Activity

The docking results indicate the presence of a suitable active compound for inhibiting ER-α, and this study shows that the active compounds of *A. altilis* can be an ER-α inhibitor. The results revealed that the compound isocyclomorusin is the most effective compound to be used as an anti–breast cancer treatment because it has the highest binding affinity value compared to the other compounds (Figure 1). The more negative the free energy binding, the better the bond stability level between the ligand and the receptor, since the bond formed is also stronger.

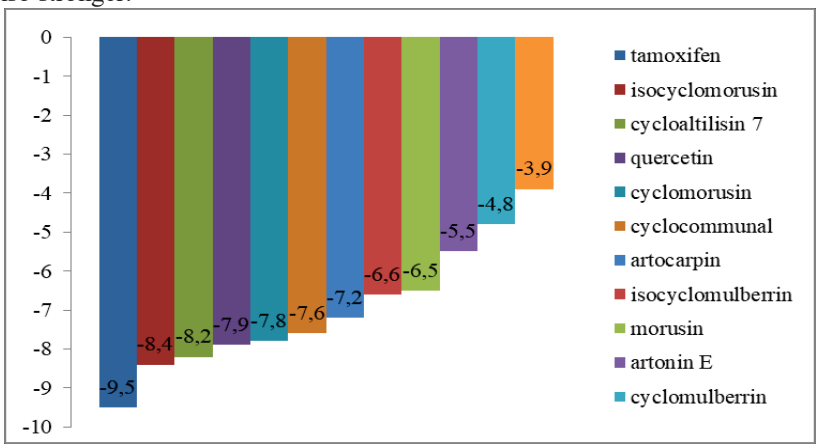

Figure 1. Ligan of flavonoid compound from *A. altilis* bind to estrogen receptor alpha

Differences in the affinity binding values of each compound are influenced by the type of interaction. The hydrogen bond has an important role in determining the size of the affinity binding value generated from the docking process because it has a stronger energy than the hydrophobic bond. According to Hernandez and Appu,[18] hydrogen bonds have higher energy than hydrophobic interactions with values of 1–7 kcal/mol versus 1 kcal/mol. Shiau et al.[19] and Anita et al. [20] also added that hydrogen supplements that predicted an important role in determining the strength of ligand interactions with ER-α are when the hydrogen bond binds to the amino acid residues Glu 353, Arg 394, Thr 347, and Asp 351.

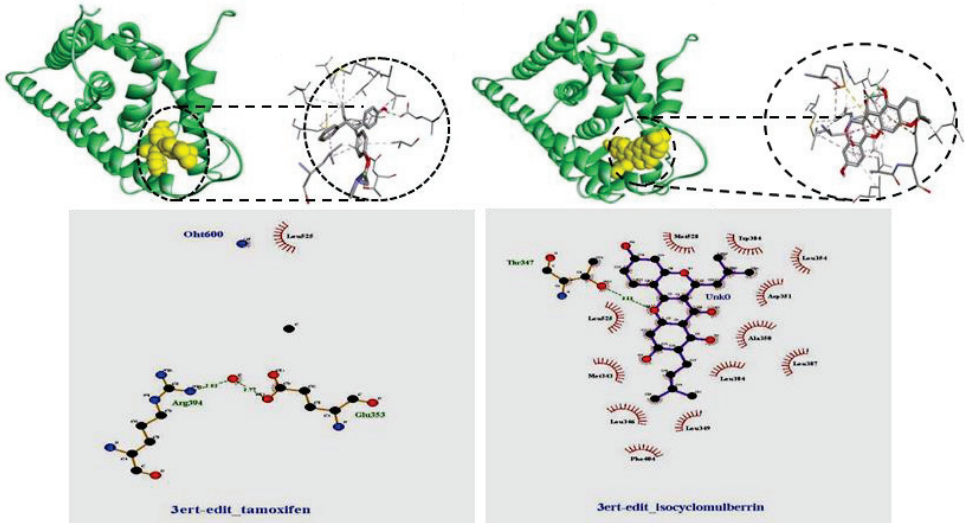

Figure 2. Molecular interaction analysis of docking (a) tamoxifen (b) isocyclomorusin

Tamoxifen as a control has 2 hydrogen bond within distance 2.77 Å of residue: Glu A: 353 and 2.82 Å of Arg A:394. Tamoxifen also had hydrophobic bond on residues Leu A: 525. While the isocyclomorusin has 1 hydrogen bond within distance 3.04 Å on residue Thr A: 347. Isocyclomorusin also has hydrophobic bond of residues Met A:343, Leu A 346, Ala A:350, Asp A: 351, Leu A: 384, Trp A: 383, Leu A: 387, Leu A: 391, Met A: 421, Leu A: 525, Met A: 528, Leu A: 536.

Pharmacokinetic Analysis of *A. altilis* as Potential Drug Candidate

Based on the results of HIA prediction (Human Intestinal Absorption), tamoxifen and the breadfruit flavonoid derived compounds have the potential to be well absorbed in the human intestinal tract (Table 1). The values obtained show that the compounds are well absorbed in the intestinal wall because they have a value greater than 70%. This is in accordance with Nerkar's observation[21] that if a compound has a predicted intestine absorption greater than 70% it means the compound has a high-capacity absorbing ability.

Table 1. Prediction of HIA (Human Intestine Absorptio) of flavonoid compound from *A. altilis*

No	Compound	HIA (%)
	Tamoxifen[b]	97.2011
	Isocyclomorusin[b]	94.7462
	Cycloaltilisin 7[b]	94.6780
	Quercetin[a]	63.4852
	Cyclomorusin[b]	94.7473
	Cyclocommunal[b]	90.6125
	Artocarpin[b]	92.7373
	Isocyclomulberrin[b]	92.4730
	Morusin[b]	92.4722
	Artonin E[b]	88.4316
	Cyclomulberrin[b]	92.4741
	Cycloaltisin[b]	92.4466

Note: a (low absorpsion) b (high absorpsion)

The flavonoid compounds with a high absorption value will more easily reach the target breast cancer cells, making them more effective. Isocyclomorusin is predicted to absorb well in the intestinal wall, so it will more easily reach the target cancer cells. When isocyclomorusin reaches the ER-α, the drug will inhibit these receptors from binding to the hormone estrogen, which could otherwise induce the proliferation of cancer cells.[22]

When the drug is able to be well absorbed in the intestine and into blood circulation, it is distributed throughout the body tissue and must penetrate the cell membrane to reach its target receptor. It is very important to consider these factors in the pharmacological world, because drug interactions with targets are unlikely to occur if they are not focused on their targets.[23] According to the Lipinski rule of five predictions, tamoxifen, as a control, and the compounds isocyclomorusin, cycloaltilisin 7, quercetin, cyclomorusin, cyclocommunal, artocarpin, isocyclomulberrin, morusin, artonin E, cyclomulberrin, and cycloaltilisin each have a molecular weight of not more than 500 Da. The H-donor and H-acceptor values obtained are also not greater than 5 and 10. The compounds also have a logP value of no more than 5, except cycloaltilisin 7 and artocarpin (Table 2).

Table 2. Lipinski Rule of Five (Ro5) of flavonoid compound from ***A. altilis***

No	Ligan	Moleculer weight (Da)	H-donor	H-Acceptor	LogP
1	Tamoxifen	388	2	2	4.284598
2	Isocyclomorusin	418	2	6	4.995601
3	Cycloaltilisin 7	406	2	5	5.715001
4.	Quercetin	302	5	7	2.010900
5.	Cyclomorusin	418	2	6	4.995601

No	Ligan	Moleculer weight (Da)	H-donor	H-Acceptor	LogP
6.	Cyclocommunal	352	3	6	3.516999
7.	Artocarpin	436	3	6	5.823903
8.	Isocyclomulberrin	420	3	6	5.025701
9.	Morusin	420	3	6	5.330301
11.	Artonin E	436	4	7	5.035901
12.	Cyclomulberrin	420	3	6	5.025701
13.	Cycloaltisin	450	3	7	5.034301

Note: molecular weight≤ 500 Da, H-donor ≤ 5, H-acceptor ≤ 10, clogP ≤ 5 (Lipinski, 2004)

Table 2 shows the breadfruit flavonoid derivatives that have been tested can pass through the cell membrane because they have a molecular weight of not more than 500 Da. Chillistone and Hardman[24] suggest that the molecular weight of a drug that is too large will make it difficult to penetrate the cell membrane because it may interfere with the diffusion process. The smaller the molecular weight of the drug, the easier it will be to diffuse into the cell membrane. The flavonoid compounds tested also have an H-donor and H-acceptor value of no more than 5, allowing them to penetrate the cell membrane. According to Patrick,[25] H-donor and H-acceptor values that are too large are more prone to making hydrogen bonds and the more bonds formed, the more slowly the drug reaches its target.

If the logP is too large it complicates the drug passing through the cell membrane. According to Etkins et al.,[26] the logP value is related to the hydrophobicity of drug molecules. The greater the logP value, the more hydrophobic the molecule is. As a drug, the structure of the ligand compound should not be too hydrophobic because it might be retained in the lipid bilayer; the drug can be widely distributed in the body, which can cause the reduction of bond selectivity to the target receptor. From the data obtained, two compounds, cycloaltilisin 7 and artocarpin, have a log value greater than 5, but this does not make the two compounds incapable of penetrating the cell membrane, according to Jadhav et al.,[23] who state that a drug will be able to penetrate to the cell membrane if the drug satisfies a minimum of two rules from the Lipinski rule of five.

Biological Activity Prediction of *A. altilis*

Based on PASS test results using tamoxifen as control and breadfruit flavonoid compounds (artonin E, cycloaltilisin 7, cycloaltilisin, isocyclomulberrin, cyclomorusin, morusin, cyclocommunal, and cyclomulberrin), each of these tested compounds has a Pa value greater than 0.7 (Pa > 0.7), while the flavonoid derivative compounds isocyclomorusin, artocarpin, and quercetin have Pa values in the range 0.5 < Pa < 0.7. The results can be seen in Table 1.

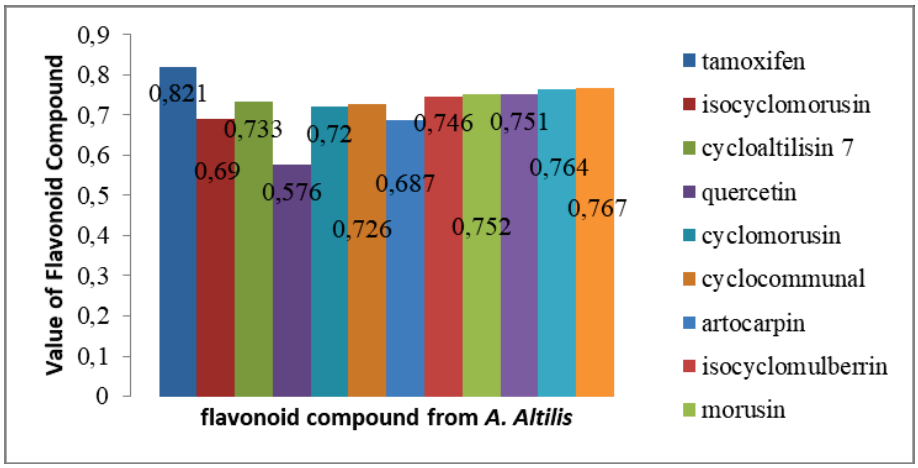

Figure 3. Antineoplastic breast cancer activity of flavonoid compound from ***A. Altilis***

The data above show that artonin E, cycloaltilisin 7, cycloaltilisin, morusin, isocyclomulberrin, cyclomulberrin, cyclomorusin, and cyclocommunal have an anticancer (neoplastic) activity that is predictable either computationally or through laboratory scale, because they have Pa values greater than 0.7 (Pa > 0.7).

Meanwhile, isocyclomorusin, artocarpin, and quercetin have good antineoplastic activity against breast cancer, but this has not been proven in the laboratory because they have Pa values in the range $0.5 < Pa < 0.7$.

CONCLUSION

It can be concluded that of the eleven flavonoid compounds from breadfruit (*Artocarpus altilis*), isocyclomorusin is the most promising candidate as an ERα inhibitor. Active compounds from breadfruit have a high potential for anticancer treatment. This study provides basic information that can be considered before testing directly in the laboratory.

ANKNOWLDGEMENTS

The authors thanks to lecturer and inbio team for supporting and facilitating this research.

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