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Molecular Docking of Gnetin C and Transresveratrol of Melinjo Seeds (Gnetum Gnemon L.) Used as the Inhibitors of Breast Cancer Cells MCF-7

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Abstract: Breast cancer is cancer caused by uncontrolled cell growth in breast tissue. In this research, molecular docking has been studied to predict the binding affinity of Gnetin C dan trans-resveratrol as active compounds of melinjo seeds to inhibit breast cancer cells Michigan Cancer Foundation-7 (MCF-7). Molecular docking was performed by autodock-vina. The result indicated that Gnetin C and trans-resveratrol could bind the same amino acid as natural ligan of MCF-7 such as VAL 54B, VAL54B, TYR 55B, TYR 216B, TRP 227B, LEU 306B, LEU 306B, and LEU 306B. The binding affinity of Gnetin C and transresveratrol was -6. and -7.9, respectively, while the natural ligan was -10.0. It means Gnetin C and trans-resveratrol can bind the protein acid of MCF-7, although the docking energy was lower than the natural ligan. Based on this research, Gnetin C and trans-resveratrol are potential anticancer and chemoprevention because they can inhibit some amino acids of cancer cells MCF-7.

Keywords: *Breast cancer, molecular docking, gnetin C, trans-resveratrol, amino acid of breast cancer cells MCF-7*

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I. INTRODUCTION

Breast cancer is a cancer caused by abnormal cell growth in breast tissue. MCF-7 is a breast cancer cell obtained from the pleural effusion of 69-year-old Caucasian breast adenocarcinoma female patients. MCF-7 cells are described as supporting the differentiated features of glandular breast epithelium. This cell is also classified as a cell that is often used in research models on female breast cancer [1].

The procedures implementation has been used to the breast cancer patients through surgical, chemotherapy, and radio therapy Surgical is the first therapy process which is used in breast cancer management. While chemotherapy is the process of administering anti-cancer drugs in the form of liquid, pills or capsules or through an infusion that aims to kill cancer cells and is usually used after surgery or before sugery when the cancer has reached the end stage. The main cases of breast cancer is recurrence rate of the disease after surgical. In fact about 90% of patients who recover after surgery are still at risk of recurrence. The effect of chemotherapy and radiotherapy are not selective but also it is very toxic because it can kill the cancer cells and the normal cells [2, 3].

Therefore, it is necessary to develop a natural compound that has the potential as an anticancer and can be used as a chemoprevention agent to optimalizing breast cancer management. Melinjo seeds (Gnetum gnemon L.) contain anticancer compounds, namely Gnetin C and

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tranveratrol [4]. The problem is is not known which one is more selective in MCF-7 which is inhibited by melinjo seeds.

One of the most recent drug development methods is based on a computational approach (in silico). Computational chemistry methods have been developed and are widely used for the development of pharmacological hypotheses and testing [5]. Computational chemical methods provide test results that are more adequate than theoretical predictions, easy to use, cheap and safe [6, 7, 8]. Molecular docking is the main tool to predict the model that binds to the ligand in the dominant region known as protein in three-dimensional structures. So that the most potential compounds obtained in melinjo seed extract as chemoprovention of breast cancer.

A. *Breast Cancer*

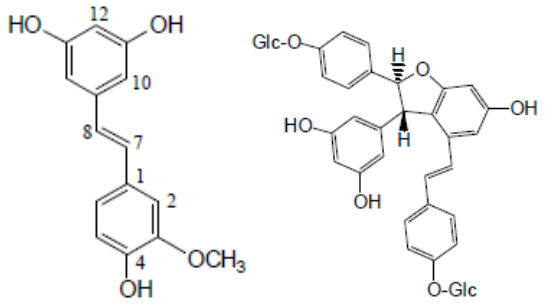
Breast cancer is a cancer caused by uncontrolled cell growth in breast tissue. Most breast cancers begin in the cells that line the duct (ductal cancer), some begin in lobules (lobular cancer), and a small percentage begin in other tissues. Symptoms of breast cancer generally also appear from the swelling in one breast, pulling on the nipple or nipple itching, and pain. In stage breast cancer further, bone pain, arm swelling, skin ulceration, or weight loss [9]. MCF-7 is a breast cancer cell obtained from the pleural effusion of 69-year-old Caucasian breast adenocarcinoma female patients. MCF-7 cells are described as supporting the differentiated features of glandular breast epithelium. This cell is also classified as a cell that is often used in research models on female breast cancer [1, 10].

The process of cancer is divided into 3 stages, firstly initiation is the process where normal cells turn premalignant. Secondly promotion where there is frequent exposure, conditions can change gene expression such as hyperplasia, enzyme induction, induction of differentiation. Thirdly progression is the process of activation, mutation, or loss of genes. In this progression benign changes become premalignant and malignant [11].

B. *Melinjo Seeds*

Melinjo seeds are effective as anticancer because at 37.3 ± 0.9 μg/mL it is able to kill cancer cells. Melinjo seed extract has chemopreventive and anticancer activities which can increase cell apoptosis MCF-7/ASPP1 (apoptosis stimulation protein of p53) and inhibit cancer cell proliferation [12].

Structure of each type of compound that is efficacious as an anticancer in melinjo seeds [13]:



Reserveratrol Gnetin C

Fig. 1. Reserveratrol and gnetin C

C. *Moleckular Docking*

Molecular docking is a combination of alogritma and scoring functions. This algorithm uses a cavity detection algorithm to detect the place of protein binding as a potential area on the active side to bind to the drug (ligand). Screening the most stable forms of ligands combined with MM2 and conformational searching to produce a positional ligand that was consistent with the active side shape of the protein. Docking is done with standard non-Ligand settings on Molegro Virtual Docker. The scoring function can know the bond affinity between ligands and proteins. The scoring function used the Gibbs free energy theory (ΔG). A strong and stable bond is seen from a small value of ΔG while a weak value bond is from a large value of ΔG [14].

II. METHODOLOGY

This research is a non-experimental research based on computational chemistry using computer devices.

A. *Hardware*

The hardware for this study used an ASUS computer which was set with an Intel Core i3 processor, 4GB RAM memory, type X455LAB with a Windows 10 double boot operating system.

B. *Software*

There are several types of software used, namely Marvin Sketch for drawing structures, Autodock Tools 4.2 where this software is used to change the file format from .pdb to .pdbqt, then PyRx-Vina where this software is used for the docking process of test ligands and compound molecules test, software Pymole used for visualization, software Chimera used to separate proteins from test ligands and water molecules.

C. Preparation

The protein used in this study is MCF-7 which was downloaded from www.pdb.org. The breast cancer cell protein used has a GDP ID: 4X06, this protein file is downloaded in the .pdb format. After the protein is downloaded and stored in the .pdb format the protein is prepared using the Chimera program. Chimera program to show the geometry stability of these 3D protein molecules.This preparation is carried out by removing water molecules and releasing test ligands from proteins. Removal of water molecules serves to accelerate the process of calculating the docking because if the water molecule is still attached to the protein the calculation process will take a long time. Release of the test ligand was carried out aiming that the test compound used can be tethered to the breast cancer cell receptor to replace the test ligand. After the process of removing water molecules and releasing the test ligand, the file is saved in the .pdb format and then displays the polar hydrogen atom because it can potentially produce bonds in the protein and stored in the Pymol format. This process can be done using the PyRx-AutoDock-Vina program.

D. Method Validation

Separation of Ligands and Target Proteins, Protein preparation results are separated between protein components and existing ligands. This can be done using the Chimera program. Conversion of crystallographic ligand and target protein formats using the PyRx-AutoDock-Vina (.pdbqt) program. Furthermore, the validation of molecular docking and target proteins still uses the same program program, PyRx-AutoDock-Vina.

The analysis of the results of molecular docking ligand validation was carried out by observing conformation and 3D position as shown by the results of molecular docking between ligands and target proteins with crystallographic ligand conformation and target proteins. The closeness of the ligand structure was assessed before and after molecular docking using the Root-Mean-Square Deviation (RMSD) parameter using the PyMOL program. RMSD results less than 2.5 indicate that the method can be used.Identification of Docking Results of Comparative Ligands and Test LigandsCollection of comparable ligands and test ligands by searching for drug molecules that have been known to be potentially as MCF-7inhibitors.

Collection of comparable ligands in 3D format obtained from www.pdb.org. While the test ligand was done by drawing a structure on melinjo extract using Marvin Sketch. Screening comparable ligands and test ligands using physico-chemical parameter descriptors include logP, 3D surface area, chiral C number, number of H donors, number of H acceptors, and molecular weights. The chemical physical properties of comparative ligands based on the ligand properties of crystallographic results. The test ligands used have four types of core structures. Docking of comparable ligands and test ligands with target proteins to obtain conformational molecules resulting from interactions between ligands and proteins.

E. Molecular Docking with Autodock Vina

After all the ligand and protein preparation processes have been completed, the process of tethering the test compound is completed. Before that, determine the location of the gridbox using the Autodock Tools program. Grid Box is the location of the mooring space of a ligand that will be docked and has a grid setting that includes center_x, center_y and center_z. To determine the magnitude of a grid box is arranged by using spacing (amstrong). Determining the location of the Grid box is based on the location of the test ligand and the active side of alpha estrogen protein, namely. In the mooring process, storage of protein files and ligands is carried out in the format .pdbqt in one folder “autodock vina”. The docking process is run using the Command Prompt with the command:

After the docking process is complete, the file “log.txt” and “logall.pdbqt” will appear in the vina autodock folder. The file “log.txt” is a file that contains docking results in the form of RMSD value and affinity value of the bond as a result of docking between ligands and proteins. While the file “logall.pdbqt” is a file that contains the conformation results of tethered ligands. The results of the docking process can be visualized using the Ligplot and Pymol program to see the interactions between proteins and ligands.

F. Data Visualization

Data that has been obtained from the download process that will be used for the preparation stage, geometry optimization, energy minimization and molecular belaying are displayed using visualization-based programs, namely plip.

G. Data Analysis

Data that has been obtained from the docking process of stylbenoid and transreservatol compounds will be compared between the Docking values, RMSD of the two compounds and can be seen the interactions that occur between ligands and proteins. If the docking value of each test compound produces docking energy lower than the docking energy value produced by the original receptor ligand, then the two test compounds have the ability as inhibitors to inhibit the growth of cancer cells that can be

used as recommendations for alternative anticancer drug ingredients.

III. RESULTS AND DISCUSSION

Analysis of molecular tethering data carried out in this study include docking energy data, RSMD and the presence or absence of ligands that bind to MCF-7 breast cancer amino acid residues. The interaction was conducted by examining MCF-7 and its natural ligand to determine its RSMD value. The RSMD results determine the validity of the method used, if < 2.5 Åindicates the method is declared valid and can be further investigated. The RSMD obtained is 0,000 which indicates that the method used can be used for molecular docking testing. The best RMSD value is a value close to 0. So that the first conformation in each ligand compares the value of the conformation with itself as the best conformation. Furthermore, it is applied to ERα with test compounds, namely transreserveratrol and gnetin C. Finishing is done in the same way as the validation method on pyrx using optimized gridbox. Analysis of the docking results in this study include the value of bGbind and the interaction of ligands with protein residues. The conformation of each ligand of docking results is ranked based on the value of indGbind from the smallest to the largest. The small indGbind value indicates that the formed conformation is stable, while the large Gbind value indicates a less stable complex formed. Therefore, Binding affinity is chosen at 0.000 RSMD, as follows:

TABLE 1
BINDING AFFINITY

Ligan	Binding Affinity	RSMD
Ligan Native	-10.0	0.000
Gnetin C	-6.3	0.000
Reserveratrol	-7.9	0.000

The results of the binding energy value (affintiy Binding) on gnetin C are -6.3 and the energy that binds to transreseveratrol is -7.9 it shows that gnetin C is capable of binding to MCF-7 but its energy is lower than its natural ligand. And then the docking results in PyRx-vina is saved in the pdb format and seen by their interaction with the Pymol software and visualized with plip.The bonds formation from the gnetin C and transreserveratrol tests on the MCF-7 are hydrophobic bonds, hydrogen bonds and bonds π (Fig. 2).

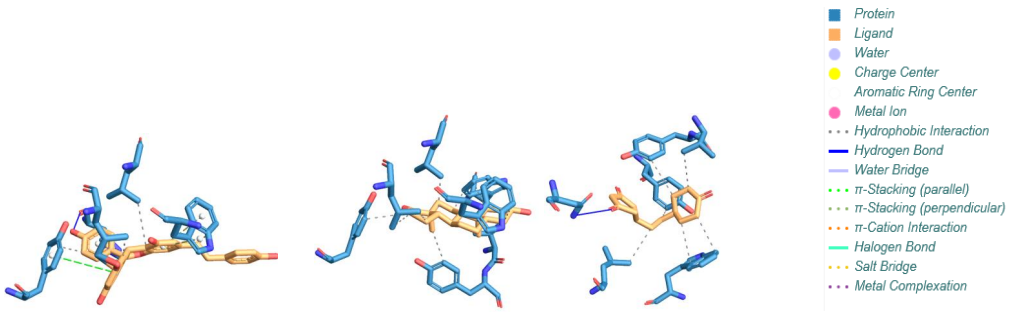


Fig. 2. Results of visualization of Gnetin C and transresveratrol docking in MCF-7 cells

There are 8 bonded gnetin C and trans-resveratrol bound which are bound to the amino acid MCF-7 according to the native ligand:

TABLE 2
THE RESIDU OF AMINO ACID INVOLVED IN THE INTERACTION MODEL

	The residu of amino acid involved in the interaction model		
Ligan	Ibinding pi	Binding Hydrogen	Interacting Hydrofobik
Ligan Natif	-	-	VAL 54B, VAL54B, TYR 55B, TRP 86B, TRP 86B, TYR 216B, TRP 227B, LEU 306B, LEU 306B, LEU 306B
Gnetin C	HIS 117B, TYR216B, TRP227B,TRP227B	HIS117B	TYR216B, TRP227B, LEU 306B, LEU 306B, LEU308B
transreserveratrol	-	ASN167B	TYR24B, TYR24B, VAL 54B, TYR 55B, TRP 227B, LEU 306B

The docking results visualized with the plip application show 8 bonds to the amino acid MCF-7 which are similar to natural ligands, where the bonds that interact are hydrogen bonds and pi bonds. The presence of these bonds means that gnetin C and transresveratrol belong to active binding sites in accordance with amino asama residues in theMCF-7.

The more interactions occur between the test compounds with amino asthma the more stable the bonds are formed in the MCF-7 protein. So that it can be said that ligands can inhibit cancer cell growth from a receptor. Based on the results of analysis and visualization of Gnetin C and transresveratrol compounds, transresveratrol has a stronger binding energy than gnetin C, but gnetin C forms more binding between ligands and amino acids than trans-resveratrol.

IV. CONCLUSION

1. Gnetin C and transveratrol have potential energy that can be used as anticancer compounds of the breast, seen from the docking energy and the bonds formed between ligands and proteins.

2. Transveratrol has higher docking energy (Binding Affinity) than gnetin C. The energy that binds to transreseveratrol is -7.9 while gnetin C is -6.3. This shows that transveratrol and gnetin C are able to bind to MCF-7 but their energy is lower than their natural ligands

3. transresveratrol has a stronger binding energy than gnetin C but gnetin C forms more bonds between ligands and amino acids than trans-resveratrol. There are 8 gnetin C bonds with amino acid residues similar to the bonds between natural ligands and MCF-7 amino acid residues, namely TYR216B, TRP227B, LEU 306B, LEU 306B, LEU308B, TYR216B, TRP227B, TRP227B, whereas transresveratrol only has 4 similar bonds with bonds between natural ligands and MCF-7 amino acid residues, namely VAL 54B, TYR 55B, TRP 227B, LEU 306B

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