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Molecular Docking of Xanthone Compounds of Mangosteen Fruits Peel (*Garcinia mangostana* L.) as Beta-OG Pocket Binding Inhibitor in Dengue Virus Envelope

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Abstract*—Objectives: Dengue Hemorrhagic Fever is a disease caused by the dengue virus (DENV) which is transmitted through the mosquitoes Aedes aegypti and Aedes albopictus. There are four widely known serotypes of dengue virus, namely DENV-1, DENV-2, DENV-3, and DENV-4. The dengue virus genome is composed of three structural genes (encoding C, prM / M, E). In dengue virus there is an Envelope / E section which has an important role in mediating the entry of the virus into the host cell. The crystal structure of the Envelope / E protein shows the part of the connection between Domain I and Domain II in the form of a "pocket" that can be occupied by ligands. The ligands to be used are seven xanthone compounds in the peel of the mangosteen fruit. The in silico approach was carried out using UCSF Chimera® 1.12, AutoDock® Vina and PyMOLTM 2.3.2 software to predict the potential and affinity of these xanthones as inhibitors of β-OG pocket binding. The results showed that the seventh xanthones compounds had greater affinity compared to the comparative ligand, n-octyl-β-D-glucoside. The affinity of the seventh compounds is as follows: Alpha-mangostin -7,3 kcal / mol, Beta-mangostin -7,2 kcal / mol, Gamma-mangostin -7,7 kcal / mol, Gartanin -7,0 kcal / mol, Mangostanol -8,3 kcal / mol, Mangostinone -8,6 kcal / mol, Trapezifolixanthone -7,8 kcal / mol while the comparative ligand is -6,3 kcal / mol. This shows that seventh xanthone compounds found in the peel of mangosteen fruit can be used as candidates for new drugs as inhibitors of β-OG pocket binding on the envelope of the dengue virus.

Keywords: dengue virus, β-OG pocket binding, xanthones, mangosteen peel, molecular docking

I. INTRODUCTION

Dengue Hemorrhagic Fever (DHF) is one of the diseases categorized as a public health problem in Indonesia. In 1954 this disease first occurred in Southeast Asia, namely in the Philippines, the first case occurred in Indonesia in 1968 in Jakarta and Surabaya, there were 58 clinical cases with 24 fatalities. Until 1991 a total of 260,769 cases with 10,104 deaths were approved in 24 provinces in Indonesia [1]. The number of cases that increased increased from 2.2 million in 2010 to 3.2 million in 2015. In 2015, 126,675 DHF sufferers were reported in Indonesia, and 1,299 people were eliminated worldwide, the number reported the previous year, namely as many as 100,347 DHF sufferers and 907 patients died in 2014 [2].

A disease caused by a virus called the dengue virus. The dengue virus belongs to the family Flaviridae and genus Flavivirus, has 4 serotypes namely DEN-1, DEN-2, DEN-3 and DEN-4. This virus enters the human body through the main vector, namely the *Aedes aegypti* mosquito, and the *Aedes albopictus* garden mosquito. Both species of mosquitoes are found throughout Indonesia except at an altitude of 1.000 meters above sea level [3]. Dengue Fever or Dengue Hemorrhagic Fever (DHF) is a disease that causes dengue virus that causes manifestations of bleeding and causes shock and death [4].

Dengue virus has a single chain RNA of 3,391 amino acids and consists of 10 viral proteins which are divided into 2 groups namely structural proteins and nonstructural proteins. Structural protein consists of Envelope / E, membrane precursor / prM and Capsid / C, while nonstructural protein consists of 7 parts, namely protein NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5 [5].

In dengue virus is part of the Envelope / E which protein in the Envelope / E section has an important role in mediating the process of entry of the virus into the host cell. In addition, the crystal structure of the Envelope / E protein inserts into the junction between Domain I and Domain II which consists of "pockets" that can be occupied by n-octyl-beta-D-glucoside (β-OG) ligands [6].

Mangosteen (*Garcinia mangostana* L.) is a native fruit from Indonesia. The mangosteen fruit is nicknamed "Queen of Fruit" which due to the features and deliciousness of the mangosteen fruit [7].

In the mangosteen fruit contains a lot of high nutritional value and the skin of the mangosteen fruit contains bioactive compounds that are collected as a therapeutic agent such as

xanthone compounds. This xanthhone compound has a variety of biological effects such as antioxidants, antimicrobial, and anti-inflammatory [8]. Based on this background, research will be carried out to find out the potential of xanthones associated with mangosteen rind against antiviral activity as β-OG inhibitors in the Envelope / E section of dengue virus by the Molecular Docking method.

II. MATERIAL AND METHOD

A. Procedure

Tools

The tools used in this study are software in the form of bioinformatics applications, namely UCSF Chimera® 1.12 (https://www.cgl.ucsf.edu/chimera/), AutoDock® Vina and PyMOLTM software. UCSF Chimera® software 1.12. The software is a computational chemistry program with high image quality that enables interactive visualization and analysis of molecular structures, and displays densities, docking results, bonds, and molecular conformations.
GDP data on the three-dimensional crystallization structure of dengue virus envelope can be downloaded from the GDP warehouse in the Research Collaboratory for Structural Bioinformatics Protein Data Bank through the website address http://www.rcsb.org/pdb/ using a computer connected to the internet.

Material

The repositories used are Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Banks (http://www.rcsb.org/pdb/home/home.do) and Pubchem (https://pubchem.ncbi.nlm.nih.gov/). The crystallization structure of dengue virus envelope protein is obtained from PDB with code 1OKE. A total of 7 xanthon compounds were used in this trial based on research by Kutumbarao et al, and n-octyl-beta-D-glucoside as a comparison, the structure of the ligand was obtained from Pubchem.

Visualization of β-OG pocket binder Envelope Virus Dengue

Three dimensional crystallization structure of envelope is visualized by software UCSF Chimera® 1.12 to see the location of the β-OG pocket binder. The input entered is PDB envelope virus envelope data in format.cif (common intermediate format).

Geometry Optimization and Energy Minimization of the Three Dimensional Structure of the Dengue Virus Envelope

Geometry optimization and energy minimization of the three-dimensional structure of the dengue virus envelope is carried out using the UCSF Chimera® 1.12 software. The algorithm uses the AMBER ff14SB molecular mechanics force field parameter. Then the hydrogen polar and the Gasteiger load are added, while the non-polar hydrogen is merged. The file is saved in .pdb (protein data bank) format.

Geometry Optimization and Energy Minimalization of Three Dimensional Ligand Structures

The ligand structure is obtained by accessing the PubChem warehouse (https://pubchem.ncbi.nlm.nih.gov/), then searching for the required ligand, copying the Canonical SMILES code into the UCSF Chimera® 1.12> Tools> Structur Editing> Build Structure> software program. Smile strings> Apply. Then the stages of geometry optimization and energy minimization are the same as the dengue envelope virus. Ligand files are stored in .pdb (protein data bank) format.

Docking

The Envelope.pdb and ligan.pdb files are opened with the UCSF Chimera® 1.12 software program, then the next step is docking using Autodock® Vina. A grid on the β-OG pocket binder section with the grid box dimensions is -13.1523 x 57.4627 x 4.45881. The number of bond positions is 10, the exhaustiveness value is 8, and the maximum energy difference is 3 kcal / mol.

B. Data Analysis

Analysis of the docking results includes the scoring function, the selection of the conformation of the ligand-envelope docking results and the bonding that occurs on the results of the ligand-envelope docking.

III. RESULTS AND DISCUSSION

Search for three-dimensional structure and visualization of dengue virus envelopes

In the search for the three-dimensional structure of the dengue virus envelope, the Research Collaboratory for Structural Bioinformatics Protein Data Bank repository can be accessed via the internet at the site address http://www.rcsb.org/pdb/. This repository contains data in PDB format where the PDB format can be used and by inputting in the molecular docking stage. There is a lot of GDP data that is on the site http://www.rcsb.org/pdb/, so we need keywords in the form of protein names or PDB code of the object we are looking for. The search for the PDB protein code is done by entering keywords related to the dengue virus envelope virus in the search field on the site's home page. Code 1OKE was chosen which is the structure of dengue virus envelope protein which in code 1OKE is a three-dimensional crystal structure determined by the x-ray diffraction method and was published in 2003. In the structure of dengue virus envelope protein with code 1OKE there is also a protein envelope binds to the comparison ligand, n-octyl-beta-D-glucoside.

Selection of ligands as β-OG pocket binder inhibitors in dengue virus envelopes

For the selection of ligands as β-OG pocket binder envelope dengue virus inhibitors are natural ligands derived from mangosteen (*Garcinia mangostana* L.) which mostly

contain xanthone compounds, especially in the skin of the fruit. The xanthone compounds that will be tested are seven compounds namely; alpha-mangostin, beta-mangostin, gamma-mangostin, gartanin, mangostanol, mangostinone and trapezifolixanthone. The reason for choosing the xanthone compound is that xanthone compound is a natural compound which is still not well known for its function as an antiviral and also natural compounds can function as the main structure for the development of new drug molecules.

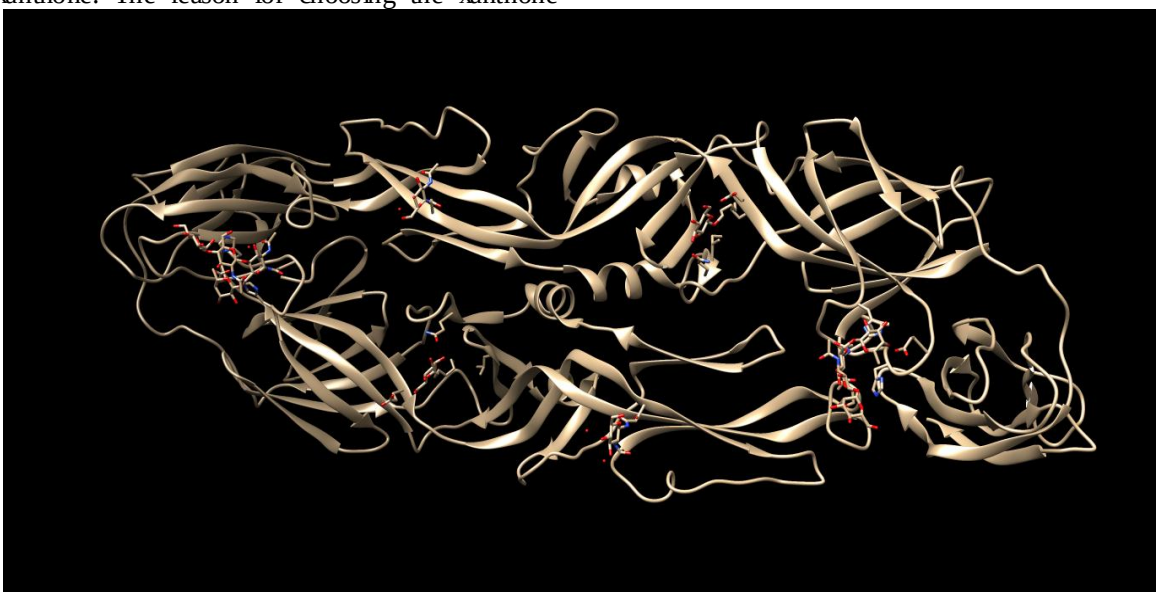


Fig.1. Visualization of the structure of dengue virus envelope proteins

Geometry optimization and energy minimization of the three-dimensional structure of dengue virus ligands and envelopes

The process of geometry optimization and energy minimization is performed using the UCSF Chimera® 1.12 software. Before the process is run, there are several parameters that must be chosen including force fields, algorithms and residual restrictions. The process is carried out using a molecular mechanics system with an AMBER force field with gasteiger residue parameters suitable for ligands, proteins and DNA. The results of geometry optimization and minimization of ligands and envelope proteins can be seen in Figure 2 and Figure 3. The use of algorithms in energy optimization and minimization of the three-dimensional structure of proteins aims to make the process run faster and more efficient because dengue virus envelope proteins are macromolecules with large atom numbers. Although the three-dimensional structure of protein is obtained from the Protein Data Bank, energy optimization and minimization must be carried out because the crystallization process of proteins or enzymes is carried out in a vacuum so that the results of crystallization in the Protein Data Bank cause a shift in the length and angle of the bonds in the three-dimensional structure. Similarly, for ligands obtained optimization is done in the same way.

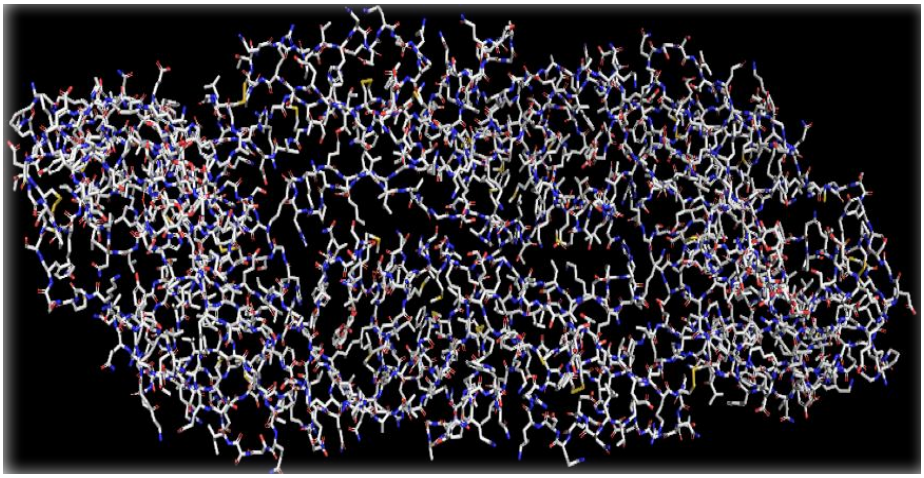


Fig. 2. Results of geometry optimization and energy minimization in the structure of dengue virus envelope protein

The purpose of the process of geometry optimization and energy minimization is to eliminate bad contact from structures, namely irrational interactions that arise in the molecular system by referring to the real state of a particular system so that the geometry of the structure will be obtained that is appropriate or close to the actual state in conditions at natural or real conditions. This process uses mathematical equations to determine the best combination of bond lengths and bond angles that produce the lowest energy. This process allows the geometrical structure of the ligand and protein envelope to reach the state of the system with the minimum energy so that the most stable structural conformation is obtained.

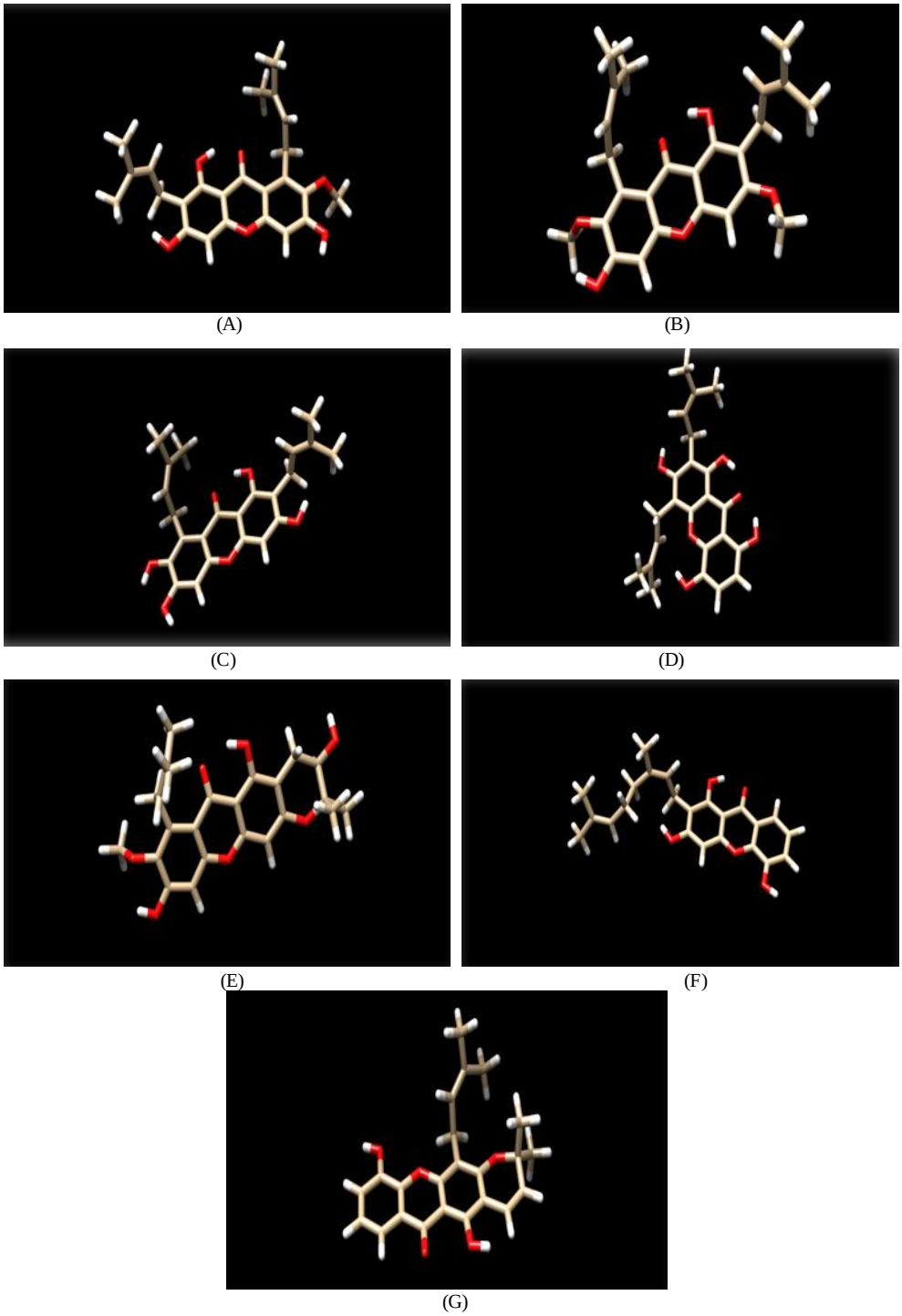


Fig. 3. Results of geometry optimization and energy minimization on the structures of seven xanthone (A) Alfa-mangostin, (B) Beta-mangostin, (C) Gamma-mangostin, (D) Gartanin, (E) Mangostanol, (F) Mangostinone, (G) Trapezifolixanthone

Molecular docking

The type of molecular docking used in this study is the semi-flexible type, where the torsional angle of the ligand is made flexible, and the protein envelope is made rigid because if both are flexible, more complicated software is needed and a very long time in the docking process. The purpose is to do gridding with center coordinates -13.1523 x 57.4627 x 4.45881 and size 22.7781 x 24.366 x 15.6481 in the β-OG pocket binder section of the dengue virus envelope is marking the location where the ligand binding or activation occurs because the part will be included in the calculation and map electrostatic each atom of the ligand with receptors. The dimensions of the grid box used must not be too small because it will be difficult to obtain optimal results and also not too wide or large because there will be a bond in an irrational position that may appear on the results of molecular docking. The settings in the receptor option column, the addition of hydrogen is selected and then given a charge and remove non-polar hydrogen, remove non-binding atoms and water and non-standard residues, this arrangement is done because it can interfere with the docking process when the ligand will bind to the receptor. The settings in the advance option column are selected as many as 10 ligand-receptor bonds, exhaustiveness of 8, and a maximum energy difference of 3 kcal/mol, this arrangement is done to avoid the bonding that should not be possible to occur in the process of docking ligands with protein envelopes offline selected local.

TABLE 1: DOCKING RESULTS OF THE SEVEN XANTHONE COMPOUNDS

Ligand	Bond energy (kcal / mol)	Molecular Weight (g / mol)	Number of hydrogen bonds	Number of hydrogen bonds	Number of hydrogen bonds	Number of hydrogen bonds
Alfa-mangostin	-7.3	410.446	2	23	66	8
Beta-mangostin	-7.2	424.493	2	25	66	8
Gamma-mangostin	-7.7	396.439	2	19	56	8
Gartanin	-7.0	396.439	4	15	28	8
Mangostanol	-8.3	426.465	4	29	49	6
Mangostinon	-8.6	380.44	5	20	60	8
Trapezifolixanthone	-7.8	378.424	4	25	40	4
n-octyl-β-D-glucoside *	-6.3	292.372	6	18	74	13

**** Comparative ligands***

Scoring

The function of the score function is to determine the affinity of the xanthone ligand complex with the β-OG pocket binder protein in the dengue virus envelope formed. This identification is based on Gibbs' free energy theory (ΔGbind). The small Gibbs free energy value states that the conformation formed is stable, while the large Gibbs free energy value states that the complex formed is less stable. The more negative the value produced, the better the affinity of the ligand-protein complex, so that the activity and its potential are better. Meanwhile, the use of algorithms plays a role in determining the most stable conformation (docking pose) of complex formation between xanthone ligands and β-OG pocket binder proteins in dengue envelope viruses.

The data shows that the test ligand has a bond energy value that is equal to or smaller than the comparison ligand (-6.3 kcal / mol). From the results of molecular docking of the seven xanthones compounds, all compounds have a smaller Gibbs free bond energy value (ΔGbind) compared to the comparative compound namely n-octyl-β-D-glucoside, the smaller Gibbs free bond energy states that the conformation is smaller. formed is stable. The more negative the value produced, the better the affinity of the ligand-protein complex, so that its activity is expected to be better. The best results are shown by the compounds Mangostinon and Mangostanol with the value of Gibbs free bond energy (ΔGbind) of -8.6 kcal / mol for Mangostinone and -8.3 kcal / mol for Mangsotanol.

Conformation of Ligands on the Binding Site

The ligand conformation to the binding site of protein envelope was visualized using PyMOLTM software. The results of molecular docking showed that mangostanol and mangostinone ligands possess the appropriate shape and conformation when compared to comparable ligands. In mangostanol and mangostinone ligands compared to comparative ligands there are similarities in aliphatic side chains that can interact with amino acid residues on the inside of the β-OG pocket binder, whereas mangostanol and mangostinone ligands have aromatic chains that can interact with amino acid residues in the outside of the β-OG pocket binder. This overall shows that the conformation of the mangostanol and mangostinone ligands corresponds to the binding site in the β-OG pocket binder of the dengue virus envelope protein. This conformation compatibility is in accordance with the lock-and-key theory, so mangostanol and mangostinone ligands can have a potential inhibitory effect that is able to inhibit the conformational activity of the dengue virus envelope protein. The results of conformation can be seen in Figure 5.

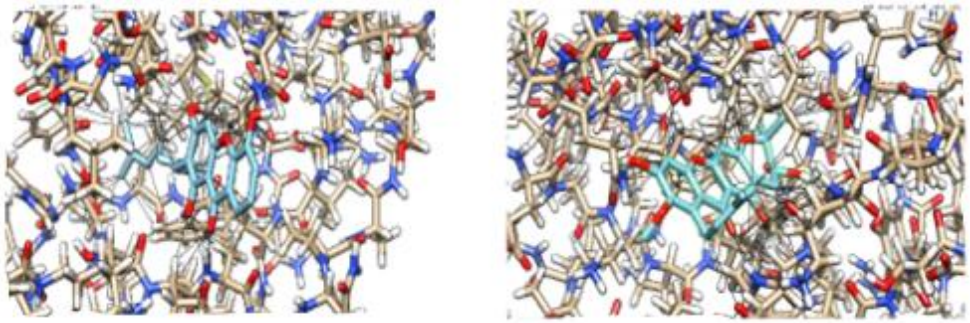
Intermolecular Interaction

Intermolecular interactions that occur in the comparative protein-ligand complex and mangostinone and mangostanol protein can be seen using the help of UCSF Chimera® 1.12 software. Intermolecular interactions that occur between ligands with proteins can increase the affinity of ligand bonds with proteins, it is necessary to know the intermolecular interactions that occur. There are 3 types of intermolecular interactions that are likely to occur, namely:

1. Hydrogen bonds, which are bonds between hydrogen atoms and amino acid protein envelope residues. The condition for hydrogen bonding is if the distance between hydrogen and the electronegative atom.
2. Electrostatic interactions, namely interactions between oxygen ligand atoms and amino acid protein envelope residues. This electrostatic interaction can occur when negatively charged atoms interact with positive partial molecules or vice versa, the magnitude of this electrostatic interaction depends on the relative molecular mass.
3. Van Der Waals Interaction, namely the interaction between carbon atoms in ligands with amino acid residues of protein envelopes. Van Der Waals interactions occur between molecules caused by polarity in the molecule caused by the molecule itself or polarity that occurs due to molecules induced by other atoms which are charged so that the molecule is polar for a moment spontaneously.

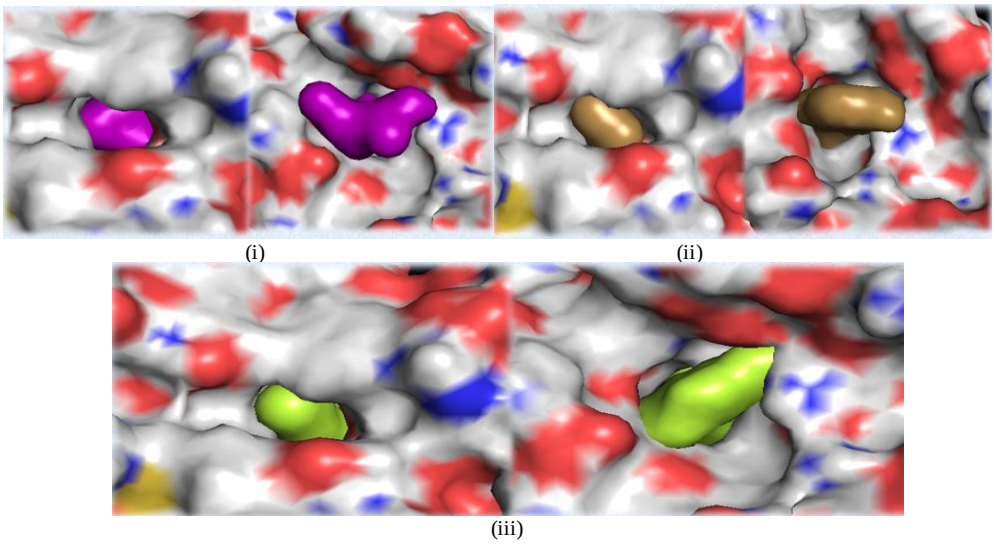
The amount of energy generated from hydrogen bonds and electrostatic interactions is greater than the energy generated by Van Der Waals interactions. This is caused by the presence of atoms which have both charge on hydrogen bonds and electrostatic interactions.

In the mangostanol and mangostinone compounds hydrogen bonds occur less than the comparative ligands, but for electrostatic interactions that occur more than the comparative ligands. The amount of electrostatic interaction which is quite a lot is one of the factors causing the energy binding of mangostanol ligands (-8.3 kcal / mol) and mangostinone (-8.6 kcal / mol) smaller than the comparative ligand (-6.3 kcal / mol).



(i) (ii)

Fig. 4. Contact of the Mangostinone
(i) ligand and the Mangostanol
(ii) ligand with the residue amino protein envelope of the dengue virus in the β-OG pocket binder



(i) (ii)

(iii)

Fig. 5. Conformation and orientation of the Mangostanol
(i), Mangostinone, (ii) and comparative ligand, (iii) ligands in the β-OG pocket binder section

IV. CONCLUSION

This in silico study used seven xanthone compounds from mangosteen rind as a ligand. The n-octyl-β-D-glucoside compound is a compound used as a comparison because it has inhibitor activity in the β-OG pocket binder contained in the dengue virus protein envelope. The molecular docking study aims to predict the strength of a ligand's affinity for protein because the potential of a compound as a ligand is influenced by its affinity. From the results obtained, the seven xanthone compounds have the ability to inhibit protein envelope conformation in dengue virus because they are able to interact with binding sites in the β-OG pocket binder section contained in dengue virus envelope protein and molecular docking results show that all xanthone ligands have affinity and potential better inhibition compared to comparative ligands with the best results of mangostanol and mangostinone compounds with binding energy of -8.3 kcal / mol for mangostanol and -8.6 kcal / mol for mangostinone. There is a conformation of the conformation of mangostanol and mangostinone ligands with binding sites in the β-OG pocket binder contained in the dengue virus envelope protein so that it can act as a potential inhibitor with the best affinity.

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