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Anti-Malarial Activity in Plasmepsin II Inhibitors Using Molecular Docking

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Abstract – The biggest challenge for indonesians is finding more efficient malaria drugs to overcome malaria by utilizing indonesian medicinal plants. Nowadays, increased computing capabilities are the solution to finding new drugs that use the insilico approach. In this study will be conducted an analysis of antimalarial activity between plasmepsin II receptors and ligands that act as inhibitors through the method of study in silico. The ligands used are derived from the filtering of the Chemical Database namely Cyanidin 3,5-di-(6-malonylglucoside); Isoscutellarein 4'methyl ether 8-(6"-n-butylglucuronide); Cyanidin 3-(6"-malonylglucoside)- 5-glucoside; Delphinidin 3-(2-rhamnosyl-6-malonylglucoside); Cyanidin 3-[6-(6-sinapylglucosyl)-2-xylosylgalactoside; and Delphinidin 3-(6- malonylglucoside)-3',5'-di-(6-p coumaroylglucoside). This research method is divided into three stages. The first is the preparatory stage, at this stage the downloaded plasmepsin II receptors are then prepared using Pymol software. The second stage is docking simulation. The software used in doing this simulation is autodock vina software. The third stage is the Analysis stage. The docking simulation results were then analyzed using Pymol and VMD software to see the activity resulting from docking simulations performed between receptors and ligands. The results of the study are among the six ligandssimulated in plasmepsin II receptors then the six ligands are stable if used as candidates for new drugs, this is because the free energy produced from docking is low and negative value. This means that the reaction that occurs is a spontaneous reaction. The free energy value between the six ligands is the difference between the smallest and the largest not too far in the range of only about -2 kcal/mol. This means the six could be used as drug candidates.

Keywords – Malaria, Docking, Drug, Plasmepsin II, Ligand.

I. INTRODUCTION

Malaria is an infectious disease caused by Plasmodium consisting of many species, but what generally causes malaria are *Plasmodium vivax, Plasmodium falciparum, Plasmodium malariae,*and *Plasmodium ovale.* Malaria is transmitted by the *Anopheles* mosquito which in its body contains *Plasmodium.* The spread and endemicity of Malaria is strongly influenced by the presence of the anopheles mosquito sanctuary as an infectious vector. Malaria is one of the infectious diseases other than HIV AIDS and Tuberkolusis whose control is part of the Sustainable Development Goals (SDGs) as a global commitment that must be achieved by the end of 2030. At the national level, malaria elimination program is determined through the Decree of the Minister of Health of the Republic of Indonesia Number 293/Menkes/SK/IV/2009 dated April 28, 2009 concerning "Malaria Elimination in Indonesia". The target of malaria elimination program is that all regions in Indonesia are free of malaria by 2030. Malaria elimination assessment starts from district/city level.

In 2019 there are three provinces whose entire districts /cities have been declared malaria-free, namely DKI Jakarta, Bali, and East Java. Five provinces in eastern Indonesia do not have malaria elimination districts/cities, namely East Nusa Tenggara, Maluku, North Maluku, West Papua, and Papua. The malaria pain rate is illustrated by the Annual Parasite Incidence (API) indicator per 1,000 inhabitants, which is proporsi among malaria positive patients against at-risk populations in the region with a constant of

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1,000. API malaria in Indonesia in 2019 increased compared to 2018, from 0.84 to 0.93 per 1,000 inhabitants. This is still a challenge in eliminating malaria in Indonesia (Kementerian Kesehatan Republik Indonesia, 2020).

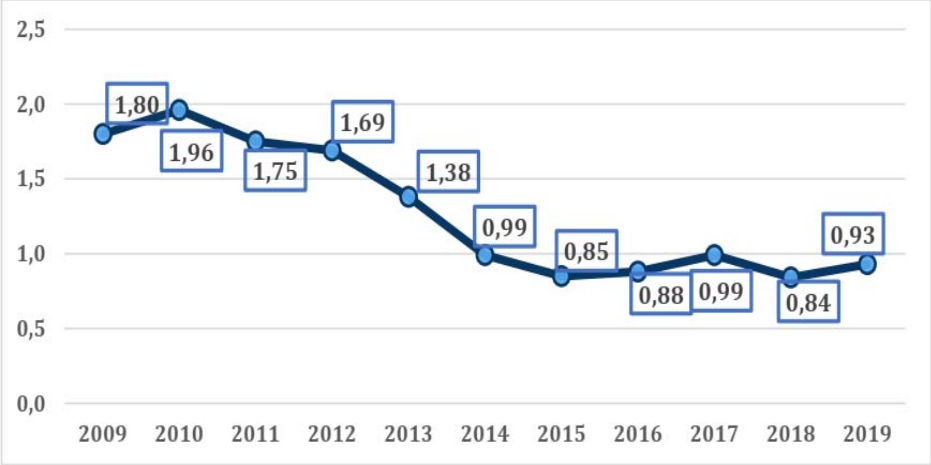


Figure 1. *Annual Paracite Incidence* (API) Per 1,000 Residents in 2009-2019

The government views malaria as still a threat to public health status, especially in people living in remote areas. This is reflected by the issuance of Presidential Regulation Number: 2 of 2015 on Medium-Term Development Plan nasional year 2015 - 2019 where malaria is a priority disease that needs to beaddressed. One of the biggest challenges in malaria treatment efforts in Indonesia is the decrease in efficacy in the use of some anti-malarial drugs, there is even resistance to chloroquine. This can be due in part to the irrational use of anti-malarial drugs (Kementerian Kesehatan Republik Indonesia, 2017).

Plasmodium falciparum is the most dangerous type of plasmodium cause of malaria (White, 2004). There are three stages of the life cycle *of Plasmodium falciparum one stage when in the body of mosquitoes and two stages in the human body, namely in the liver* (pre-erythrocytes) and blood (erythrocytes). Usually there are symptoms of malaria that is when merozoite is released from the liver to the blood so that merozoite will attack red blood cells. In red blood cells, parasites will degrade hemoglobin in order to produce amino acids that will be used for the development of *Plasmodium falciparum* (Khan and Waters, 2004). In eucariot, *aspartic protease* of *Plasmodium falciparum,*involved in the process of degradation of hemoglobin into large fragments that will later be further degraded into amino acids. Plasmepsin is an aspartic protease enzyme of *the Plasmodium falciparum* species that has a major role in the initial division process of hemoglobin and is then followed by other protease enzymes (francis et.al., 2011)

Because of the vital role of plasmepsin in the life cycle *of plasmodium falciparum* in the infected human body makes this enzyme suitable for analysis as a potential drug target in the search for new antimalarial drugs (Banerjee et al., 2002; Coombs et al., 2001). Based on previous research, 10 types of plasmepsin have been identified, of which plasmepsin II is the best isoform plasmepsin enzyme that has been studied (Ersmark et al., 2006).

Medical chemistry is a science that studies methods in finding new chemical drug compounds, optimizations and development processes that ultimately find drug molecules that are efficient and useful in treating a particular disease. The steps taken to achieve that goal are medical chemists in addition to designing and synthesizing new molecules as well as ensuring the interaction of these molecules with the biological macromolecules that become their receptors. In addition, it should also be known the relationship between the structure and biological activity and evaluate the process of metabolic changes caused (Nogrady, 2005).

The use of computers as a tool in the discovery of new drugs is an efficient, easy and inexpensive solution. By utilizing computers as preliminary research it will help researchers to streamline their work and cut the cost of research because if done by conventional methods it would be very expensive. Exponentially improved computing capabilities are opportunities to develop simulations and calculations in drug design. *In silico* is a method of approaching a real condition or state into a computer simulation using a specific program. (Geldenhuys, 2006).

Today, the field of Health has begun to look at plants as the basic ingredients of making medicines. This is because the use of synthetic chemical drugs has an impact or side effects that damage the body. So that the use of plants as medicine or commonly called herbal medicine globally experienced a significant increase (Oladunmoye & Kahinde, 2011).

Therefore, this review emphasizes on various local plants of Indonesia as medicinal plants that have the potential to be used in the prevention of malaria. In this study, an analysis of antimalarial activity between plasmepsin II receptors and ligands that act as inhibitors through the method of study in *silico*. The ligands used are derived from the filtering results from the Plant Chemistry Database that has been done before. The ligands used are *Cyanidin 3,5-di-(6-malonylglucoside); Isoscutellarein 4'methyl ether 8-(6"-n-butylglucuronide); Cyanidin 3-(6"-malonylglucoside)- 5-glucoside; Delphinidin 3-(2-rhamnosyl-6-malonylglucoside); Cyanidin 3-[6-(6-sinapylglucosyl)-2-xylosylgalactoside;* and *Delphinidin 3-(6- malonylglucoside)-3',5'-di-(6-p-coumaroylglucoside)* (Eko, 2017).

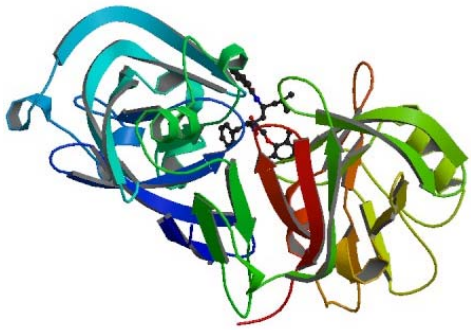
II. Research Methods

2.1 Hardware and Software

Docking simulation conducted using Lenovo brand laptop with windows 10 operating system with eight gigabyte RAM (Random Access Memory) specification, Ryzen series 4000 processor. The software used for docking simulation preparation is VMD (Visual*Molecular Dynamics Program)*version 1.9.1, Autodock tools. For docking simulation process using Autodock vina 1.1.1 software and for analysis of simulation results using VMD software (Visual*Molecular Dynamics Program)*version 1.9.1, Autodock tools, and Pymol.

2.2 Materials

In this study, the aspartic protease receptor analyzed was plasmepsin. The chosen plasmepsin is plasmepsin II because it is a widely researched plasmepsin (Friedman & Caflisch, 2007). The plasmepsin receptors used in this study were downloaded from protein data bank with http://www.rscb.org/pdb site with the identity of 1LEE in the form of monomers (Figure 2). Plasmepsin receptors in the form of experimental data results of X-Ray *Diffraction* is the coordinate data of the *three-dimensional* structure of the enzyme Plasmepsin. Meanwhile, ligands used as candidates for new malaria drugs are ligan from medicinal plants in Indonesia contained in the Indonesian Medicinal Plants Database (Table 1). The ligand compound used is a three-dimensional structure downloaded from the PubChem *Database* with the https://pubchem.ncbi.nlm.nih.gov site.



[Source: downloaded from http://rcsb.org]

Figure 2. Struktur crystalline plasmepsin II of Plasmodium falciparum withN inhibitor R36

Table 1. Ligan Compounds from Indonesian Medicinal Plant Database

o.	**ompounds**
	yanidin 3-(6"-malonylglucoside)- 5-glucoside
	oscutellarein 4'methyl ether 8-(6"-n-butylglucuronide)
	yanidin 3,5-di-(6-malonylglucoside)
	elphinidin 3-(2-rhamnosyl-6-malonylglucoside)
	yanidin 3-[6-(6-sinapylglucosyl)-2-xylosylgalactoside
	elphinidin 3-(6- malonylglucoside)-3',5'-di-(6-p-coumaroylglucoside)

(Eko et al, 2017)

2.3 Method

The study was divided into three stages. The first is the preparatory stage, at this stage the downloaded plasmepsin II receptors are then prepared using Pymol software. It turns out that the three-dimensional structure of the downloaded plasmepsin II receptor is a three-dimensional structure that has bonded with its natural ligand. So at this stage, the natural ligand is removed first. In addition there are still solvents in the three-dimensional structure of plasmepsin II receptors. Then the solvent must also be removed. Then the predicted three-dimensional structure of plasmepsin II is stored in .pdb format. After that, the three-dimensional structure of receptors and ligands is converted using Autodock tools (ADT) software into files with a .pdbqt extension. Then prepared box place receptors and ligands when conducted docking simulation. The grid box is a measure that limits ligands when docking with receptors. The specified grid box size is adjusted to the size of the receptor. In this study the size of the boss grid used are:

center_x = 32.336
center_y = 45.963
center_z = 22.641
size_x = 82
size_y = 118
size_z = 94

The second stage is docking simulation. The software used in doing this simulation is autodock vina software. In the simulation stage docking required config file, the contents of which is the size of the grid box that has been determined at the preparation stage. In addition, receptor files and ligands have been prepared in the first stage. Docking simulations are performed at *Command Prompt* by using commands to perform simulations. After the simulation is completed, the final result obtained from this simulation is several docking modes between receptors and ligands along with Affinity (kcal/mol) values. Repeated 5 times to get consistent and stable results.

The third stage is the Analysis stage. The docking simulation results were then analyzed using Pymol and VMD software to see the activity resulting from docking simulations performed between receptors and ligands. The observed parameter is gibbs free energy produced when simulating docking between ligands and receptors.

III. Results And Discussion

3.1 Receptors

Plasmepsin II receptors downloaded from the database of protein data banks (PDB) namelythree-dimensional trucktur enzymes with pdb code 1ILEE, this three-dimensional structure is obtained from the crystallization using x-ray diffraksi with a resolution of 1.9 Amstrong and has residue 331 (Asojo, 2002). From the visualization using VMD 1.9.3 software, it was found that the three-dimensional structure of plasmepsin II receptors has 1 chain, namely A. In receptors bound to one ligand, R36.

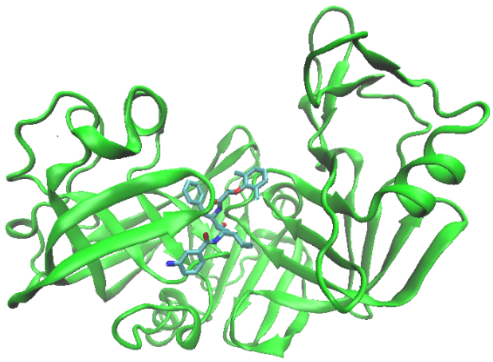


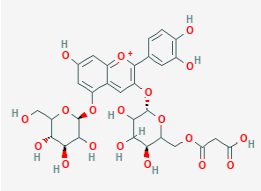
Figure 3. Three-dimensional structure of Plasmepsin II receptors with R36 ligands

3.2 Ligands

1. Cyanidin 3-(6”-malonylglucoside)- 5-glucoside

The compound *Cyanidin 3-(6''-malonylglucoside)- 5-glucoside* is a flavonoid compound of anthocyanin class derived from the family Lamiaceae with a plant species of origin namely *Thymus serpyllum* or known by the name of serpili. Anthocyanin compounds are known to have antimalarial activity (Yanuar et al, 2011).

Table 2. Cyanidin Compound 3-(6''-malonylglucoside)- 5-glucoside

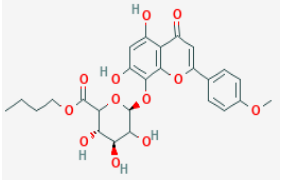
PubChem CID	44256758
IUPAC Name	3-[[(3*S*,6*S*)-6-[2-(3,4-dihydroxyphenyl)-7-hydroxy-5-[(2*S*,4*S*,5*S*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxychromenylium-3-yl]oxy-3,4,5-trihydroxyoxan-2-yl]methoxy]-3-oxopropanoic acid
Structure	
Molecular Formula	$C_{30}H_{33}O_{19}^{+}$
Chemical Names	Cyanidin 3-(6''-malonylglucoside)-5-glucoside LMPK12010153
Molecular Weight	697.6 g/mol
Hydrogen Bond Donor Count	11
Hydrogen Bond Acceptor Count	18

(PubChem : https://pubchem.ncbi.nlm.nih.gov/compound/44256758)

2. Isoscutellarein 4’methyl ether 8-(6”-n-butylglucuronide)

The compound *Isoscutellarein 4'methyl ether 8-(6''-n-butylglucuronide)* is a flavonoid glucuronide derived from the family *Sterculiaceae* with a plant species of origin namely *Helicteres isora* or puteran (Sunda). Natural flavonoids as well as synthesis show antimalarial activity (Yanuar et al, 2011).

Table 3. Compound Isoscutellarein 4'methyl ether 8-(6''-n-butylglucuronide)

PubChem CID	44258582
IUPAC Name	butyl (3*S*,4*S*,6*S*)-6-[5,7-dihydroxy-2-(4-methoxyphenyl)-4-oxochromen-8-yl]oxy-3,4,5-trihydroxyoxane-2-carboxylate
Structure	

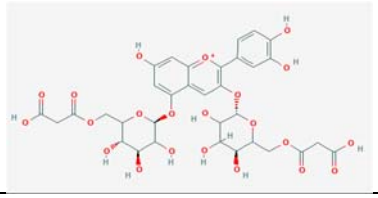
Molecular Formula	$C_{26}H_{28}O_{12}$
Chemical Names	Isoscutellarein 4'-methyl ether 8-(6"-n-butylglucuronide) LMPK12111355
Molecular Weight	532.5 g/mol
Hydrogen Bond Donor Count	5
Hydrogen Bond Acceptor Count	12

(PubChem : https://pubchem.ncbi.nlm.nih.gov/compound/44258582)

3. Cyanidin 3,5-di-(6-malonylglucoside)

The compound *Cyanidin 3,5-di-(6-malonylglucoside)* is a flavonoid compound of anthocyanin class derived from the family Lamiaceae with a plant species of origin namely *Thymus serpyllum* or known by the name of flakes. Anthocyanin compounds are known to have antimalarial activity. One of them is found in *Corchorus olitorius* containing anthocyanins and is known to inhibit malaria parasites namely *Plasmodium falciparum* above 96% (Morris & Wang, 2007).

Table 4. Cyanidin Compound 3,5-di-(6-malonylglucoside)

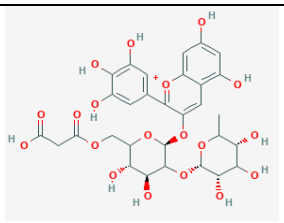
PubChem CID	**44256761**
IUPAC Name	3-[[(3*S*,4*S*,6*S*)-6-[3-[(2*S*,5*S*)-6-[(2-carboxyacetyl)oxymethyl]-3,4,5-trihydroxyoxan-2-yl]oxy-2-(3,4-dihydroxyphenyl)-7-hydroxychromenylium-5-yl]oxy-3,4,5-trihydroxyoxan-2-yl]methoxy]-3-oxopropanoic acid
Structure	
Molecular Formula	$C_{33}H_{35}O_{22}^{+}$
Chemical Names	Cyanidin 3,5-di-(6-malonylglucoside) LMPK12010156
Molecular Weight	783.621 g/mol
Hydrogen Bond Donor Count	11
Hydrogen Bond Acceptor Count	21

(PubChem : https://pubchem.ncbi.nlm.nih.gov/compound/44256761)

4. Delphinidin 3-(2-rhamnosyl-6-malonylglucoside)

Delphinidin compound 3-(2-rhamnosyl-6-malonylglucoside) is a flavonoid compound of anthocyanin class derived from the family Fabaceae with a plant species of origin namely Clitoria ternatea or known as blue flower (Maluku). Anthocyanin compounds are known to have antimalarial activity.

Table 5. Delphinidin Compound 3-(2-rhamnosyl-6-malonylglucoside)

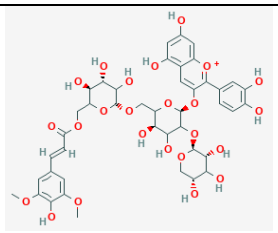
PubChem CID	44256924
IUPAC Name	3-[[(3*S*,4*S*,6*S*)-6-[5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)chromenylium-3-yl]oxy-3,4-dihydroxy-5-[(2*S*,3*S*,5*R*)-3,4,5-trihydroxy-6-methyloxan-2-yl]oxyoxan-2-yl]methoxy]-3-oxopropanoic acid
Structure	
Molecular Formula	$C_{30}H_{33}O_{19}^{+}$
Chemical Names	Delphinidin 3-(2-rhamnosyl-6-malonylglucoside) LMPK12010319
Molecular Weight	697.6 g/mol
Hydrogen Bond Donor Count	11
Hydrogen Bond Acceptor Count	18

(PubChem : https://pubchem.ncbi.nlm.nih.gov/compound/44256924)

5. Cyanidin 3-[6-(6-sinapylglucosyl)-2-xylosylgalactoside

Cyanidin compound 3-[6-(6-sinapylglucosyl)-2-xylosylgalactoside is a flavonoid compound class anthocyanin derived from the family Apiaceae with plant species of origin namely Foeniculum vulgare or known by the name of fenor (Java) and Apium graveolens or known as Saladri (Sunda). Anthocyanin compounds are known to have antimalarial activity.

Table 6. Cyanidin Compound 3-[6-(6-sinapylglucosyl)-2-xylosylgalactoside

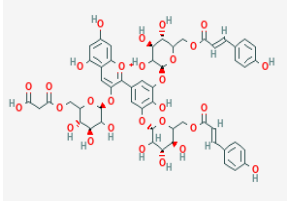
PubChem CID	44256710
IUPAC Name	[(3*S*,4*S*,6*R*)-6-[[(3*R*,6*S*)-6-[2-(3,4-dihydroxyphenyl)-5,7-dihydroxychromenylium-3-yl]oxy-3,4-dihydroxy-5-[(2*S*,3*R*,5*R*)-3,4,5-trihydroxyoxan-2-yl]oxyoxan-2-yl]methoxy]-3,4,5-trihydroxyoxan-2-yl]methyl (*E*)-3-(4-hydroxy-3,5-dimethoxyphenyl)prop-2-enoate
Structure	
Molecular Formula	$C_{43}H_{49}O_{24}^{+}$
Chemical Names	Cyanidin 3-[6-(6-sinapylglucosyl)-2-xylosylgalactoside] LMPK12010105
Molecular Weight	949.8 g/mol
Hydrogen Bond Donor Count	13
Hydrogen Bond Acceptor Count	23

(PubChem : https://pubchem.ncbi.nlm.nih.gov/compound/44256710)

6. *Delphinidin 3-(6- malonylglucoside)-3',5'-di-(6-p-coumaroylglucoside)*

Delphinidin compound 3-(6- malonylglucoside)-3',5'-di-(6-p-coumaroylglucoside) is a flavonoid compound of anthocyanin class derived from the family Fabaceae with a plant species of origin namely Clitoria ternatea or known as blue flower (Maluku). Anthocyanin compounds are known to have antimalarial activity.

Table 7. Delphinidin Compound 3-(6- malonylglucoside)-3',5'-di-(6-p-coumaroylglucoside)

PubChem CID	44256933
IUPAC NAME	3-[[(3*S*,4*S*,6*S*)-6-[5,7-dihydroxy-2-[4-hydroxy-3,5-bis[[(2*S*,4*S*,5*S*)-3,4,5-trihydroxy-6-[[(*E*)-3-(4-hydroxyphenyl)prop-2-enoyl]oxymethyl]oxan-2-yl]oxy]phenyl]chromenylium-3-yl]oxy-3,4,5-trihydroxyoxan-2-yl]methoxy]-3-oxopropanoic acid
Structure	
Molecular Formula	$C_{54}H_{55}O_{29}^{+}$
Chemical Names	Delphinidin3-(6-malonylglucoside)-3',5'-di-(6-p coumaroylglucoside)

	LMPK12010328
Molecular Weight	1168 g/mol
Hydrogen Bond Donor Count	15
Hydrogen Bond Acceptor Count	28

(PubChem : https://pubchem.ncbi.nlm.nih.gov/compound/44256933)

3.3 Docking Analysis

Gibbs free energy (ΔG *binding)* is one of the parameters that can be used to determine the stability of the bond between receptors and ligands. If the free energy of an interacting molecule is lower so the bond formed is stable and the reaction occurs is a spontaneous reaction. Conversely, if free energy is getting higher so the reaction is unstable and the reaction that arises is a non-spontaneous reaction. This is known as thermodynamic equilibrium, i.e. if free energy is negative then there will be a spontaneous reaction, meaning that the conformation formed is more stable (Nelson *et al.* 2008).

Table 8. Energy Bond Receptors and Ligands

o.	**ompounds**	**ibbs free energy (ΔG*binding)***
	yanidin 3-(6"-malonylglucoside)- 5-glucoside	- 9.5
	oscutellarein 4'methyl ether 8-(6"-n-butylglucuronide)	- 8.7
	yanidin 3,5-di-(6-malonylglucoside)	- 8.5
	elphinidin 3-(2-rhamnosyl-6-malonylglucoside)	- 10.5
	yanidin 3-[6-(6-sinapylglucosyl)-2-xylosylgalactoside	- 9.9
	elphinidin 3-(6- malonylglucoside)-3',5'-di-(6-p-coumaroylglucoside)	- 10.2

In table 8, it can be seen that from between the 6 ligands that are distilled with plasmepsin II receptors, the six ligands produce free energy that is negative value, meaning that all ligands are stable and the reaction is spontaneous. The value of free energy between the six ligands is also not very large which is only about -2 kcal / mol from among the lowest and the highest. If analyzed the six ligands used it turns out that the six ligands are flavonoid compounds that are known to have good activity as antibacterial or anti-malarial. In contrast to the compound *Lycopene* that has also previously been studied free energy bonding on plasmepsin II receptors namely - 5.9 kcal / mol. This value is lower than the six ligands studied in this study. This is because the compound *Lycopene* is an isoprenoid compound and not a flavonoid compound, this results in the compound *Lycopene* has antimalarial activity compared to the six ligands studied (Malau, 2020). The most stable ligand among the six ligands that are diocking is the *Delphinidin 3-(2-rhamnosyl-6-malonylglucoside)* ligand which has free energy of -10.5 kcal/mol. While the most unstable ligand among the six ligands that are diockingkan is the *3.5-di-(6-malonylglucoside) Cyanidin* ligand with free energy of -8.5 kcal/mol.

IV. Conclusion

Among the six ligands that are attached to plasmepsin II receptors, the six ligands are stable if used as candidates for new drugs, this is because the free energy produced from docking is low and negative. This means that the reaction that occurs is a spontaneous reaction. If analyzed based on the free energy of the six dockings it was found that the most stable ligand compound is the ligand *Delphinidin 3-(2-rhamnosyl-6-malonylglucoside)* which has free energy of *-10.5* kcal /mol. While the most unstable ligand among the six ligands that are diockingkan is the *3.5-di-(6-malonylglucoside) Cyanidin* ligand with free energy of -8.5 kcal/mol. The free energy value between the six ligands is the difference between the smallest and the largest not too far in the range of only about -2 kcal/mol. This means the six could be used as drug candidates.

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