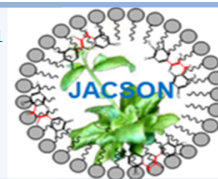




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### ANALYSIS DOCKING OF *PLASMODIUM FALCIPARUM* ENOYL ACYL CARRIER PROTEIN REDUCTASE (PFENR) WITH ORGANIC COMPOUNDS FROM VIRTUAL SCREENING OF HERBAL DATABASE

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**Abstract.** Malaria is one of problematic infectious diseases worldwide. The absence of an effective vaccine and the spread of drug resistant strains of *Plasmodium* clearly indicate the necessity for the development of new chemotherapeutic agents. Recent method being developed is searching a new drug of antimalarial using in silico screening, or also known as virtual screening. One of enzyme target that important for growth of the malaria parasite is *Plasmodium falciparum* Enoyl Acyl Carrier Protein Reductase (PfENR). Inhibition of this enzyme cause the fatty acid biosynthesis type II will be terminated. In this research, in silico screening was performed using AUTODOCK VINA software to find inhibitor candidates of PfENR by using ligands from the database of Medicinal Plants in Indonesia. On the AUTODOCK VINA software molecular docking experiments were performed between ligands and macromolecule target PfENR. This target that has been optimized with residue removal and charges addition. Ligand is expected to be the PfENR inhibitors.

**Keywords:** docking, PfENR, malaria, ligand, receptors

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## 1. Introduction

Malaria is an infectious disease that can be found in almost all parts of the world, especially in tropical and sub-tropical countries. According to the World Health Organization (WHO), in 2012 there was a spread of malaria in 99 countries from 104 countries which in the WHO data, Indonesia was one of the 99 countries (WHO, 2012). According to the Indonesian Ministry of Health's Directorate of Animal-Based Disease Control, in 2012 there were 417,819 positive cases of malaria in Indonesia.

The efforts made to control and eradicate malaria have been many, however, the disease has developed again due to increased cases of resistance to some antimalarial drugs, vector resistance to insecticides, and human migration from one region to another (Fauci et al, 2008). More than 40% of the world's population lives in malaria endemic areas at risk of contracting malaria (Aguir, 2012). Malaria can directly cause anemia and can reduce work productivity. This disease is also still endemic in most parts of Indonesia. According to 2014 Ministry of Health data, the incidence of malaria in Indonesia in 2014 was 1%. The five provinces with the highest malaria incidence include Papua (29.57%), West

Papua (20.85%), NTT (12.81%), Maluku (6.0%), and North Maluku (3.32 %) (Indonesian Ministry of Health, 2011).

Malaria is an erythrocyte infectious disease caused by protozoan parasites from the *Plasmodium* genus. The protozoa enters the human body through the intermediaries of the female *Anopheles* mosquito. There are four *Plasmodium* species which commonly infect humans, that is *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. Of the four species, the most common cause of severe disease and even death is *P. falciparum*, the disease is commonly called tropical malaria / cerebral malaria (Katzung, 2006) while other species generally only cause fever and sometimes anemia (Fauci et al, 2008 & Brunton, 2006).

To overcome infections caused by plasmodium, it is necessary to provide safe, effective, practical and economically affordable anti-malaria drugs to reduce deaths from malaria. There are several malaria drugs that are developed by inhibiting or killing the asexual forms of malaria parasites in human erythrocytes, such as quinine, chloroquine, pyrimethamine, sulfonamides, sulfones and artemisinin derivatives. To overcome infections caused by plasmodium, it is necessary to provide safe, effective, practical and economically affordable anti-malaria drugs to reduce deaths from malaria. There are several malaria drugs

that are developed by inhibiting or killing the asexual forms of malaria parasites in human erythrocytes, such as quinine, chloroquine, pyrimethamine, sulfonamides, sulfones and artemisinin derivatives. The problem is that lately the above drugs mostly fail in the healing process caused by parasites that become resistant to malaria drugs (Harijanto et al, 2009). Some suspected causes of malaria resistance to antimalarials, in this case chloroquine is a parasite does not have an active side to bind to chloroquine so that chloroquine malaria drugs cannot be concentrated in erythrocytes (Pribadi & Muljono, 2004)

Several enzymes that play an important role in the life cycle of plasmodium include haematin, hemozoin, plasmepsin, falsipain, falsicillin, dihydrofolate reductase, dihydroperoxide synthase, Plasmodium falciparum Enoyl-ACP reductase (PfENR), and others. Inhibition of these enzymes is a precursor to the development of antimalarial drugs (Sullivan & Krishna, 2005).

In the last decade, there have been potential targets for antimalarials. This target is the type II fatty acid biosynthesis pathway that takes place in Plasmodium falciparum, with a specific target of Plasmodium falciparum Enoyl Acyl Carrier Protein Reductase (PfENR). This target was chosen because it has an important role in the synthesis of fatty acids in the Plasmodium falciparum body (Tasdemir, 2006).

For developing countries such as Indonesia, this clearly requires its own strategy so that the discovery of new drugs that are useful for the community can be achieved but not costly. These efforts are not only carried out by parties engaged in the economic sector, but also by scientists and technologists. Today there is an alternative way to find new drugs raised by computer experts and information technology. During this time the drugs were tested before being marketed using the in vitro and in vivo methods, whereas now the third method that is no less important is the in silico method or inside the computer. The use of computers in the discovery of new drugs aims to improve the efficiency of the simulation process and calculations in drug design. The computer offers an in silico method as a complement to in vitro and in vivo methods commonly used in drug discovery processes. The science that plays a role in this process is bioinformatics. One branch of bioinformatics is in silico screening or in silico screening, which involves a database with relevant molecular structures that are tethered to the target protein. The assessment results are then used as identification of structures with potential further physiological bonds and activities that can be evaluated in experiments (Pripp, 2006).

In this study, an analysis of the mechanism and interaction between Plasmodium falciparum Enoyl Acyl Carrier Protein Reductase (PfENR) and ligands will act as an inhibitor through the in silico study method. The ligand used was derived from the results of screening from the Plant Chemical Database that has been done before. Based on the

results of in silico antimalarial screening using the Indonesian Medicinal Plant Database on the target enzyme Plasmodium falciparum Enoyl Acyl Carrier Protein Reductase (PfENR). These chemical compounds are Kaempferol 3-rhamnosyl- (1-3) -rhamnosyl- (1-6) -glucoside or known as tea plants (Berwi, 2011). The compound is examined for its bonding ability with enzymes through bond-free energy ( $\Delta G$ ) and the resulting inhibition constant ( $K_i$ ). The in silico method that will be carried out to examine these interactions is by molecular docking. This method is also used to save costs and time needed during research. (Wolff, 1996)

## 2. Materials And Methods

The material used is a two-dimensional structure of the Kaempferol 3-rhamnosyl- (1-3) -rhamnosyl- (1-6) -glucoside ligand or tea plant is known that you get online on the database <https://pubchem.ncbi.nlm.nih.gov/>. While the macromolecule file selected as the receptor is the enzyme Plasmodium falciparum Enoyl Acyl Carrier Protein Reductase (PfENR) with the code 1NHG which can be downloaded from the Bank Data Protein (GDP) database at the site <http://www.rscb.org>. The tool used consists of hardware and software. The hardware consists of a computer with the HP Pavilion dv2 laptop specifications (Amd Athlon neo dual core, 2GB ddr2 RAM, 250 seagate HDD, and VGA ati radeon x1200). The software used consists of Autodock Vina, ADT 1.5.6 (The Scripps Research Institute, America, LigPlot + 4.5.3 (Rowan Lawkowski), OS. Ubuntu 14.04 lts.

### Receptor Identification

The protein used as a receptor is the enzyme Plasmodium falciparum Enoyl Acyl Carrier Protein Reductase (PfENR) (GDP code: 1nhg) which was obtained online through the site <http://www.rscb.org/pdb> (Figure 1). The identification process was carried out using Discovery Studio Visualizer 4.0 software based on information obtained from the 1nhg coded Plasmodium falciparum Enoyl Acetyl Carrier Protein Reductase (PfENR) crystallization data conducted by Perozzo et al. (2002).

### 3D Receptor Structure Geometry Optimization

The 1nhg-coded receptor contains the coordinates of all residual atoms, crystallographic structural factors and XRD experimental data from the Plasmodium falciparum Enoyl Acyl Carrier Protein Reductase (PfENR) enzyme so that it can determine the quality of the protein three-dimensional structure. The geometry optimization process was done with ADT 1.5.6 software. The initial stage was the removal of water molecules (H<sub>2</sub>O) around proteins, hetero atoms and natural ligands. Next was the addition of Gasteiger and Hydrogen charges. Files were stored in PDBQT format.

### 3D Ligand Geometry Optimization

The ligand that will be used is Kaempferol 3-rhamnosyl- (1-3) -rhamnosyl- (1-6) -glucoside or known tea plants downloaded online at the database

<https://pubchem.ncbi.nlm.nih.gov/>. The optimization process was done using ADT 1.5.6. The initial stage was nonpolar merged hydrogen gives Gasteiger charge and all files were stored in PDBQT format.

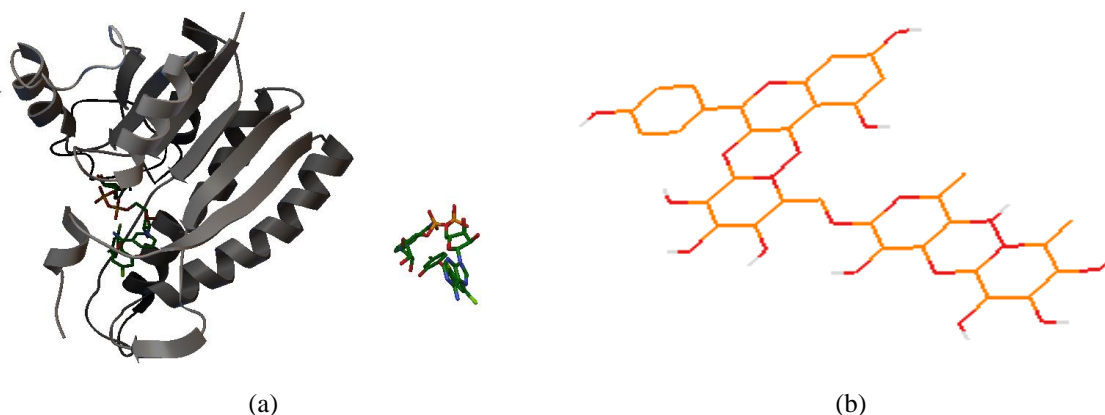
### Molecular docking process

Grid Process and docking parameter validation are carried out with ADT 1.5.6, molecular docking is carried out with AutoDock Vina (Scripps Research Institute, USA) and it is assumed that all rotatable bonds (cyclic bonds) of ligands can rotate (flexible) and the receptor is fixed (rigid) ( Morris et al. 1998). The following parameters are parameters that have been validated. The selected grid box size is 64 x 52 x 64 Å with spacing centered 1,000 Å at the receptor, center\_x

= 46,572, center\_y = 82,254, center\_z = 38,472 and the box area covers the entire receptor structure. Exhaustiveness is set to number 8. The vina folder is placed on the C: \ Vina drive and then filled in the CONF.TXT file with the above parameters according to the center and size numbers. Execute the docking command using commands via the CMD window, call Vina.exe then type the command "C: \ vina --config conf.txt --log log.txt", then press enter and wait for the process to complete. Ligands that have the smallest Gibbs free energy ( $\Delta G$ ) from the list are stored in PDBQT format (ADT 1.5.6) and converted to PDB format with DSV 4.0.

### Analysis

The docking results in the analysis of bond energy ( $\Delta G$ ), hydrogen bond, RMSD value, binding residue, using Ligplot + 4.5.3, for 2D visualization using DSV 4.0 and ADT 1.5.6.



**FIGURE 1.** Visualization of 2D and 3D structures (a). Plasmodium falciparum receptor Enoyl Acyl Carrier Protein Reductase (PfENR) (b). Ligand of Kaempferol 3-rhamnosyl- (1-3) -rhamnosyl- (1-6) -glucoside compound

## 3. Results And Discussion

### Receptor Identification

Three-dimensional structure of the enzyme Plasmodium falciparum Enoyl Acyl Carrier Protein Reductase (PfENR) downloaded through the Protein Data Bank. Three-dimensional structure of Plasmodium falciparum Enoyl Acyl Carrier Protein Reductase (PfENR) enzyme, from the results of crystallization carried out by Perozzo et al. (2002) coded PDB of 1nhg. Visualization of PDB 1nhg coded protein is done using DSV 4.0 software. The visualization results show that there are 4 chains, namely A, B, C and D and there are two natural ligands of TCL and NAD (Figure 1). Both natural ligands must be removed because the enzyme that will be docking must be a pure enzyme. Furthermore, the Ligan used is Kaempferol 3-rhamnosyl-(1-3) -rhamnosyl-(1-6) -glucoside or tea plant known.

### Bonding Energy (Gibbs free energy)

Gibbs free energy ( $\Delta G_{binding}$ ) is a parameter of conformational stability between ligands and receptors.

Thermodynamically the metabolic reactions in the body take place exergonically and endergonically. Exergonic reaction is a reaction that produces Gibbs free energy, which is the energy used to work at a fixed temperature and pressure. Exergonic reactions cause the free energy of the reagent molecules to fall, because their free energy is released during the reaction (Nelson et al. 2008). Therefore, free energy products become lower than reagent free energy. The lower the free energy of a molecule, the more stable the molecule is and the reaction goes spontaneously. This is called thermodynamic equilibrium, the more negative the free energy the reaction will be more spontaneous or will quickly form a stable conformation (Nelson et al. 2008). The bonding energy of the molecular docking results for the Kaempferol 3-rhamnosyl- (1-3) -rhamnosyl- (1-6) -glucoside compound produces a final value of -12.4 kcal / mol consisting of 9 ligand modes. This means that the Kaempferol 3-rhamnosyl- (1-3) -rhamnosyl- (1-6) -glucoside compound is stable to be used as an inhibitor of the enzyme

Plasmodium falciparum Enoyl Acyl Carrier Protein Reductase (PfENR) because the lower the free energy of a molecule, the molecule the more stable and the reaction goes spontaneously.

**TABLE 1.** The bond energy of the Ligan docking result

Mode	Affinity (kcal/mol)	dist from best mode	
		rmsd l.b.	rmsd u.b
1	- 13.4	0.000	0.000
2	- 13.3	2.141	8.775
3	- 13.3	1.152	3.452
4	- 13.3	2.788	9.052
5	- 12.7	2.926	9.106
6	- 12.7	2.603	8.862
7	- 12.7	2.424	9.173
8	- 12.4	1.973	4.318
9	- 12.4	1.379	2.193

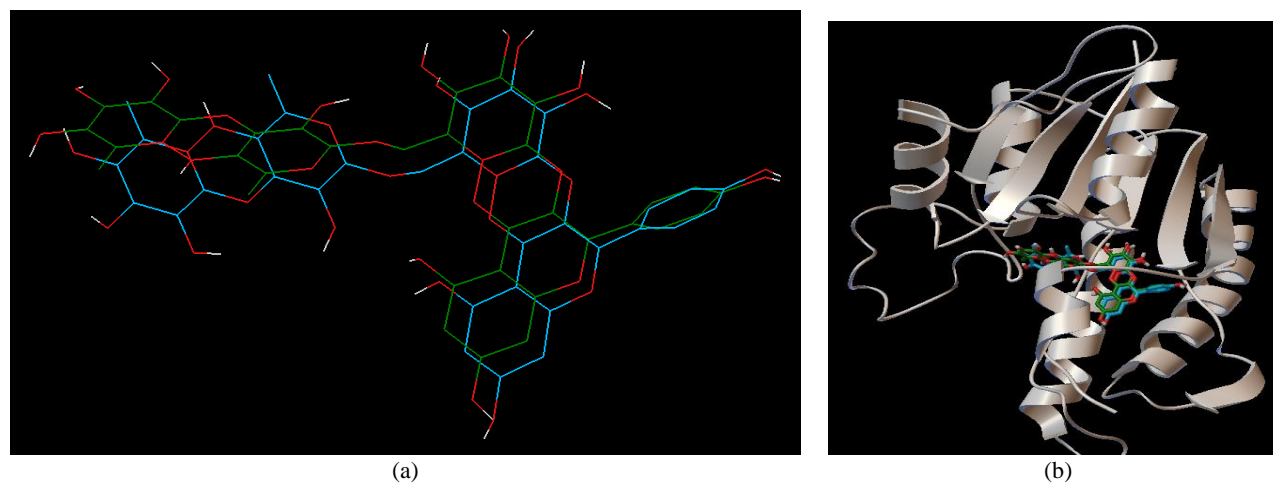
When viewed from the value of RMSD (Root Means Standart Deviation) the final value of RMSD from Kaempferol 3-rhamnosyl- (1-3) -rhamnosyl- (1-6) -glucoside is 1,375 Angstrom for RMSD l.b and 2,193 Angstrom for RMSD u.b. This means that the Kaempferol 3-rhamnosyl-

(1-3) -rhamnosyl- (1-6) -glucoside compound is stable to be used as a ligand because the RMSD value is below 2.5 Angstrom.

When viewed from the ligand obtained, two modes are most similar to the original ligand position and which is the mode with the best poses, mode 2 and mode 7. Therefore, the compound Kaempferol 3-rhamnosyl- (1-3) -rhamnosyl- (1-6) -glucoside is stable to be used as a ligand of the enzyme receptor Plasmodium falciparum Enoyl Acyl Carrier Protein Reductase (PfENR).

### Hydrogen Bond

Hydrogen bonds are intermolecular attractions or between dipoles formed between two partial charges with opposite polarity. Hydrogen bonds occur when an atom gives its hydrogen covalent bond (donor) to an electronegative atom (acceptor), such as oxygen in -OH (Ser, Thr, Tyr, carbohydrate), H<sub>2</sub>O and Nitrogen in -NH<sub>3</sub><sup>+</sup> (Lys, Arg) or -NH- (found in many peptide bonds, Trp, His, Arg, nucleotide bases) are all types of donors (Jefrey & Mart 1997).



**FIGURE 2.** Pose Ligand compound Kaempferol 3-rhamnosyl- (1-3) -rhamnosyl- (1-6) -glucoside (a). Ligand Mode 2 and Mode 7 (b). Ligand Mode 2 and Mode 7 with reseptor

Based on the hydrogen bond data obtained, then among the 9 ligand modes produced, mode 3 has the most hydrogen bonds, there are 3 hydrogen bonds that bind to the residues of Gly106, Tyr111, and Gly112. The next one that has a hydrogen bond 2 is mode 1 which is the hydrogen bond with a ligand and hydrogen bonds with a residue of Leu315. For ligands that have only 1 hydrogen bond present in mode 4 ligand which has a hydrogen bond at Ser317 residue, mode 5 has hydrogen bonds only at the residue Ala17 and mode 9 which has 1 hydrogen bond at the residue of Ala217. Whereas in mode 2, mode 6, mode 7 and mode 8 do not have hydrogen bonds. The large number of hydrogen bonds determines the strength of the interaction. So that in ligand mode 3 is considered to have the highest stability between other fashion ligands. In addition, the overall ligand of

Kaempferol 3-rhamnosyl- (1-3) -rhamnosyl- (1-6) -glucoside is stable to be used as an inhibitor in the enzyme Plasmodium falciparum Enoyl Acyl Carrier Protein Reductase (PfENR) because there are hydrogen bonds that will increase stability.

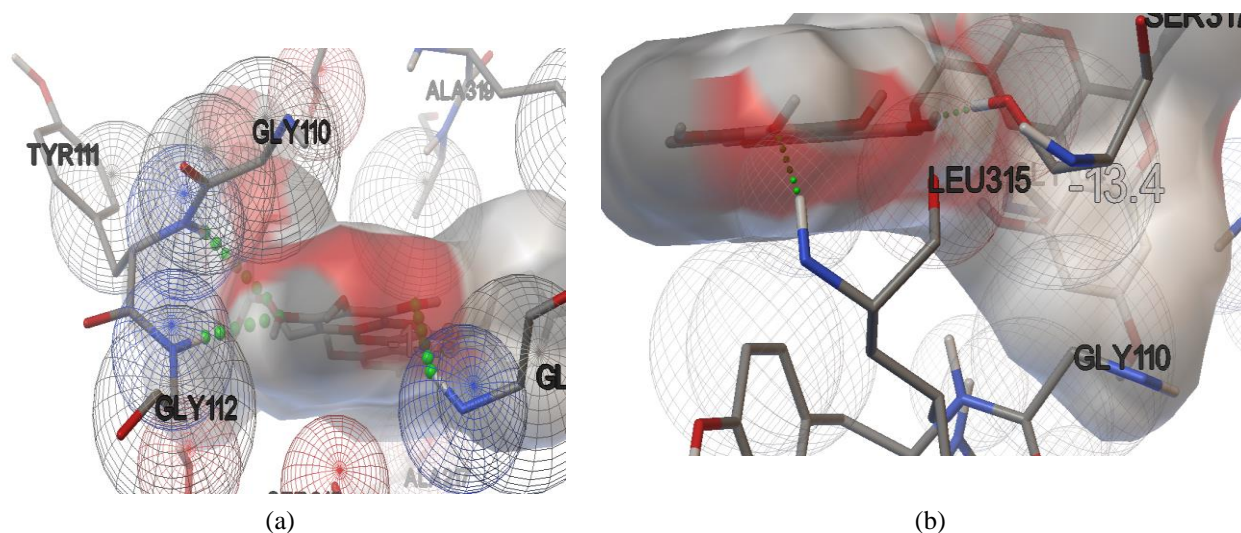
### Ligands Interaction and Receptors

Hydrophobic interactions also play an important role in the stability of the ligand against the receptor. Hydrophobic interaction is an interaction that avoids the liquid environment and tends to group in the inner part of the globular protein structure to minimize interaction with water which can damage the structure of the protein and cause the enzyme to lose its activity (Lin & Brasseur 1995). In ligand mode 1, there are residues that form hydrophobic interactions, namely residues Leu315 and Ala217. From this

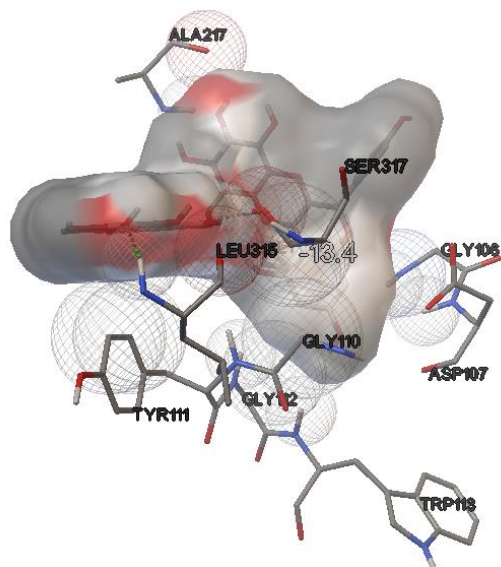


interaction it can be seen that the ligand Kaempferol 3-rhamnosyl- (1-3) -rhamnosyl- (1-6) -glucoside is stable to be used as an inhibitor in the enzyme Plasmodium falciparum Enoyl Acyl Carrier Protein Reductase (PfENR) because there are hydrophobic interactions that will increase stability. The visualization of electrostatic interactions has not been able to be done by the software used in the study. But this interaction can be analyzed through residues that tend to form electrostatic interactions. Electrostatic interaction is an interaction between atoms caused by polarity. This

interaction is a weak and non-covalent interaction so that it is easy to escape, but in large numbers, this interaction can have a large influence on stability (Sharp & Honig 1990). Polar residues are charged at the receptor if the interaction with ligand mode 1 is observed, namely, Asp107. From this interaction it can be seen that the ligand of Kaempferol 3-rhamnosyl- (1-3) -rhamnosyl- (1-6) -glucoside is stable to be used as an inhibitor in the enzyme Plasmodium falciparum Enoyl Acyl Carrier Protein Reductase (PfENR) because there are electrostatic interactions that will increase stability.



**FIGURE 3.** Hydrogen bonds Ligand compounds Kaempferol 3-rhamnosyl- (1-3) -rhamnosyl- (1-6) -glucoside (a). Ligan Mode 3 (b). Receptors and Ligand Mode 1



**FIGURE 4.** Interaksi Ligan mode 1 senyawa *Kaempferol 3-rhamnosyl-(1-3)-rhamnosyl-(1-6)-glucoside* dengan reseptor *Plasmodium falciparum Enoyl Acyl Carrier Protein Reductase (PfENR)*

## CONCLUSION

Kaempferol 3-rhamnosyl- (1-3) -rhamnosyl- (1-6) -glucoside compounds have good stability against the enzyme Plasmodium falciparum Enoyl Acyl Carrier Protein Reductase (PfENR). This is evident from the low bond energy, which is -12.4 kcal / mol which consists of 9 ligand modes. This means that the Kaempferol 3-rhamnosyl- (1-3) -rhamnosyl- (1-6) -glucoside compound is stable to be used as an inhibitor of the Plasmodium falciparum Enoyl Acyl Carrier Protein Reductase (PfENR) enzyme because it is increasingly low in free energy of a molecule, the molecule is more stable and the reaction runs spontaneously, besides that, Kaempferol 3-rhamnosyl- (1-3) -rhamnosyl- (1-6) -glucoside compound has a good bond between the ligand and the receptor stability of the enzyme Plasmodium falciparum Enoyl Acyl Carrier Protein Reductase (PfENR). The hydrogen bond formed is seen in three hydrogen bonds, namely at the residue of Gly106, Tyr111, and Gly112 in the 3 ligand mode. The interaction between ligands and receptors is also formed a stable is seen in the hydrophobic interactions seen at Leu315 and Ala217 residues and electrostatic interactions at Asp107 residues. From this interaction it can be seen that the ligand of Kaempferol 3-rhamnosyl- (1-3) -rhamnosyl- (1-6) -glucoside is stable to be used as an inhibitor in the enzyme Plasmodium falciparum

Enoyl Acyl Carrier Protein Reductase (PfENR) because there are electrostatic interactions that will increase stability.

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