

Potential of Gossypetin Compounds and Organic Acids of Rosella Kombucha Tea as Immunomodulators in Malaria Cases: in Silico Study

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Abstract. Malaria is an infectious disease caused by Plasmodium. Cases of resistance to Artemisin-based Combination Therapy (ACT) have been found. Therefore, complementary therapy is needed. The content of flavonoids (gossypetin) and organic acids (acetic acid, gluconic acid, and lactic acid) in Rosella Kombucha tea may be used as complementary therapy for the treatment of malaria. The In Silico study used gossypetin, acetic acid, gluconic acid, and lactic acid as ligands. While the target protein is the crystal structure of the enzyme TCR - MHC II Complex. Applications used for docking insilico are AutodockTools 1.5.6, Autodock 4.2 and Discovery Studio Visualizer. The results showed that gossypetin and organic acids had the potential to increase the activity of immunocompetent cells because these compounds were able to bind to the TCR – MHC II complex receptor. Gossypetin is more potent than organic acid compounds. gossypetin has Free Energy Binding ($\Delta G_{\text{binding}}$) value (-2.26 kcal/mol), inhibition constant value (22.21 nM) and RMSD value < 5 (2.58). Meanwhile, the most potent organic acid was lactic acid with a value of Free Energy Binding ($\Delta G_{\text{binding}}$) (-1.76 kcal/mol), an inhibition constant value (51.69 nM) and an RMSD value < 5 (4.1).

1 Introduction

Plasmodium is an endoparasite that causes malaria. In the life cycle, Plasmodium will infect human erythrocytes and can cause changes in the number of blood cells. The process of transmission from person to person through the bite of a female Anopheles mosquito. Plasmodium that commonly causes malaria in Indonesia are *P. falciparum*, *P. malariae*, *P. ovale*, and *P. vivax*. In 2012, Plasmodium knowlesi was found as a specific cause of malaria in the area of South Kalimantan. The different variations of Plasmodium that infect cause variations in the type of malaria. Even infection by more than one type of Plasmodium can cause complications of malaria [1].

Treatment of malaria uses ACT (Artemisin-based Combination Therapy) [2]. Resistance to Artemisin has occurred in the Greater Mekong, Southeast Asia in 2011 [3]. In addition, it is also found in several Southeast Asian countries, namely Thailand, Cambodia, and Myanmar [4]. So other methods are needed in the treatment of malaria.

The use of herbal plants as companion therapy in the treatment of malaria is still being carried out. Some herbal plants that can be candidates for malaria companion therapy include *Fraxinus graffithi* stem extract, *Piper sucatum* leaf extract, and *Eucalyptus globulus* stem extract [5]. According to the World Health Organization (WHO) to avoid the occurrence of resistance to the use of malaria drugs, it should be supported by companion therapy [4]. Research conducted by Ulfah [6] showed that the immunomodulatory activity of fermented Rosella Kombucha tea could increase the proliferation of mice lymphocyte cells. Increased proliferation of lymphocyte cells can improve the body's immune system in the process of immunotherapy. The presence of immunomodulatory activity allows roselle kombucha tea to facilitate companion therapy for infectious diseases including malaria.

The ability of roselle kombucha tea to boost the immune system is thought to be due to the presence of flavonoids (quercetin, hibiscetin, and gossypetin) and organic acids (acetic acid, gluconic acid, and lactic acid). Research conducted by Nerdy [7] shows that the results of in silico docking of flavonoid compounds can act as antimalarials in inhibiting the action of plasmepsin I and plasmepsin II, which are the main enzymes that play a role in the plasmodium life cycle. Another study by Ulfah [6] showed that the immunomodulatory activity of rosella kombucha tea increased on the 10th day of fermentation which was thought to be due to an increase in acid content.

Bioinformatics through the in silico screening method is currently widely used in drug and therapeutic design. In silico is used to complement research data in vivo and in vitro. To observe the immunomodulatory activity of rosella kombucha tea in silico, the ligands to be used were the compound gossypetin and 3 organic acids namely acetic acid, gluconic acid, and lactic acid. While the target protein to be used is the TCR protein $\alpha\beta$ – MHC II complex with code (PDB: 4GRL). This protein was chosen because of its role as a receptor that causes T lymphocyte cells to be able to recognize antigens that will trigger the body's immune response [8]. For this reason, the in silico approach in this study was carried out as a scientific basis to see the potential of rosella kombucha tea as a candidate in supporting therapy for the treatment of malaria.

2 Materials and Methods

The equipment used in this study was the hardware Acer Aspire E1-410 laptop with Windows 7 operating system, N2820 2.39 GHz processor, with 2GB RAM specifications and a cellphone laptop with 14-bs0xx laptop specifications (4096MB RAM) with Windows 10 operating system. along with the software used are Autodock tools 1.5.6, Autodock 4 and Discovery Studio Visualizer 2020.

The crystal structure of the enzyme TCR $\alpha\beta$ - MHC II Complex (PDB code: 4GRL) was obtained from the Protein Data Bank (PDB) (<http://www.pdb.org/pdb/home/home.do>). The ligand is a 3D molecular structure of lactic acid with CID code: 612, acetic acid with CID code: 176, gluconic acid with CID code: 10690, and the 3D structure of Gossypetin with CID code: 5280647 which were downloaded on the PubChem page (<http://PubChem.ncbi.nlm.nih.gov>).

Ligands and proteins that have been downloaded are prepared so as not to interfere with the process of docking macromolecules with the test ligands during the docking process. The preparation was carried out to remove unnecessary residues during the docking test. The preparation process was carried out using the Discovery Studio Visualizer application.

Docking simulation using AutoDock tools 1.5.6. and Autodock 4. To get the best docking results, the docking simulation process was repeated 3 times. After the docking results were obtained, they were analyzed using the ADT 1.5.6 application. to see the Binding Energy parameter, the Inhibition Constant value and the RMSD value. Negative binding energy, small Inhibition Constant values, and RMSD values ≤ 2 or at least ≤ 5 indicate that the docking results are valid [9]. To see the number of hydrogen bonds and the interaction of the ligand with the receptor, a visualization of the docking results was carried out using the Discovery Studio Visualizer 2020.

3 Result

This research was conducted to see the content of flavanoid compounds (Gossypetin) and organic acids contained in rosella kombucha drink. The research method uses computerized in silico-based software. In the process, binding is carried out between the target protein and the ligand compound. The target protein used is the TCR $\alpha\beta$ -MHCII complex. Flavanoid compounds (Gossypetin), acetic acid, gluconic acid and lactic acid, were used in this study as ligands.

3.1 Identification of Target Proteins

The target protein in the form of TCR $\alpha\beta$ -MHCII complex was downloaded from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB). The TCR $\alpha\beta$ -MHCII complex consists of four protein chains, namely: A, B, C, and D chains and has one natural ligand, namely N-acetyl-D-Glucosamic (NAG). (Fig. 1.) (Table 1.).

The receptor of human naïve lymphocyte cells is the protein TCR $\alpha\beta$. Immunity will be triggered to increase when exposed to antigens. The TCR $\alpha\beta$ protein recognizes antigens through APC intermediaries. Naive T lymphocyte cells that are in a dormant phase, will be activated and proliferate due to the presence of antigen signals activated by APC and MHCII(B7). Therefore, the TCR $\alpha\beta$ -MHCII complex was chosen as the target protein in this study [8].

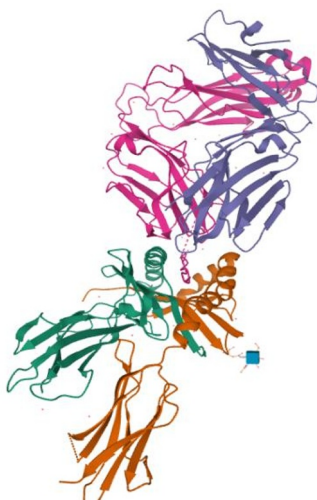



Fig. 1. Structure of the TCR $\alpha\beta$ -MHCII complex (PDB:4GRL)

Tosca : A Chain
Brown: B Chain
Purple : C Chain
Pink : D Chain
Blue : Natural Ligand
Dots : Water Molekuls

Table 1. Target Protein ligands of the TCR $\alpha\beta$ -MHCII complex

ID	Chains	Name / Formula / InChi key	2D diagram
NAG	B	N-ACETYL-D-GLUCOSAMINE C ₈ H ₁₅ N O ₆	

3.2 Ligand Identification

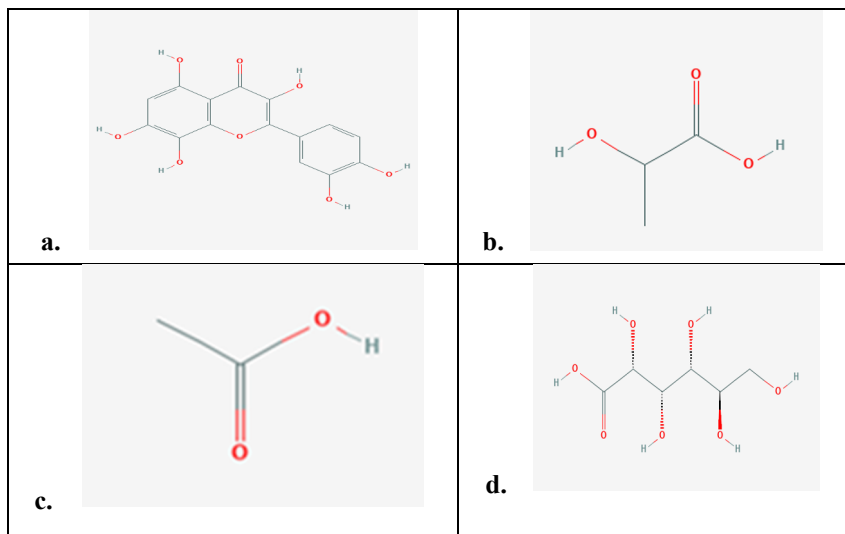


Fig. 2. 2D Design a. Gossypetin; b. Lactic acid; c. Acetic acid; d. Gluconic Acid

In this study the ligands used were flavanoid compounds in the form of Gossypetin and three organic acid compounds namely acetic acid, gluconic acid, and lactic acid. The structure of Gossypetin and organic acid compounds obtained from the NCBI website.

The validation stage is carried out before the ligand docking process. Validation was carried out by redocking natural ligands. Below is a table of natural ligand redocking results:

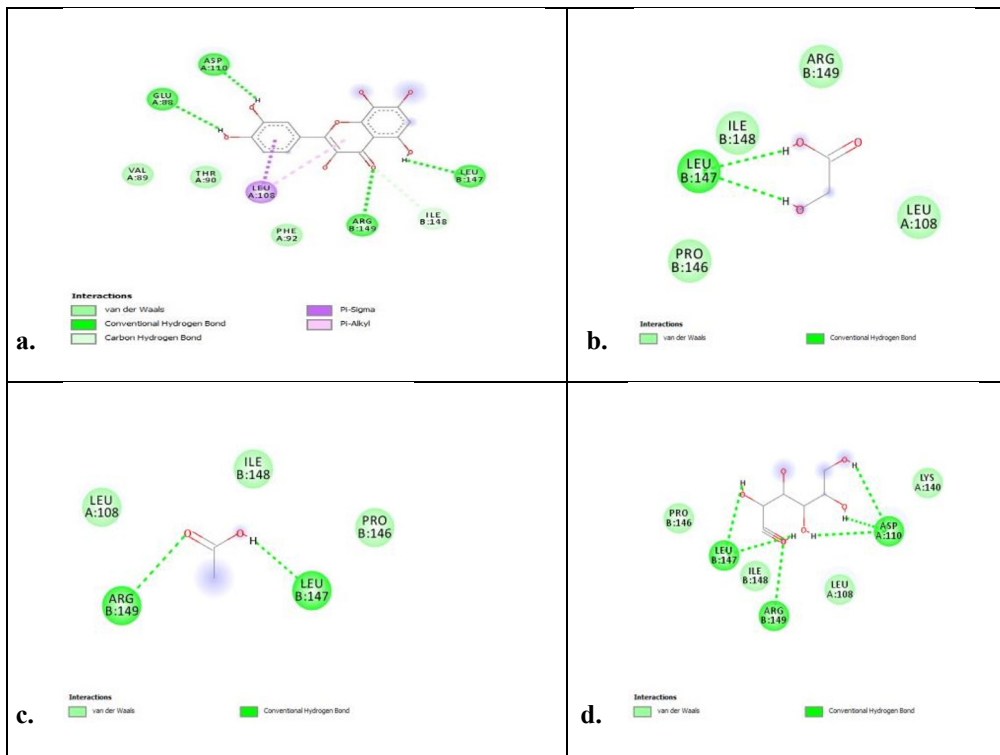
Table 2. Redocking result of natural ligand against TCR $\alpha\beta$ -MHCII complex

Ligand	Residual interaction	Free energy binding (kcal/mol)	Constanta inhibition mM	Cluster RMSD Å	Reference RMSD Å
Natural Ligand	ASN B:19	-1.34	104.37	0.00	1.74

Gossypetin ligand and organic acid docking simulation aims to observe the interaction of the compound to the receptor protein. In addition to seeing the value of the best binding affinity for the receptor. Table 3. is the result of docking of the Gossypetin compound and organic acids against the TCR $\alpha\beta$ -MHCII complex receptor.

Table 3. Gossypetin and organic acids docking scores for the TCR $\alpha\beta$ -MHCII complex

Ligand	Interaksi residu	Free energy binding (kcal/mol)	Konstanta inhibisi mM	Cluster RMSD Å	Reference RMSD Å
<i>Gossypetin</i>	ASP A:110 GLU A:88 A:88 LEU B:147 ARG B:149	-2.26	22.21	0.0	2.58
Asam Laktat	LEU B:147	-1.76	51.69	0.0	4.10
Asam Asetat	LEU B:147 ARG B :149	-1.60	67.60	0.0	4.25
Asam Glukanat	AP A:110 LEU B:147 ARG B :149	-1.12	150.66	0.0	2.26

**Fig 3.** Visualization of (a) Gossypetin; (b) Lactic acid; (c) Acetic acid; and (d) Gluconic acid against the TCR $\alpha\beta$ -MHCII complex

Based on the analysis of in silico docking results, it appears that the Gossypetin compound and these organic acids have the potential to bind to the TCR $\alpha\beta$ -MHCII complex receptor or

it can be said that Gossypetin and these organic acids have the potential to increase the activity of immunocompetent cells.

4 Discussion

The immune response can be triggered by the Gossypetin compound and organic acids which act as antigens which are then recognized by their receptors, in this case T cells and B cells. Recognition of antigens by naive T cells through their receptors $TCR\alpha\beta$. In general, $TCR\alpha\beta$ found on the cell surface of naive T lymphocytes will recognize antigens with the help of APC. Antigen from MHCII is a surface molecule of APC (dendritic cells) which has a role as APC to activate and proliferate T lymphocyte cells [8]. Therefore, in this study, molecular docking was carried out on the target protein $TCR\alpha\beta$ -MHCII complex.

Gossypetin compounds and kombucha rosella organic acids will activate naïve T lymphocyte cells and experience proliferation when exposed to antigens presented by MHCII. Furthermore, naive T cells will turn into Th0 cells [8]. The presence of diacylglycerol and the high concentration of Ca^{2+} during the process of activation and proliferation of lymphocyte cells will stimulate Th0 cells to produce and secrete IL-2 cytokines [6]. IL-2 will trigger the proliferation and differentiation of T cells, B cells, and Natural Killer (NK) cells [8].

When the body is infected with Plasmodium, Th0 cells will receive signals from the infected cells, then Th0 cells change to Th1 and Th2. Furthermore, Th1 cells will secrete $IFN-\gamma$ type cytokinins. These cytokinins will activate macrophages (phagocytes) in the effector phase. Activated phagocytes will destroy Plasmodium at the erythrocytic stage as a form of cellular immune response. In the Th2 pathway, Th2 cells will produce IL-4 and IL-5 cytokinins and then secrete them to help the development of B cells into plasma cells. These plasma cells will secrete antibodies against Plasmodium in the exoerythrocytic or erythrocytic stages. Apart from that, this phase of the immune response also produces memory cells which will be able to recognize Plasmodium more quickly when it re-infects the body [8].

5 Conclusion

Based on the results of the study, the Gossypetin compound and organic acid compounds in the form of acetic acid, gluconic acid, and lactic acid from kombucha rosella have the potential to increase the activity of immunocompetent cells *in silico*, because this can be seen from the bond between the test ligands and $TCR\alpha\beta$ -MHCII complex.

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