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Original Article

Antimalarial activity of curcumin and kaempferol using *structure-based drug design* method

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ABSTRACT

Malaria is a major global health problem caused by plasmodium parasites. The alarming spread of drug resistance and its limited availability underscores the importance of discovering new antimalarial compounds. This study aims to examine the interaction of curcumin and kaempferol on the *triose-phosphate isomerase* receptor of *P. falciparum* strain through an *in-silico* trial using a structure-based drug design method. Molecular docking was performed using crystalline protein *triose-phosphate isomerase* (PDB ID: 1O5X) from *P. falciparum* TIM complexed into *2-phosphoglycerate* from protein database (https://www.rcsb.org/). Furthermore, curcumin and kaempferol compounds have better free energy binding values, which are -8.57 and -9.02 kcal/mol compared to *2-phosphoglycerate* which is -5.97 kcal/mol. Considering the value of the inhibition constant, curcumin and kaempferol compounds also had a lower value of 0.523 and 0.244 µM, compared to *2-phosphoglycerate*, which was 41,970 µM. In addition, curcumin compounds have five interactions with *2-phosphoglycerate*, one of which is a *hydrogen bonding interaction* on the amino acid GLY171 and it interacts with the hydroxy group on the structure of curcumin. Kaempferol compounds have six interactions with *2-phosphoglycerate*, two of which are *hydrogen bonding interactions* including amino acids GLY232 and VAL212 which interact with the hydroxy group on the structure. These two compounds have antimalarial activity through competitive inhibition of *2-phosphoglycerate* against *triose-phosphate isomerase* receptors. They are also better than *2-phosphoglycerate* when assessed from the results of *hydrogen bonding interaction*, inhibition constants, and types of amino acid interactions obtained from *the in-silico trial* using the *structure-based drug design* method.

Keywords: Antimalarial, Curcumin, Kaempferol, In-silico, Structure-based drug design method

Introduction

Malaria is an endemic infection caused by parasitic protozoa of the genus *Plasmodium* [1]. In 2020, WHO reported that there were 229 million cases of infection with 409,000 deaths in the world [2]. The high number of cases was caused by the emergence of various obstacles in the eradication of malaria. These include the resistance of malaria parasites to available antimalarial drugs, increased immunity of Anopheles mosquitoes to chemicals, and the discovery of side effects of these drugs [3]. Currently, the growth and spread of resistance to all the first-line antimalarial drugs used in the treatment and prevention of malaria created many problems in the control programs [4-6]. The study to obtain new synthetic and naturally derived antimalarial drugs is continuing. One of which is through the exploration of active compounds from natural ingredients, specifically medicinal plants traditionally used to treat malaria in various endemic areas in the world. This study aims to obtain new antimalarial compounds that have mild side effects with low toxicity [7]. One of the active compounds from natural ingredients that have antimalarial activity is curcumin and kaempferol [8-10]. In a previous *in-silico* antimalarial study, *2-phosphoglycerate* was reported as a new substrate that activates the glycolysis process in *Plasmodium falciparum*. When the ligand interacts with the triose-phosphate isomerase receptor, a

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glycolysis process will occur in *P. falciparum* [11]. Therefore, new potential antimalarial compounds should be developed by examining the interaction of curcumin and kaempferol compounds with *triose-phosphate isomerase* receptors in replacing the *2-phosphoglycerate* substrate position, *in-silico* using the *structure-based drug design* method.

Materials and Methods

Protein and grid preparation

Molecular docking studies have been performed to investigate protein-ligand interactions. The crystal structure of protein *triose-phosphate isomerase* (PDB ID: 1O5X) from *Plasmodium falciparum* TIM complexed into *2-phosphoglycerate* was obtained from the protein database (https://www.rcsb.org/) [11]. In this preparation, the water molecule at the receptor was also removed to minimize aberrations in the resulting *hydrogen bond interactions*, and after the separation, further preparation was conducted using Autodock 4.0.1 software. The receptor and ligand were added for *Kollman charge* (charge added for the receptor) and *Compute gasteiger charge* (charge added for native ligand). Furthermore, the charge increased the interaction between the ligand and receptor. The *polar hydrogen* was then added to the protein molecule while the *non-polar hydrogen* was added to the ligand molecule. Then each of the molecules was stored in a *protein data bank partial charge* (.pdbqt) format. In the next step, a *grid parameter file* (.gpf) and a docking parameter file (dpf) was prepared by combining the data of ligand.pdbqt and receptor.pdbt to set other docking parameters (100 GA Runs and energy 2500000). In the final stage, redocking was conducted using the *command prompt* (cmd) and interpreting the data from the validation results of the docking method [12].

Ligand preparation

In this study, the antimalarial activity of curcumin and kaempferol was tested using the *structure-based drug design* (SBDD) method. The 1O5X receptor (*triose phosphate isomerase*) which is known to have regulation in the glycolytic pathway was used as the test target, while the activator substrate (*2 -phosphoglycerate*) was used as a guide compound [11]. In addition, curcumin and kaempferol compounds were drawn using Chem Draw 2D, and energy was minimized using MM2 0.01. The results obtained after *minimizing energy* are then stored in the format (.pdb). Subsequently, preparation continued using Autodock 4.0.1 to add *compute gasteiger charge* and *non-polar hydrogen*, which was merged to both structures and compounds. In the final stage, grid (gpf) and docking parameter files (dpf) were created by combining each test compound with the target receptor [13, 14].

Molecular docking

The *in-silico* docking process was conducted on molecules with 1O5X receptors using Autodock 4.0.1 to predict the binding affinity between the ligand and the receptor. A *discovery studio visualizer* was implemented to depict various intermolecular interactions such as hydrogen bonds, hydrophobic Vander Waals interactions, and pi-pi interactions. Furthermore, molecular docking involves selecting the three-dimensional active binding site of the receptor molecule and calculating the binding affinity and energy of the resulting orientation [15]. The bond affinity value was determined by the highest bond affinity or the lowest bond energy (more negative values) indicating the most favorable conformation [16].

Results and Discussion

Identification of receptors and lead compounds

Triose-phosphate isomerization receptor (1O5X) was obtained using the X-ray diffraction (XRD) method. This method is considered better than others such as *nuclear magnetic resonance* (NMR). The receptor was obtained from the *P. falciparum* parasite, while the resolution value parameter representing the similarity of the structure was obtained with the original receptor. Generally, this receptor has a resolution value of 1.10 Å, and it is expected to be <2 [17]. **Table 1** showed the results of the identification of receptors and lead compounds.

Table 1. Identification of receptors and lead compounds.

PDB Code	Receptors	Classification	Native ligand	Method	Organism	Resolution
1O5X	Triose-phosphate isomerase	Isomerase	2-phosphoglycerate	XRD	*Plasmodium falciparum*	1.10 Å

Molecular docking method validation

The validation of the docking method conducted on the 1O5X receptor emphasized the *root mean standard deviation* (RMSD) value. It represents the difference in the native ligand position before docking and after redocking (with a requirement of <2 Å) [18]. Based on the test data, the 1O5X receptor has results that fulfill the requirements of having an RMSD value of 1,552. Meanwhile, in the validation results of the docking method, the *free energy binding* value for the *2-phosphoglycerate* ligand is -5.97 kcal/mol, with an inhibition constant of 41.97 μM, it has *hydrogen bonding interaction* on the amino acids GLY232, GLY173, ASN233, THR172, ALA234, VAL212, and *non-hydrogen bonding interaction* on SER211, LYS12, and GLY171. The value of the results was used as a standard to assess the antimalarial activity of the test compounds in molecular docking screening [19] as seen in **Table 2**.

Table 2. Molecular docking method validation

Receptors (Resolution)	Amino acid interactions	Free energy (ΔG Gibs)	Inhibition constants (μM)	RMSD
1O5X (1.10 Å)	**GLY232, GLY173, ASN233, THR172, ALA234, VAL212,** SER211, LYS12, GLY171	-5.97 kcal/mol	41.97 μM	1.522 Å

Virtual compound screening test

The results showed that the curcumin and kaempferol compounds have better *free energy binding* values of -8.57 and -9.02 kcal/mol compared to native ligan *2-phosphoglycerate*, which is -5.97 kcal/mol. This indicates that the smaller the binding free energy value, the lower the activation energy. Therefore, the potential for interactions between compounds and receptors takes place more quickly (*spontaneous reactions*) [13, 20, 21]. Based on the value of the inhibition constant, curcumin and kaempferol compounds also had lower values of 0.523 and 0.244 μM compared to native ligand *2-phosphoglycerate*, which was 41,970 μM. These values represent the ability of the test compound to inhibit receptors or enzymes, where a relatively low value is considered to have great power. This is because a low concentration can sufficiently have a large inhibitory ability [22]. Besides considering the value of *free energy binding* and inhibition constants, one of the parameters to determine the activity of a compound is the interaction between the structure and amino acids at the receptor. Furthermore, the curcumin compound has five interactions in common with the *2-phosphoglycerate*, and the *hydrogen-bonding interactions* on the amino acid **GLY171** interact with the hydroxy group on the structure. The kaempferol compound has six interactions with the *2-phosphoglycerate*, two of which are *hydrogen bonding interactions* including **GLY232** and **VAL212.** These amino acids interact with the hydroxy groups in the kaempferol structure. *Hydrogen bonding interactions* are reversible and relatively stronger than other types [23, 24]. **Table 3** along with **Figures 1 and 2** showed the results of the interaction of kaempferol and curcumin with the *triose-phosphate-isomerase* receptor.

Table 3. Virtual compounds screening test.

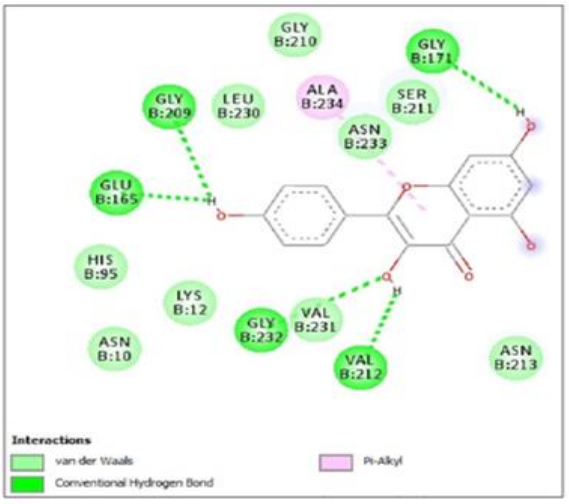
Compounds	Amino acid interactions	Free energy (ΔG Gibs)	Inhibition constants (μM)
2-phosphoglycerate (Native ligand)	**GLY232, GLY173, ASN233, THR172, ALA234, VAL212,** SER211, LYS12, GLY171	-5.97 kcal/mol	41.970 μM
Curcumin	**GLU165, LYS12, GLY171, LYS237, ASN213,** HIS95, ASN233, ALA234, GLY232, VAL212, LEU230, VAL231, GLY210, GLY209	-8.57 kcal/mol	0.523 μM
Kaempferol	**GLY232, VAL212**, **GLU165, GLY209**, **GLY171,** ALA234, LEU230, GLY210, ASN233, SER211, ASN213, LYS12, ASN10, VAl231, HIS95	-9.02 kcal/mol	0.244 μM

Description:

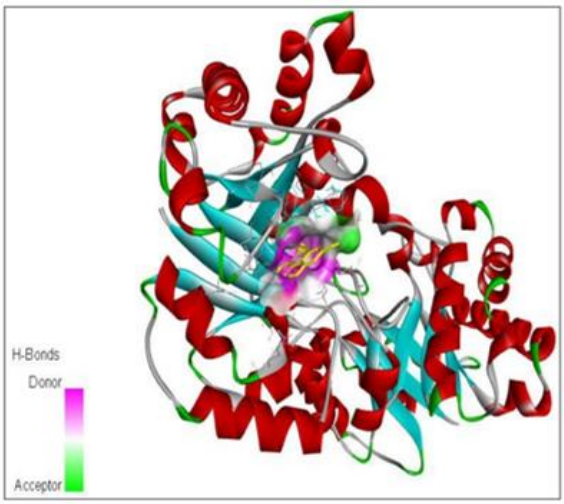
Hydrogen bonding interaction

- *2-phosphoglycerate*: **GLY232, GLY173, ASN233, THR172**, **ALA234, VAL212.**
- Curcumin: **GLU165, LYS12, GLY171, LYS237, ASN213.**
- Kaempferol: **GLY232, VAL212, GLU165, GLY209, GLY171.**

Similar interactions between curcumin and kaempferol with *2-phosphoglycerate* represent the activity in binding to 1O5X receptors. The interaction in the two test compounds with the active site of the 1O5X receptor can competitively prevent the activation of these receptors by *2-phosphoglycerate* (a competitive inhibitor) [25, 26]. Furthermore, this mechanism can inhibit the *glycolytic pathway* in *P. falciparum* [11]. The *in-silico* comparison of the two test compounds considered kaempferol to be better than curcumin judging from its lower *free energy binding*, lower inhibition constant, and a large number of specific *hydrogen-bonding interactions*.

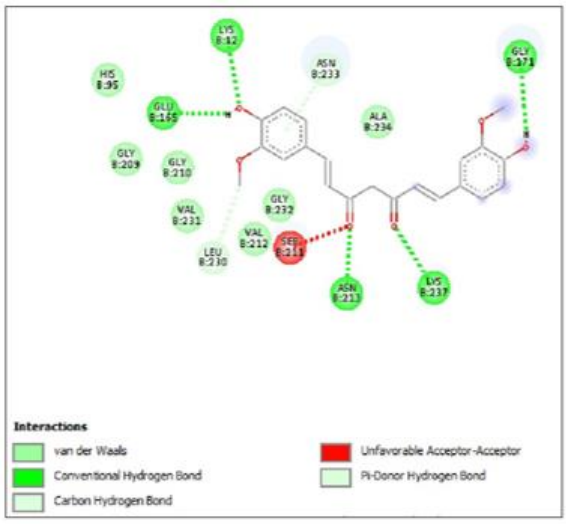


a)

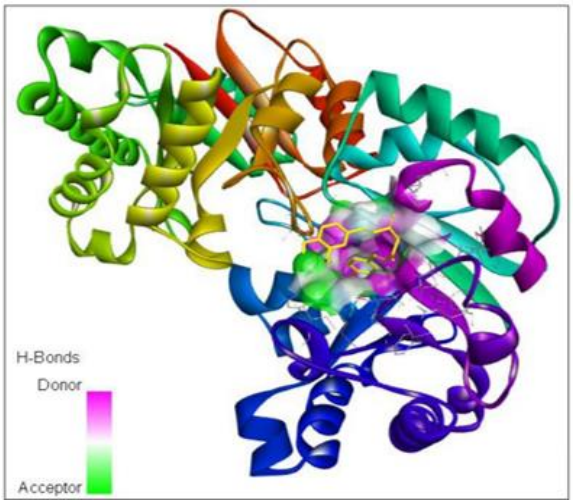


b)

Figure 1. Kaempferol interaction with *triose-phosphate-isomerase* receptors. a) 2D visualization; b) 3D visualization.



a)



b)

Figure 2. Interaction of curcumin with *triose-phosphate-isomerase* receptors. a) 2D visualization; b) 3D visualization.

Conclusion

This study showed the antimalarial activity of curcumin and kaempferol compounds through competitive inhibition of the native ligand *2-phosphoglycerate* against the *triose-phosphate isomerase* receptor (1O5X). Furthermore, these compounds are better than native ligand *2-phosphoglycerate* considering the results of *hydrogen bonding interaction*, inhibition constants, and types of amino acid interactions obtained from *in-silico* using the *structure-based drug design* method.

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Conflict of interest: None

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Ethics statement: None

References

1. Alkandahri MY, Berbudi A, Utami NV, Subarnas A. Antimalarial activity of extract and fractions of Castanopsis costata (Blume) A.DC. Avicenna J Phytomed. 2019;9(5):474-81. doi:10.22038/AJP.2019.13188
2. WHO. World malaria report 2020; 2020. Available from: http:// www.who.int/malaria/publications/world_malaria_report_2020/ report/en/wmr2020_full_report.pdf. Accessed: August/2020.
3. Witmer K, Dahalan FA, Delves MJ, Yahiya S, Watson OJ, Straschil U, et al. Transmission of artemisinin-resistant malaria parasites to mosquitoes under antimalarial drug pressure. Antimicrob Agents Chemother. 2021;65(1):1-17. doi:10 .1128/AAC.00898-20.
4. Alkandahri MY, Maulana YE, Subarnas A, Kwarteng A, Berbudi A. Antimalarial activity of extract and fractions of Cayratia trifolia (L.) Domin. Int J Pharm Res. 2020;12(1):1435-41.
5. Sergeevna SM, Efimovna LE, Irina K. Pharmaceutical consultation as a basis for drug care continuity. Pharmacophore. 2020;11(4):76-82.
6. Faller EM, Hernandez MT, Hernandez AM, Gabriel JR. Emerging roles of pharmacists in global health: An exploratory study on their knowledge, perception, and competency. Arch Pharm Pract. 2020;11(1):40-6.
7. Budiarti M, Maruzy A, Mujahid R, Sari AN, Jokopriyambodo W, Widayat T, et al. The use of antimalarial plants as traditional treatment in Papua Island,

Indonesia. Heliyon. 2020;6(12):1-10. doi:10.1016/j.heliyon.2020.e05562.

8. Ounjaijean S, Benjasak N, Sae-lao S, Somsak V. Kaempferol addition increases the antimalarial activity of artesunate in experimental mice. J Trop Med. 2020:1-4. doi:10.1155/2020/6165928
9. Lwin KH, Mon HM, Myint KH. Evaluation of the antimalarial activity of Curcuma longa Linn., singly and in combination with Eupatorium odoratum Linn. J Ayurvedic Herb Med. 2017;3(1):11-4.
10. Somsak V, Damkaew A, Onrak P. Antimalarial activity of kaempferol and its combination with chloroquine in Plasmodium berghei infection in mice. J Pathog. 2018:1-7. doi:10.1155/2018/3912090.
11. Parthasarathy S, Eaazhisai K, Balaram H, Balaram P, Murthy MR. Structure of Plasmodium falciparum triose-phosphate isomerase-2-phosphoglycerate complex at 1.1-A resolution. J Biol Chem. 2003;278(52):52461-70. doi:10.1074/jbc.M308525200.
12. Morris GM, Lim-Wilby M. Molecular docking: in Molecular modeling of proteins. Springer; 2008. 365-82 p.
13. Morris GM, Huey R, Olson AJ. Using autodock for ligand-receptor docking. Curr Protoc Bioinforma. 2008;24(1):8-14. doi:10.1002/0471250953.bi0814s24.
14. Goodsell DS. Computational docking of biomolecular complexes with AutoDock. Cold Spring Harb Protoc. 2009;4(5):1-6. doi:10.1101/pdb.prot5200.
15. Perez-Castillo Y, Lima TC, Ferreira AR, Silva CR, Campos RS, Neto JBA, et al. Bioactivity and molecular docking studies of derivatives from cinnamic and benzoic acids. BioMed Res Int. 2020:1-13. doi:10.1155/2020/6345429
16. Umar AB, Uzairu A, Shallangwa GA, Uba S. Design of potential anti-melanoma agents against SK-MEL-5 cell line using QSAR modeling and molecular docking methods. SN App Sci. 2020;2(815):1-18. doi:10.1007/s42452-020-2620-8.
17. Gupta M, Sharma R, Kumar A. Docking techniques in pharmacology: How much promising?. Comput Biol Chem. 2018;76:210-7. doi:10.1016/j.compbiolchem.2018.06.005.
18. Santosa H, Putra GS, Yuniarta TA, Budiati T. Synthesis and molecular docking studies of N'-benzoylsalicylhydrazide derivatives as antituberculosis through InhA enzyme inhibition. Indonesian J Pharm. 2018;29(4):198-205. doi:10.14499/indonesianjpharm29iss4pp198
19. Shivanika C, Kumar D, Ragunathan V, Tiwari P, Sumitha A, Devi B. Molecular docking, validation, dynamics simulations, and pharmacokinetic prediction of natural compounds against the SARS-CoV-2 main-protease. J Biomol Struct Dyn. 2020:1-27. doi:10.1080/07391102.2020.1815584.
20. Menzinger M, Wolfgang R. The meaning and use of the arrhenius activation energy. Angew. Chemie Int Ed English. 1969;8(6):438-44. doi:10.1002/anie.196904381.
21. Butt SS, Badshah Y, Shabbir M, Rafiq M. Molecular docking using chimera and Autodock Vina software for nonbioinformaticians. JMIR Bioinform Biotech. 2020;1(1):1-25. doi:10.2196/14232
22. Chou TC. Relationships between inhibition constants and fractional inhibition in enzyme-catalyzed reactions with different numbers of reactants, different reaction mechanisms, and different types and mechanisms of inhibition. Mol Pharmacol. 1974;10(2):235-47.
23. van der Lubbe SCC, Guerra CF. The nature of hydrogen bonds: A delineation of the role of different energy components on hydrogen bond strengths and lengths. Chem Asian J. 2019;14:2760-9. doi:10.1002/asia.201900717
24. Coppola F, Perrella F, Petrone A, Donati G, Rega N. A not obvious correlation between the structure of green fluorescent protein chromophore pocket and hydrogen bond dynamics: A choreography from ab initio molecular dynamics. Front Mol Biosci. 2020;7(569990):1-17. doi:10.3389/fmolb.2020.569990
25. Muchtaridi M, Syahidah HN, Subarnas A, Yusuf M, Bryant SD, Langer T. Molecular docking and 3D-pharmacophore modeling to study the interactions of chalcone derivatives with estrogen receptor alpha. Pharmaceuticals. 2017;10(4):1-12. doi:10.3390/ph10040081.
26. Megawati G, Herawati D, Musfiroh I. Binding affinity of omega 3 fatty acid as an agonist PPAR-γ and GPR120 receptor for obesity using molecular docking and ADME prediction. Eur J Mol Clin Med. 2021;7(10):1686-95.