Allium sativum as Antimalaria Agent via Falcipain Protease-2 Inhibitor Mechanism: Molecular Docking Perspective

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ARTICLE INFO

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<https://doi.org/10.21776/ub.crjim.2021.002.01.4>

Received on Feb 15th, 2021;
Revised on Mar 15th, 2021;
Accepted on Apr 2nd, 2021

ABSTRACT

Background: Malaria is an endemic disease that can lead to death. Malaria control is a threatening cause of resistance to antimalarial drugs so that renewable therapies are needed to overcome this disease. The chemical compounds of garlic have potential as antimalarial agents, but the mechanism is still unknown. **Aim:** This research will predict the compounds' molecular mechanism in garlic (*Allium sativum*) using the in-silico method. **Methods:** The In-silico method using chemical compounds in *Allium sativum* were obtained from PubChem, and Falcipain protease-2 was obtained from the Protein Data Bank. Then performed a docking simulation between ligand-protein and analyzed it in 3D. We were used PyRx, Pymol, and DS (Discover Studio) software for analysis and visualization of the interaction of ligand-protein. **Results:** The results we got, the Alliin compound contained in *Allium sativum* has the strongest bond with Falcipain protease-2. Alliin has fulfilled Lipinski Rule, so Alliin drug-likeness potentially. Alliin has antimalarial activity with its inhibition mechanism against Falcipain protease-2. **Conclusion:** We recommend this study as a reference for further research on Alliin compounds as antimalarials through in vitro and in vivo methods.

Keywords: Antimalarial, Falcipain protease-2 inhibitor, *Allium sativum*, in Silico

INTRODUCTION

Malaria is an endemic disease that can lead to death. Malaria is caused by infection with the parasite *Plasmodium sp.* which is transmitted through the bite of an Anopheles mosquito. According to the latest data from the World Health Organization (WHO), there were 212 million malaria cases in 2015, which caused 429,000 deaths, and around 3.2 billion people

were at risk of malaria. The incidence rate is estimated at 214 million cases, meaning 91 new cases per 1,000 people at risk. Most cases in 2015 are estimated to have occurred in the African Region (88%), followed by Southeast Asia (10%).^[1]

Malaria control is threatened by antimalarial drug resistance. In recent years,

Cite this as:

Syaban M.F.Rizki, Rachman H.A, Arrahman, A.D, Hudayana Nur, Khamid J.P, Pratama, F.A. *Allium Sativum* As Antimalaria Agent Via Falcipain Protease-2 Inhibitor Mechanism: Molecular Docking Perspective. Clinical and Research Journal in Internal Medicine. 2021;2(1):130-135. DOI: <https://doi.org/10.21776/ub.crjim.2021.002.01.4>

artemisinin resistance has been detected in 5 countries in the Southeast Asia region, including Indonesia. This is due to a genetic mutation of *P. falciparum* that causes drug resistance. Patients recover when treated with ACT if there is no resistance to the drug combination.^[2-4]

Based on these facts, a new therapeutic strategy is needed for malaria infection. Plasmodium protease has an essential role in the life cycle of parasites. So that Plasmodium protease has the potential as a target for antimalarial drugs. Falcipain protease-2 (FP-2) from *P. falciparum* is a papain-family (C1A) cysteine protease that plays an essential role in parasites' life cycle by reducing erythrocyte protein especially hemoglobin.^[5] Disruption of FP2 causes a marked decrease in hemoglobin hydrolysis by trophozoite.^[6] The breakdown of hemoglobin is carried out in a particular parasitic organelle, the food vacuole, by a complex ~ 200 kDa protein, one of which contains the FP2. Genetic analysis has shown many of these components, including FP2, to be essential for parasite survival. The maturity of FP2 is a 27 kDa papain-type protease, which is responsible for cleaving hemoglobin into small peptides. Apart from having hemoglobinase activity, FP2 is involved in lowering the ankyrin protein skeletal erythrocyte in the process required for red blood cells to rupture at the end of the parasite's intra-erythrocytic cycle. The optimum pH for the FP2-mediated degradation on specific substrates is different with hemoglobinase. The activity occurs at pH 5-6 as found in food vacuoles, while ankyrin degradation and self-activation of FP2 by autoproteolysis takes place at neutral or slightly alkaline pH. This suggests that FP2

activity may depend on the local cellular environment, thereby providing a substrate discrimination mechanism.^[5,7-9]

Allium sativum, better known as garlic, is easy to grow in mild climates and easily found in Indonesia. Analysis of garlic steam distillation carried out more than a century ago shows that garlic contains Allicin, Alliin, Diallyl disulfide, Diallyl trisulfide, Ajoene S-Allyl-Cysteine compounds are compounds contained in *Allium sativum*. The compounds in *Allium sativum* are known to inhibit the CSP process and prevent the invasion of sporozoites in vitro. The in vivo results also showed that mice injected with Allicin experienced a decrease in Plasmodium infection.^[10-13]

METHODS

Ligan and Protein Preparation

The active compound of garlic was based on a literature search. 3D structure samples of these compounds were obtained from PubChem (pubchem.ncbi.nlm.nih.gov). While the Falcipain protease-2 protein was obtained from the Protein Data Bank (PDB) (rcsb.org) with a protein code (6ssz).^[7]

Pharmacokinetic analysis

The active compound obtained from the database was analyzed pharmacokinetically by analyzing it based on Lipinski rules of five using the SwissAdme (swissadme.ch) webserver.^[14,15]

Protein-Ligand Interaction Analysis

The molecular docking test between the receptor protein and its ligands was carried out using the Vina Wizard application with the PyRx virtual screening tool program. The receptor and ligand files were analyzed in the

PDBQT file format. Analysis of protein-ligand bonds based on the interaction and the type of bond of the active compound *Allium sativum* binds to the protein Falcipain protease-2.

The ligand-protein interactions were assessed using the Discovery Studio program. The program will provide a representative 2D schematic representation of the complex bond between the ligand and the receptor after docking using the previous Vina Wizard.^[16,17]

RESULTS & DISCUSSION

Based on literature searches, we found six compounds in *Allium sativum*, namely Allicin, Alliin, Diallyl disulfide, Diallyl trisulfide, Ajoene, and S-allyl-cysteine. The ligands sought included: Allicin (Pubchem ID: 65036), Alliin (Pubchem ID: 87310), Diallyl disulfide (Pubchem ID: 16590), Diallyl trisulfide (Pubchem ID: 16315), Ajoene (Pubchem ID: 5386591), and S-allyl-cysteine (Pubchem ID: 9793905). The 3D structure of the ligands is stored and prepared for molecular docking tests.^[7]

The active compound from *Allium sativum* obtained from Pubchem is downloaded in 3D with the format (.sdf) and then converted into (.pdb) to analyze the 3D structure further. The compound is then predicted to be a drug-like molecule through the SwissAdme website based on the Lipinski rule of five. Analysis of Lipinski's Rule of Five was conducted to assess the pharmacokinetic ability of the compound. If a compound contains a rule from Lipinski, the compound can be said to be a drug. The rule is molecular weight ≤ 500 daltons, high lipophilicity ≤ 5 , hydrogen bond donor ≤ 5 , hydrogen bond acceptor ≤ 10 , molar refractivity 40-130.^[18]

The prediction results show that all compounds present in *Allium sativum* have drug-like molecules **Table 1**, so that these compounds can be further analyzed in their interaction with the target protein falcipain protease-2 in terms of strength and type of bond.

Table 1. Druglikeness prediction result from *Allium sativum* Compound

Compound	Molecular Weight	Hydrogen Bond Donor	Hydrogen Bond Acceptor	Log P	Lipinski Criteria
<i>Allicin</i>	162.72 g/mol	0	1	1.61	Yes
<i>Alliin</i>	177.22 g/mol	2	4	-1.33	Yes
<i>Diallyl disulfide</i>	146.27 g/mol	0	0	2.39	Yes
<i>Diallyl trisulfide</i>	178.34 g/mol	0	0	2.68	Yes
<i>Ajoene</i>	234.40 g/mol	0	1	2.53	Yes
<i>S-allyl-cysteine</i>	161.22.38 g/mol	2	3	-0.45	Yes

Note: Logp: Lipophilicity

Biomolecular Analysis *Falcipain protease-2*

Table 2. Binding affinity Between *Allium sativum* compound and *Falcipain protease-2*

Natural Compound	Binding Affinity (Kcal/mol)
Alliin	-4.2
Ajoene	-3.8
S-allyl-cysteine	-3.8
Diallyl trisulfide	-2.9
Diallyl disulfide	-2.8
Allicin	-3.1

Molecular docking is a study that studies the bonds between ligands and target proteins based on the analysis of their bond energies. The target protein is prepared using

Pymol to remove water molecules to make them more stable.^[19]

Molecular docking study uses a blind docking method because the functional domain of the target is unknown^[20]. Compounds derived from *Allium sativum* as ligands and Falcipain protease-2 (6ssz) were simulated using the PyRx software with a grid docking center X: 18.004 Y: -34,949 Z: 5,797 with dimensions (Å) X: 13 Y: 13 Z: 13. The docking results using PyRx will be visualized using Pymol and Discover studio software (**Figure 2,3**).

Figure 2. Visualization of the molecular interaction of the *Allium sativum* (yellow) compound and the *Falcipain protease-2* protein (green)

The content of the compound *Allium sativum* has the strongest bond with Falcipain protease-2 is Alliin with a bond energy of -4.2 kcal/mol. Strong protein-ligand bonds can affect a protein target's biological activity so that this compound is predicted to have activity in inhibiting the Falcipain protease-2. The

lowest bond energy will produce a molecule that has a constant temperature and pressure^[21]. The amino acid residues also influence it in the target protein's binding domain and the type of chemical interactions¹³. Alliin has the potential to be

further analyzed in terms of bond position and the type of chemical interactions formed.

We analyze chemical compounds with the lowest bond energies to determine molecular interactions and the type of bonds formed using the Discovery studio software.

Alliin compounds interact with Falcipain protease-2 with hydrogen and hydrophobic bond type domains. Alliin binds to the amino acids GLN19, ASP18, TRP189 by forming hydrogen bonds (green line) and ALA140 and TRP189 by forming hydrophobic bonds (yellow, purple, cyan lines) (**Figure 3**).

Figure 3. Chemical interaction of Alliin compound and *Falcipain protease-2* protein

The types of chemical bond interactions formed by ligands have a role in influencing the bond energy; the bond interactions formed such as hydrogen and hydrophobic^[22,23]. The residual amino acid produced by the interaction between ligand-protein is essential to determine compound molecules, probability as drug candidates. Alliin has a candidate as an antimalarial drug with its resistance to the protein Falcipain protease-2 with low bond energy with hydrophobic and hydrogen bonding interactions.

CONCLUSION

Alliin compounds contained in *Allium sativum* have the strongest bonds with Falcipain protease-2. Alliin compounds meet the Lipinski rules so that they can be used as drug candidates in the treatment of falciparum malaria infection. Based on the molecular docking test results, we recommend it as a reference for further research related to the anti-malarial effect of Alliin compounds through in vitro and in vivo tests

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