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Research Article

IN SILICO SCREENING OF ANTIMALARIAL FROM INDONESIAN MEDICINAL PLANTS DATABASE TO PLASMEPSIN TARGET

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ABSTRACT

Objective: Malaria is a disease that impacts millions of people annually. Among the enzymes, plasmepsin is the main enzyme in the plasmodium life cycle that degrades hemoglobin during the erythrocytic phase in the food vacuole. Recently, pharmaceutical industries have been trying to develop therapeutic agents that can cure malaria through the discovery of new plasmepsin inhibitor compounds. One of the developing approaches is the *in silico* method.

Methods: The chosen *in silico* screening method in this experiment is a structure-based screening using GOLD software and the Indonesian medicinal plants database.

Results: From ten *in silico* screening runs, three of the compounds always ranked in the top ten. These three compounds are trimyristin, cyanidin 3,5-di-(6-malonylglucoside), and isoscutellarein 4'-methyl ether 8-(6"-n-butylglucuronide). Another compound that emerged with high frequency is cyanidin 3,5-di-(6-malonylglucoside).

Conclusions: Based on the results obtained from this screening, 11 inhibitor candidates are expected to be developed as antimalarial. These compounds are trimyristin; cyanidin 3,5-di-(6-malonylglucoside); isoscutellarein 4'-methyl ether 8-(6"-n-butylglucuronide); cyanidin 3-(6"-malonylglucoside)-5-glucoside; multifloroside; delphinidin 3-(2-rhamnosyl-6-malonylglucoside); delphinidin 3-(6-malonylglucoside)-3',5'-di-(6-p-coumaroylglucoside); cyanidin 3-[6-(6-sinapylglucosyl)-2-xylosylgalactoside; kaempferol 3-glucosyl-(1-3)-rhamnosyl-(1-6)-galactoside; sanggenofuran A; and lycopene with a GOLD score range from 78.4647 to 98.2836. Two of them, Asp34 and Asp214, bind with all residues in the catalytic site of plasmepsin.

Keywords: Antimalarial, *In silico* screening, Indonesian medicinal plants database, Plasmepsin.

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INTRODUCTION

Every year, malaria, a disease caused by plasmodium, affects billions of people; over 2.5 million lose their lives. There are four species of plasmodium, namely, *Plasmodium falciparum, Plasmodium ovale, Plasmodium vivax,* and *Plasmodium malariae.* Of those four, *P. falciparum* accounts for over 95% of all malaria cases, mainly in Africa, and children under 5 years of age are among the most vulnerable to malaria infection [1]. It has been shown that hemoglobin catabolism, which takes place in an acidic food vacuole, is essential for parasite survival, both in culture and in animal models [2-4]. Besides asparticproteases, three cysteine proteases (falcipain-1, -2, and -3) and one metalloprotease (falcilysin) have also been identified to digest hemoglobin in the food vacuole [5-8]. To break hemoglobin down, several enzymes are involved, but plasmepsins are known to be vital for this function, as they initiate the catalytic breakdown of hemoglobin [9]. Two aspartic proteases of *P. falciparum* have been implicated in the initial steps of the hemoglobin degradation process. The first protease, plasmepsin I (Plm I), appears to make an initial strategic cleavage that presumably leads to an unraveling of the native hemoglobin structure such that further proteolysis can rapidly proceed. Plasmepsin II (Plm II), the second aspartic protease, is capable of cleaving native hemoglobin, but is more active against denatured or fragmented globin, such as that produced by the action of Plm I [10-12]. Plm I and Plm II make the first strategic cleavage of hemoglobin between Phe33 and Leu34 of the R-chain, resulting in protein unfolding and release of the heme moiety. Subsequent degradation steps are catalyzed by the cysteine protease falcipain, the metalloprotease falcilysin, and cytoplasmic aminopeptidases [13-16]. These complexes reveal key conserved hydrogen bonds between the inhibitor and the binding-cavity residues, notably with the flap residues Val78 and Ser79, the catalytic dyad Asp34 and Asp214, and the residues Ser218 and Gly36 that are in proximity to the catalytic dyad [17]. The catalytic mechanism of plasmepsin II is that the Asp34 and Asp214 residues coordinate a water molecule that following abstraction of a proton by Asp214, attacks the Phe33-Leu34 peptide bond of the α-chain in host hemoglobin [18].

As a mega-biodiversity nation, Indonesia ranks second in the world after Brazil. If marine biota is included, Indonesia moves to the first position. About 40,000 species of plants live on Earth, of which 30,000 species live in the Indonesian archipelago. Among the 30,000 plant species in the Indonesian archipelago, at least 9,600 species of plants are known to have pharmacological activity [19]. Previous research showed that the medicinal plants database and three-dimensional structure of the active compounds from medicinal plants in Indonesia have been prepared [20]. This database is supposed to be valuable compounds as an inhibitor for various diseases and targets, including plasmepsin in malaria.

In silico screening of small-molecule compounds against protein targets implicated in a disease of interest has been widely used to discover potential inhibitors. The efficacy and efficiency of multi-target *in silico* screening provides the potential to considerably reduce time, effort, and cost to obtain promising candidates for drug development [21]. *In silico* screening orients and scores a library of small molecules in the binding site of a protein, ranking the compounds from best to worst. Often, only a few of the top-scoring hits selected for experimental testing actually bind [22]. Regarding these characteristics, *in silico* screening will be favorably conducted to search potential plasmepsin inhibitors from the Indonesian medicinal plants database. This research aimed to gain candidates of inhibitor compounds *in silico* screening using GOLD [23]

software and ligands of the Indonesian medicinal plants database that originated from natural products which have an activity of inhibiting plasmepsin as the initial step of the antimalarial discovery process. 11 potential compounds from the database will be ranked and considered as hits.

METHODS

This experiment was conducted using literature study and *in silico* screening by molecular docking (structure-based *in silico* screening). The applied experimental design was as follows.

Plasmepsin structure preparation

Selecting a suitable plasmepsin structure for the binding target was the initial step of this research. Plasmepsin II (1LEE) was chosen. This plasmepsin structure was obtained from the Protein Data Bank [24] website as *.pdb file format. Plasmepsin II or 1LEE has one subunit (monomer), which is subunit A, and one ligand, which is 4-amino-N-{4-[2-(2,6-dimethyl-phenoxy)-acetylamino]-3-hydroxy-1-isobutyl-5-phenyl-pentyl} benzamide (R36), that is, gained through x-ray diffraction [17]. The structure was separated from solvent and ligand or non-standard residue using UCSF Chimera [25] software, then was optimized using Vega ZZ [26], including separation of the water molecule and the addition of a hydrogen atom. Afterward, Gasteiger partial charges were added, and AutoDock force field was applied. The last step was minimization using the steepest descent and conjugate gradient 100 and 1,000 steps, respectively.

Ligand file format preparation

The Indonesian medicinal plants compounds structure were obtained from the HerbalDB [20]. Research was conducted beforehand to generate the database 3D structure using Vega ZZ script "2D to 3D." This structure was then optimized by adding hydrogen with the "generic organic" and "after each heavy atom" option. After adding hydrogen to the structure, minimization was done using the steepest descent and conjugate gradient, 1,000 steps for each method. The last step was adding Gasteiger partial charges and applying AutoDock force field with AutoDock Tools [27].

Table 1: Docking results of positive control compounds using GOLD with slow speed and plasmepsin as target

Rank based on GOLD score	Name	GOLD score average (n=5)	SD	CV (%)
1	Pepstatin*	86.89	1.89	2.17
2	R37**	73.10	4.55	6.22
3	R36**	71.04	3.42	4.82
4	TIT**	68.24	11.32	16.59
5	5FE**	66.20	8.61	13.00
6	Halofantrin*	65.82	2.06	3.13
7	5FP**	63.73	3.73	5.86
8	IH4**	63.25	3.06	4.83
9	EH5**	62.40	11.55	18.52
10	IVS**	62.20	4.75	7.64
11	Norstatin*	61.32	1.41	2.30

*Positive controls downloaded from PubChem Compound, **positive controls extracted from Protein Data Bank

Molecular docking protocol validation

As the center of molecular docking target, the ligand binding site coordinates were defined using GOLD. The coordinates of the binding site were x=31.7977; y=33.2087; and z=12.3365 in the plasmepsin target. After being defined, these coordinates were used for validating the protocol using the 11 positive control compounds stated in Table 1 by conducting preliminary docking. The positive controls used are inhibitors of plasmepsin gained from the PubChem Compound [28] website and separated ligands from the Protein Data Bank website. To conduct molecular docking, the data format of the downloaded molecule from PubChem Compound must be converted first from a 2D structure to 3D, and it eventually changes the extension type from *.sdf into *.mol. To do this conversion, Vega ZZ was employed. This preliminary docking was conducted with three different speeds (slow, medium, and fast), five times for each speed, using GOLD.

Indonesian medicinal plants database *in silico* screening

In silico screening was conducted 10 times using the GOLD wizard model with the best parameters of the positive control compounds orientation docking. Docking parameter was set at 15 Å radius, 10 GA runs, 10 numbers of solutions, GOLD Score scoring function, and the slow GA search option.

Analysis and visualization of protein-ligand interaction

The 11 best compounds screened were visualized using GOLD and PyMOL [29] software. The GOLD docking result was saved in *.conf and *.mol formats that can be opened using GOLD and PyMOL. In GOLD, the best conformation of these top 11 compounds was observed, as well as the hydrogen bond between ligand and protein. PyMOL was then employed to process the molecular docking result data to be visualized later.

RESULTS AND DISCUSSION

Validation of molecular docking protocol using preliminary docking was conducted five times (n=5) for each speed (slow, medium, and fast) with three positive control compounds from the PubChem A compound known as a plasmepsin inhibitor, and eight positive control compounds extracted from the Protein Data Bank. The results were ranked based on GOLD Score, as shown in Table 1. After obtaining the best parameter from preliminary docking, *in silico* screening was conducted using the Indonesian medicinal plants database (http://herbaldb.farmasi.

Table 2: Top 11 compounds docked by GOLD based on rank as top ten from each run

Rank	Name	n	GOLD score average	SD	CV (%)	Plant(s) source [20]
1	Trimyristin	9	85.4396	4.9025	5.7379	*Aleurites moluccana, Myristica fragrans*
2	Cyanidin 3,5-di-(6-malonylglucoside)	9	84.4627	3.6624	4.3362	*Thymus serpyllum*
3	Isoscutellarein 4'-methyl ether 8-(6"-n-butylglucuronide)	9	80.8250	1.6951	2.0972	*Helicteres isora*
4	Cyanidin 3-(6"-malonylglucoside)-5-glucoside	7	83.1239	2.2321	2.6852	*Thymus serpyllum*
5	Multifloroside	7	82.3070	2.5021	3.0399	*Jasminum multiflorum*
6	Delphinidin 3-(2-rhamnosyl-6-malonylglucoside)	6	87.8589	3.1734	3.6119	*Clitoria ternatea*
7	Delphinidin 3-(6-malonylglucoside)-3',5'-di-(6-p-coumaroylglucoside)	4	92.5770	6.0155	6.4979	*Clitoria ternatea*
8	Cyanidin 3-[6-(6-sinapylglucosyl)-2-xylosylgalactoside	4	84.5243	4.0815	4.8288	*Apium graveolens, Foeniculum vulgare*
9	Kaempferol 3-glucosyl-(1-3)-rhamnosyl-(1-6)-galactoside	4	82.1026	0.9071	1.1048	*Camellia sinensis*
10	Sanggenofuran A	4	81.1762	1.8186	2.2404	*Morus australis*
11	Lycopene	4	81.0619	1.6951	2.0911	*Brassica napus, Diospyros kaki, Momordica charantia, Psidium guajava*

ui.ac.id) (Herbal db) [20]. The top 11 compounds are provided in Table 2. The average GOLD scores range from 80.8250 to 92.5770.

From ten *in silico* screening runs, three of the compounds always ranked in the top ten. These three compounds are trimyristin, cyanidin 3,5-di-(6-malonylglucoside), and isoscutellarein 4'-methyl ether 8-(6"-n-butylglucuronide). Trimyristin is a triglyceride from the Euphorbiaceae family, notably from *Aleurities moluccana*. Trimyristin is also found in *Myristica fragrans,* which belongs to the Myristicaceae family. Trimyristin is used as a nanoparticle lipid, which is combined with the curcuminoidin malaria treatment [30]. Triglycerides also have roles in malaria treatment as oil-phase transporters of antimalarial, for example, Self-microemulsifying Drug Delivery Systems that alter into a microemulsion after passing through the oral route [31].

Another compound that emerged with the highest frequency is cyanidin 3,5-di-(6-malonylglucoside). This is a flavonoid of the anthocyanin class which belongs to Lamiaceae family, notably from *Thymus serpyllum*. Anthocyanin is known to have antimalarial properties. It is also contained in *Corchorus olitorius* and is known to inhibit the malaria parasite *P. falciparum* more than 96% [32].

The other compound with the same frequency as the above compounds is isoscutellarein 4'-methyl ether 8-(6"-n-butylglucuronide). This is a glucuronide flavonoid from Sterculiaceae family, notably *Helicteres isora*. This species is known to have cucurbitacin, flavonoids, neolignan, and a derivate of rosmarinic acid. An earlier experience [33] extracted some flavonoids from *H. isora*, which are isoscutellarein 4'-methyl ether 8-O-β-D-glucuronide; isoscutellarein 4'-methyl ether 8-O-β-D-glucuronide 6"-n-butyl ester; isoscutellarein 4'-methyl ether 8-O-β-D-glucuronide 2"-sulfate; isoscutellarein 4'-methyl ether 8-O-β-D-glucuronide 2", 4"-disulfate; and Isoscutellarein 8-O-β-D-glucuronide 2", 4"-disulfate.

Most of the top 11 compounds belong to the flavonoid group or its glycol side. Natural and synthetic flavonoids show antimalarial activity [34]. The other experiment [35] also showed that flavonoids from *Artemisia annua* have *in vitro* antiplasmodial property, with IC_{50} score 2.3-6.5×10^{-5} M. Flavonoids can also be combined with artemisinin to treat malaria by increasing artemisinin activity [36]. This can be a consideration in the development of antimalarial from flavonoid-derived compounds due to their potential to inhibit plasmepsin. It is also valuable to conduct *in vitro* research regarding this potency to find the exact correlation between these compounds with their properties as antimalarial, especially as plasmepsin inhibitor.

The best 11 candidates of plasmepsin inhibitors were visualized and analyzed using GOLD and PyMOL. This showed that delphinidin 3-(2-rhamnosyl-6-malonylglucoside) and isoscutellarein 4'-methyl ether 8-(6''-n-butylglucuronide) bind with all catalytic residues in plasmepsin, which are Asp34 and Asp214. Delphinidin 3-(2-rhamnosyl-6-malonylglucoside) also binds with Gly36, Tyr192, Ser215, and Ser218 residues. This compound has a hydrogen bond with three residues in the plasmepsin active site, which are Asp34, Gly36, and Asp214. Gly36 residue is an active site that is in proximity to the catalytic dyad, which is Asp34 and Asp214. In the hemoglobin degradation process, Asp34 and Asp214 residues coordinate a water molecule that following abstraction of a proton by Asp214, attacks the Phe33-Leu34 peptide bond of the α-chain in host hemoglobin [18]. This bond will inhibit the substrate of plasmepsin to bind with the active site of the enzyme. Due to the inhibition of two catalytic dyads of plasmepsin by delphinidin 3-(2-rhamnosyl-6-malonylglucoside), the hemoglobin degradation process will also be inhibited, and so will malaria emergence. The pose of this compound is shown in Fig. 1.

Isoscutellarein 4'-methyl ether 8-(6''-n-butylglucuronide) binds with plasmepsin in Asp34, Ser79, Asp214, Gly216, and Thr217. Like delphinidin 3-(2-rhamnosyl-6-malonylglucoside), which has a binding site in the plasmepsin catalytic dyad, isoscutellarein 4'-methyl ether 8-(6''-n-butylglucuronide) also binds with two residues in catalytic

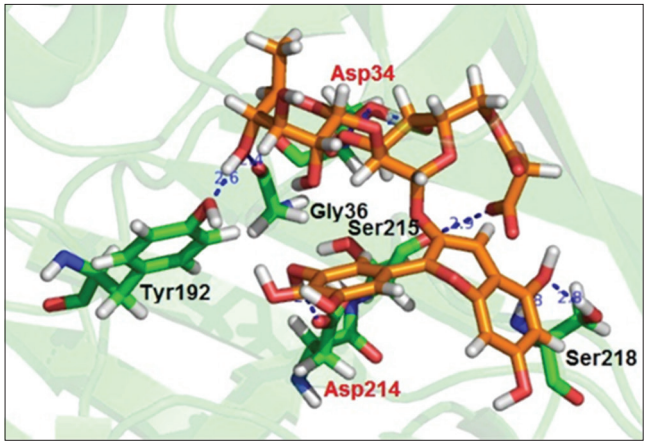

Fig. 1: Interaction of delphinidin 3-(2-rhamnosyl-6-malonylglucoside) (orange) with some amino acid residues (green) in plasmepsin. The red font shows the catalytic dyad Asp34 and Asp214

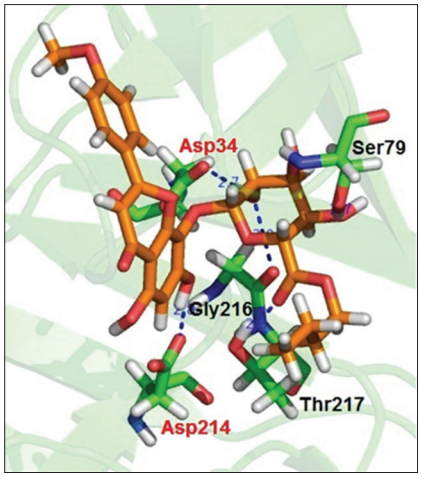

Fig. 2: Interaction of isoscutellarein 4'-methylether 8-(6''-n-butylglucuronide) (orange) with some amino acid residues (green) in plasmepsin. The red font shows the catalytic dyad Asp34 and Asp214

dyads, Asp34, and Asp214, so it can inhibit substrate binding with the enzyme. This binding disarms the enzyme so it cannot catalyze a reaction in the host hemoglobin degradation process. The pose of this compound shown in Fig. 2.

CONCLUSION

The 11 potential inhibitors against plasmepsin obtained from the *in silico* screening of the Indonesian medicinal plants database, such as trimyristin; cyanidin 3,5-di-(6-malonylglucoside); isoscutellarein 4'-methyl ether 8-(6"-n-butylglucuronide); cyanidin 3-(6"-malonylglucoside)-5-glucoside; multifloroside; delphinidin 3-(2-rhamnosyl-6-malonylglucoside); delphinidin 3-(6-malonylglucoside)-3',5'-di-(6-p-coumaroylglucoside); cyanidin 3-[6-(6-sinapylglucosyl)-2-xylosylgalactoside; kaempferol 3-glucosyl-(1-3)-rhamnosyl-(1-6)-galactoside; sanggenofuran A; and lycopene, have the prospective possibility for further investigation as leading antimalarial compounds.

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