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Antimalarial activity prediction analysis of *sticophus hermanni* on *plasmodium falciparum* hexose transporter (PfHT1)

Prawesty Diah Utami

Faculty of Medicine, Universitas Hang Tuah, Surabaya, Indonesia

*Corresponding author email: prawesty.diah@hangtuah.ac.id

R. Varidianto Yudho

Faculty of Medicine, Universitas Hang Tuah, Surabaya, Indonesia

Abstract--The objective of this study is to investigate *S.hermannii*'s antimalarial action against PfHT1 utilizing an in silico technique. In silico method, developing a protein target database by searching and collecting the protein target structures from the Protein Data Bank (PDB) and the UNIPROT databases. PfHT1 protein (PDB ID 6m20) with glucose control. Download all ligand structures from the PubChem database. Molecular docking analysis with Molegro virtual docker predicts interactions between ligands and protein targets. The last step was docking visualization to display 3D views and their interactions with the discovery studio program. The control compound beta-D-glucopyranose binds PfHT1 at the active site GLN169, GLN305, ASN341, GLY408, ASN311, and GLN305. All active compounds of *S.hermannii* were able to bind to PfHT1. This indicates that all active compounds enter the cell via hexose transporter 1 (PfHT1) receptors, such as glucose. All active substances of *S.hermannii* have antimalaria activity through PfHT1inhibition. Almost all active substances used were similar to the control binding sites, but only quinoxaline used different binding sites. The active substance of *S.hermannii* has a variety of binding affinities to PfHT1.

Keywords--bioinformatics, *S. hermannii*, natural products.

Introduction

Malaria was a global health concern that induced anemia and decreased labor productivity and mortality, especially in vulnerable populations such as newborns, young children, and childbearing women [1]. The malaria incidence declined significantly from 2000 to 2015, with progress stagnating. Based on

WHO report (2021), malaria infection reach 241 million in 2020, 6 percent rise over the previous year's data (227 million infection cases in 2019). Malaria deaths reflected a shift in the distribution of fatalities in early childhood, significantly rising than the previous year. With this updated baseline, malaria-related fatalities increased to 627,000 in 2020, up from 558,000 in 2019 [1–3]. The rise in mortality and morbidity rates was caused by service delays caused by the COVID-19 pandemic [3].

Artemisinin combination treatment (ACT) or ACT has been the principal drug used to treat various malaria infections for the past two decades. In the following ten years, the Mekong Subregion of Southeast Asia reported an increase in artemisinin resistance. Artemisinin resistance emerges as an interruption in parasite clearance following artemisinin-based therapy [4]. The new publications provide updated information on India's state of artemisinin resistance [5], and additional recent papers include details about Africa and other regions [6,7]. Malaria elimination efforts will be jeopardized if ACT activity diminishes. Discovering alternative psychosocial interventions to fight the expansion of ACT-resistant parasites has become crucial.

The action mechanism of antimalarial medications developed generally acts by interfering with the parasite's major metabolic pathway. Protein targets that match the drug or active molecule will decrease progression and enhance clinical symptoms[8]. Glucose uptake was a major metabolic pathway because malaria parasites require glucose as their principal carbon resource. Potentially antimalarial drug development is to selectively reduce parasite glucose uptake without interfering with similar physiological functions in the host. *Plasmodium falciparum* hexose transporter (PfHT1) was the primary glucose transporter responsible for glucose absorption. Thus, specific inhibition of PfHT1 represents a possible new target for identifying novel antimalarial drugs[9,10]. The ocean, which covers two-thirds of the planet's surface, is inhabited by many marine species. Ocean life contains a variety of active chemicals that have medicinal properties. One of the advantages of marine organisms over terrestrial organisms is a significant rise in phylogenetic variation. The phylogenetic variety is generated by environmental stimuli, demanding various adaptations and allowing marine organisms to develop distinct active molecules with therapeutic efficacy [11].

Sticophus hermanni, or golden sea cucumber, was prevalent in shallow seas and coral reef areas [12]. This sea cucumber was recognizable by its light yellow or greenish yellow coloration and little black or dark brown papillae dispersed on the dorsal and lateral surfaces. Sea cucumbers were previously recognized as a possible healthy food source and medicinal properties. *S.hermannii* has many therapeutic properties of its numerous components, which include fatty acids, vitamins, amino acids, glycosaminoglycans, keratin, glucosamine, triterpene glycoside, carotenoids, peptides, chondroitin, cell growth factors, mucopolysaccharides, glycosides, lectins, minerals, omega 3 and 6, also collagen, etc. [12,13]. Several studies have discovered that *S.hermannii* has biological activities such as tissue regeneration, relieving pain, antimicrobial, fungicidal, antioxidant, and anticancer [11,14]. Previous research revealed that *S.hermannii* has a high potency of antimalarial activity based on in vitro study [15]. The

occurrence of ACT resistance, elevated malaria mortality and morbidity, and the existence of bioactive components in sea cucumbers provided a stimulus to investigate the antiplasmodial action of *S. hermanni* utilizing in silico analysis [16].

Materials and Methods

The active component of *S. hermanni*

The bioactive compounds of *S. hermanni* were derived from searching for previous research/publications that investigated its active chemicals molecules.

Protein Target Database Development

In this research, we used the Therapeutic Target Database and a literature review to get the target protein database (TTD). The target protein screening procedure is carried out by conducting a keyword search with the terms "malaria" and "target protein." We selected and downloaded the target protein that matches the requirements from the Protein Data Bank, which is accessible via <http://www.rcsb.org> and available as an a.pdb file extension [17]. The target protein must fulfill the following standards such as :

- It plays a vital function in *Plasmodium* survivability, and its inhibition can result in parasite death.
- It possesses its native ligand [17].

All protein target structures were retrieved from the Protein Data Bank (PDB) and the UNIPROT databases, respectively. PfHT1 protein (PDB ID 6m20) from *Plasmodium falciparum* with glucose control [10,18,19]. Each protein's control was retrieved from the protein sample database. Each protein's control involvement is to validate docking with the control grid, and bioactive compounds/peptides interact in the same grid on a protein. Optimization of protein structure is done by cleaning the molecules that make up the solvent/solvent and attached ligands [20].

Tabel 1

Protein Target, PDB ID, Referensi dan Grid Docking

Protein	PDB ID	Refferensi	X (Å)	Y (Å)	Z (Å)	Radius
<i>Plasmodium falciparum</i> hexose transporter (PfHT1)	6m20	[10,18,19]	14.64	-28.75	- 19.93	8

Ligand Preparation

A ligand is a molecule that can interact with and forms a complex with receptors to conduct biological activities. A receptor is a protein that interacts with medications or drug metabolites in the body. Drug molecules interact with receptors to generate a reversible complex that eventually induces a reaction [21].

The ligands and receptors' structures were collected from the PDB[17]. Three-dimensional ligand structures were obtained from the National Center for Biotechnology Information's (NCBI) PubChem database: Glycine (CID 750), palmitic acid (CID 985), arginine (CID 6322), Quinoxaline (CID 7045), chondroitin sulfate (CID 10837), glutamic acid (CID 33032), glucosamine (CID 439213), arachidonic acid (CID 444899), docosahexaenoic acid (CID 445580), eicosapentaenoic acid (CID 446284), linoleic acid (CID 5280934), carotenoids (CID 14730338), and heparan sulfate (CID 53477715). The omega three and six complexes were retrieved from the NCBI PubChem database. Omega 3 complex (CID 56842239) is composed of linoleic acid, eicopentaenoic acid, and docohexaenoic acid. The omega-six complex (CID 56842208) comprises arachidonic and linoleic acid.

Molecular Docking

Molecular docking analysis is one of the most effective structure-based in silico tools for predicting interactions between molecules and biological targets. Molecular docking is usually accomplished by indicating the orientation of the ligand molecules in the receptor and then assessing their complementarity using the scoring function. Docking was carried out with the Molegro virtual docker five programs with specific protein grids on the active sites (binding cavities) of each protein (Table 1) [20]. The docking specifications for Molegro's virtual docker are Score Function Moldock Score [Grid].; with grid resolution 0.30; algorithm MolDock SE; Max iteration 1500; Number of Runs 10; max population size 50; pose generation energy threshold 100, 300; neighbor distance factor 1.00; tries 10 – 30; simplex evolution max steps; number of poses 5; 0.00energy threshold; cluster similar poses RMSD threshold 1.

Data analysis

The results of docking with Molegro virtual docking version 5 were combined with protein (superimposed) using PyMol version 2.2 software. Docking visualization to display 3D and 2D views and their interactions with the Discovery Studio program version 21.1.1

Results

The active component of *S.hermannii*

According to a previous literature study, *S.hermannii* contains several bioactive components. The following amino acids were discovered by HPLC examination of *S.hermannii* extracts: collagen (11200), glycine (37600), glutamic acid (3700), arginine (2050), and glucosamine hydrochloride (5.00) [22]. Ridhowati et al. (2018) study showed that *S.hermannii* has several fatty acids such as linoleic acid, palmitic acid, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and arachidonic acid [23]. Research by Masre et al. (2012) revealed that *S.hermannii*'s integument has many sulfated glycosaminoglycans (GAGs) like heparan sulfate and chondroitin sulfate [24,25]. Other literature research concluded that these sea cucumbers also contain quinoxaline derivates which have neuroprotective effects, and carotenoid [25,26]. Based on all previous studies, this research will

Tabel 2
Interaction of PfHT1 with Active Compound *S.hermannii*

Ligand	Interaction	Distance (Å)	Category
Control (beta-D-glucopyranose)	A:GLN169:NE2 - :10:O1	3,2115	Hydrogen Bond
	A:GLN169:NE2 - :10:O2	2,73284	
	A:GLN305:NE2 - :10:O3	2,88281	
	:10:H7 - A:ASN341:OD1	2,35401	
	:10:H9 - A:GLN169:OE1	1,89275	
	:10:H9 - A:GLY408:O	2,85165	
	:10:H10 - A:ASN311:OD1	1,97539	
Glycine	:10:H12 - A:GLN305:OE1	1,76072	Hydrogen Bond
	A:ASN311:ND2 - :10:O2	2,71009	
	:10:H3 - A:ASN435:OD1	2,68468	
	:10:H4 - A:ASN435:OD1	2,31822	
	:10:H5 - A:ASN435:OD1	1,93466	
	:10:H3 - A:TRP436	3,16152	
Palmitic acid	:10:H3 - A:TRP436	2,98164	Hydrophobic Bond
	A:ASN341:ND2 - :10:O2	2,60686	
	:10:H32 - A:GLN169:O	2,90537	
	:10:H32 - A:THR173:OG1	1,94097	
	A:THR173:CA - :10:O2	3,49147	
	A:VAL44 - :10	5,17521	
	:10 - A:ILE172	5,31389	
	:10 - A:ILE176	5,19835	
	A:PHE40 - :10	5,1428	
	A:PHE40 - :10:C13	3,95574	
	A:HIS168 - :10:C13	4,96969	
	A:TRP412 - :10	4,76151	
	A:TRP412 - :10:C13	3,92878	
A:TRP412 - :10	5,4132		
A:TRP412 - :10:C13	3,87805		
A:TRP436 - :10	4,7329		
Arginine	A:ILE172:CD1 - :10:C9	2,26068	Unfavorable
	A:ILE172:CD1 - :10:H17	1,75028	
	A:ILE172:CD1 - :10:H18	1,91413	
Arginine	A:GLN169:NE2 - :10:O2	3,14466	Hydrogen Bond
	A:GLN305:NE2 - :10:O1	2,88553	
	:10:H9 - A:GLN305:OE1	3,08824	
	:10:H10 - A:GLN169:OE1	1,96738	
	:10:H10 - A:GLY408:O	2,9973	

Ligand	Interaction	Distance (Å)	Category	
	:10:H12 - A:HIS168:NE2	2,03366	Hydrophobic	
	:10:H13 - A:HIS168:NE2	1,7916		
	:10:H5 - A:GLN305:OE1	2,37645		
	A:PHE40 - :10	4,9712		
	A:TRP412 - :10	4,78763		
	A:TRP412 - :10	4,94043		
	A:TRP436 - :10	5,12075		
	A:ILE172:CD1 - :10:N4	2,24206		Unfavorable
	A:ILE172:CD1 - :10:H14	1,92721		
Quinoxaline	A:THR145:OG1 - :10:N1	3,1533	Hydrogen Bond	
	:10:H6 - A:HIS168:NE2	2,33633		
	A:TRP412 - :10	4,89896	Hydrophobic Bond	
	A:TRP412 - :10	3,74599		
	A:TRP412 - :10	4,78065		
	A:TRP412 - :10	3,91642		
	:10 - A:ILE172	5,25652		
Chondroitin sulphate	A:ASN311:ND2 - :10:O1	2,69046	Hydrogen Bond	
	:10:H3 - A:GLN305:OE1	2,48224		
	:10:H7 - A:GLN305:OE1	2,33161		
	:10:H9 - A:ASN435:OD1	2,40942	Hydrophobic Bond	
	A:PHE403 - :10	4,7829		
	:10 - A:ILE310	4,38253		
Glutamic acid	A:GLN169:NE2 - :10:O3	2,84404	Hydrogen Bond	
	A:GLN305:NE2 - :10:O1	3,03418		
	A:GLN306:NE2 - :10:O4	3,16673		
	A:ASN311:ND2 - :10:O4	2,68423		
	A:TRP412:NE1 - :10:O1	2,99914		
	:10:H8 - A:GLN169:OE1	1,64716		
	:10:H3 - A:GLN305:OE1	2,99429	Unfavorable	
	:10:H9 - A:ASN435:OD1	1,80526		
	A:GLN306:NE2 - :10:H9	2,30299		
Glucosamine	A:GLN169:NE2 - :10:O1	2,85614	Hydrogen Bond	
	A:GLN305:NE2 - :10:O4	2,92037		
	A:ASN311:ND2 - :10:O2	2,73791		
	A:ASN341:ND2 - :10:O5	3,30691		
	:10:H9 - A:GLN305:OE1	2,80863		
	:10:H11 - A:ASN311:OD1	1,90153		
	:10:H12 - A:GLN169:OE1	1,91044		
	:10:H12 - A:GLY408:O	2,96439		Unfavorable
A:GLN169:NE2 - :10:H13	2,01685			

Ligand	Interaction	Distance (Å)	Category
Arachidonic acid	A:GLN169:NE2 - :10:O1	2,99041	Hydrogen Bond
	A:GLN169:NE2 - :10:O2	2,9983	
	:10:H32 - A:ASN341:OD1	2,25858	
	A:VAL44 - :10	4,12898	
	A:VAL314 - :10	3,46782	
	A:VAL314 - :10	5,43028	
	:10 - A:ILE310	4,89541	
	:10 - A:ILE310	4,90868	
	:10 - A:ILE172	4,53932	
	A:PHE40 - :10:C4	4,74851	Hydrophobic Bond
	A:PHE40 - :10	5,23491	
	A:PHE40 - :10	4,71643	
	A:PHE403 - :10	4,66671	
	A:TRP412 - :10:C4	4,27079	
	A:TRP412 - :10:C4	3,97856	
	A:TRP436 - :10:C4	5,02324	
	A:TRP436 - :10:C4	4,63558	
	A:TRP436 - :10	5,31643	
docosahexaenoic acid	A:GLN169:NE2 - :10:O1	2,60194	Hydrogen Bond
	:10:H32 - A:ASN341:OD1	1,99458	
	A:VAL44 - :10	5,3812	Hydrophobic Bond
	A:VAL44 - :10	4,29738	
	A:VAL314 - :10	4,58659	
	:10 - A:ILE176	4,83844	
	:10 - A:ILE310	4,03468	
	:10:C19 - A:ILE172	4,91237	
	A:PHE40 - :10	5,28126	
	A:PHE40 - :10	4,41924	
	A:PHE40 - :10:C19	3,91792	
	A:HIS168 - :10:C19	4,65416	
	A:PHE403 - :10	5,19334	
	A:TRP412 - :10	5,35138	
	A:TRP412 - :10:C19	3,61158	
	A:TRP412 - :10	5,38897	
	A:TRP412 - :10:C19	4,026	
	A:TRP436 - :10	5,26093	
A:TRP436 - :10	4,58294		
A:ILE172:CG2 - :10:C6	2,30972	Unfavorable	
A:ILE172:CG2 - :10:H11	1,26823		
A:ILE172:CD1 - :10:C18	2,35376		

Ligand	Interaction	Distance (Å)	Category	
Eicosapentaenoic acid	A:ILE172:CD1 - :10:C20	2,19017		
	A:ILE172:CD1 - :10:H28	1,69251		
	A:THR173:OG1 - :10:O2	2,64485	Hydrogen Bond	
	:10:H30 - A:THR173:OG1	2,36275		
	:10:H30 - A:ASN341:OD1	2,67095		
	A:THR173:CA - :10:O2	2,88128		
	A:VAL44 - :10	5,16279		
	A:VAL314 - :10	4,25544		
	:10 - A:ILE176	5,29192		
	:10 - A:ILE310	5,30598		
	:10 - A:ILE172	4,81958		
	:10 - A:ILE172	5,49794		
	:10 - A:ILE310	4,62271		
	:10:C18 - A:ILE172	5,40649		
	A:PHE40 - :10	4,58247	Hydrophobic Bond	
	A:PHE40 - :10:C18	4,06196		
	A:PHE403 - :10	5,10665		
	A:TRP412 - :10	5,23387		
	A:TRP412 - :10:C18	3,9404		
	A:TRP412 - :10	5,09021		
	A:TRP412 - :10:C18	4,02489		
	A:TRP436 - :10	4,92093		
	A:TRP436 - :10	4,16151		
	A:TRP436 - :10:C18	5,34962		
	linoleic acid	A:THR173:OG1 - :10:O2	2,64636	Hydrogen Bond
		A:ASN341:ND2 - :10:O2	3,03098	
		:10:H23 - A:ASN341:OD1	1,62424	
		A:VAL44 - :10	4,26614	
A:VAL314 - :10		4,4704		
A:VAL314 - :10		4,17463		
:10 - A:ILE310		3,99544	Hydrophobic	
:10 - A:ILE172		4,00999		
:10 - A:ILE172		4,81922		
A:PHE40 - :10		4,46017		
A:PHE40 - :10		4,45985		
A:TRP412 - :10		4,46854		
A:TRP412 - :10	5,01698			
Carotenoids	:10:H32 - A:GLU417:OE1	2,5101	Hydrogen Bond	
	A:PRO149 - :10	4,59508	Hydrophobic	

Ligand	Interaction	Distance (A)	Category	
	A:ILE176 - :10	4,35518	Bond	
	A:VAL314 - :10	4,19929		
	A:VAL314 - :10	4,09259		
	:10:C5 - A:VAL180	4,05648		
	:10 - A:ILE152	4,50858		
	:10 - A:ILE413	4,96052		
	:10:C18 - A:LYS161	4,22852		
	:10:C19 - A:ILE176	3,06195		
	:10:C19 - A:VAL314	2,83286		
	:10:C22 - A:PRO149	4,56878		
	:10:C22 - A:ILE152	3,08157		
	:10 - A:ILE176	4,18691		
	:10 - A:ILE310	5,40399		
	:10 - A:ILE413	4,29758		
	:10:C30 - A:ILE310	4,18734		
	:10:C32 - A:PRO409	4,41805		
	:10 - A:ILE172	4,36773		
	:10 - A:ILE172	4,09237		
	A:HIS168 - :10:C22	5,12706		
	A:HIS168 - :10	4,461		
	A:HIS168 - :10:C32	5,05538		
	A:HIS168 - :10	3,84675		
	A:PHE403 - :10:C30	4,87998		
	A:TRP412 - :10	5,37454		
	A:TRP412 - :10:C32	5,4665		
	A:TRP412 - :10	3,50781		
	A:TRP412 - :10	5,47027		
	A:HIS416 - :10:C17	5,2409		
	A:SER153:CA - :10:C18	1,82631		Unfavorable
	A:SER153:CA - :10:H26	1,5774		
	A:SER153:CA - :10:H27	1,54005		
	A:SER153:CB - :10:C18	1,71248		
	A:SER153:CB - :10:H26	0,654726		
	A:SER153:OG - :10:C18	1,74014		
	A:SER153:OG - :10:H25	1,7065		
	A:SER153:OG - :10:H26	1,1557		
	A:GLN169:CB - :10:C32	2,15224		
	A:GLN169:CB - :10:H46	1,10034		
	A:GLN169:OE1 - :10:C35	2,03599		
	A:GLN169:OE1 - :10:C37	2,09881		

Ligand	Interaction	Distance (Å)	Category
	A:GLN169:OE1 - :10:C38	1,99977	
	A:GLN169:OE1 - :10:C40	2,18432	
	A:GLN169:OE1 - :10:H50	1,68845	
	A:ILE172:CB - :10:C40	2,3748	
	A:ILE172:CB - :10:H54	1,50687	
	A:ILE172:CG2 - :10:H54	1,63084	
	A:ILE172:CD1 - :10:H54	1,76319	
	A:SER334:HG - :10:O1	1,38314	
	A:THR173:OG1 - :10:O16	2,5958	
	A:GLN306:NE2 - :10:O11	2,48772	
	A:ASN341:ND2 - :10:O17	3,0764	
	A:TRP412:NE1 - :10:O15	2,82205	
	:10:H22 - :10:O7	1,83533	
	:10:H24 - A:ASN341:O	3,04409	
	:10:H24 - A:ASN341:OD1	1,61345	
	:10:H25 - A:ASN311:O	2,43047	Hydrogen Bond
	A:GLY408:CA - :10:O18	3,64731	
	:10:H1 - :10:O17	2,96312	
	:10:H3 - :10:O14	2,19971	
	:10:H5 - A:ASN311:OD1	2,23499	
Heparan sulphate	:10:H7 - :10:O13	2,27372	
	:10:H16 - A:PHE40:O	2,97976	
	:10:H19 - :10:O13	1,93106	
	:10:H21 - A:ASN435:OD1	1,81849	
	:10:S1 - A:TRP412	5,5355	
	:10:S2 - A:PHE403	4,85451	Other
	:10:S3 - A:PHE85	5,39959	
	A:VAL44:CG1 - :10:N1	2,24152	
	A:ILE176:CD1 - :10:O2	1,8828	
	A:GLN305:OE1 - :10:O7	2,08821	Unfavorable
	A:ILE310:CG2 - :10:O12	1,59107	
	A:ASN311:OD1 - :10:O4	1,88899	
	:10:H14 - A:ASN311:OD1	0,985427	

Based on the table (table 2) and the 3-dimensional picture (figure 1) above, the control compound beta-D-glucopyranose binds PfHT1 at the active site GLN169, GLN305, ASN341, GLY408, ASN311, and GLN305. All active compounds of *S. hermanni* were able to bind to PfHT1 both with different active sites and the same as the control. Quinoxaline binds PfHT1 through other active areas of control. This indicates that all active compounds enter the cell via hexose transporter 1 (PfHT1) receptors, such as glucose. The types of bonds that

dominate are hydrogen bonds and hydrophobic interactions. In addition, some ligands found unfavorable bonds. A biomolecule's binding affinity is the potency of the binding connection between a ligand (such as a protein) with its binding partner (e.g., drug or inhibitor molecule). Inversely, the lower the affinity between receptors, the higher the binding energy value. The lower the binding energy value, the greater the affinity of the receptor and ligand.

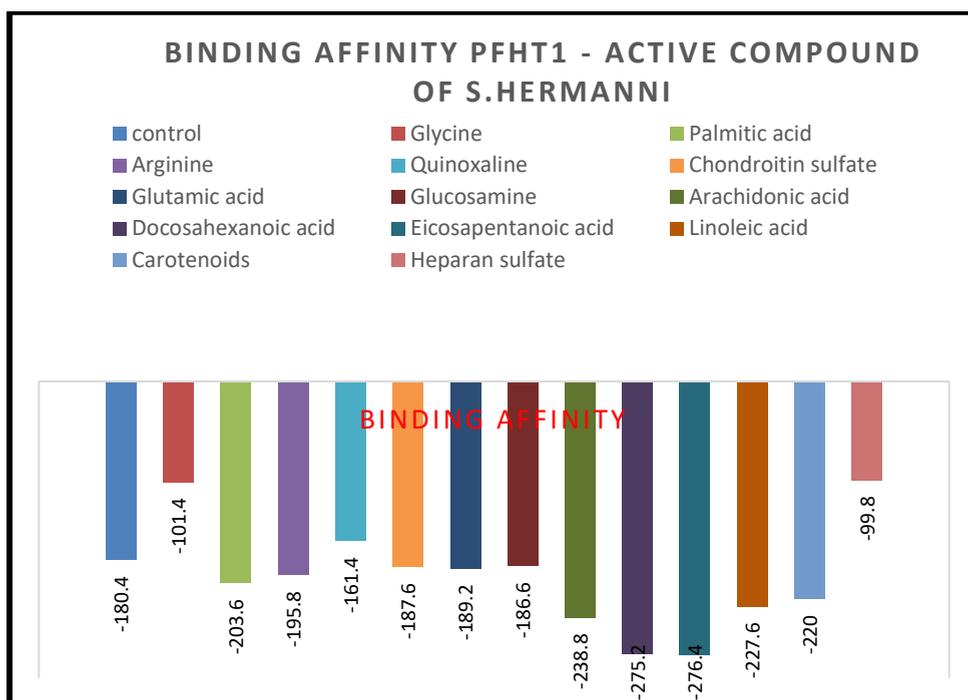


Figure 2. Comparison of Binding Affinity Between PfHT1 with Active Compounds of *S.hermanni* and Control

According to the table above, eicosapentaenoic acid has the lowest binding energy (-276.4 KJ/mol). Heparan sulfate with PfHT1 was the highest binding energy(-99.8 KJ/mol).

Discussion

According to study literature, *S.hermanni* has several active compounds that hypothesize to have antimalarial effects. Active compounds on *S.hermanni* consists of amino acids (glycine, arginine, glutamic acids); fatty acids (palmitic acid, arachidonic acid, DHA and EPA), glycosaminoglycan derivatives (Chondroitin sulfate, heparan sulfate), Quinoxaline, glucosamine, and carotenoids [22–26]. The principal energy source for the parasites, especially for the intraerythrocytic cycle, continuously need glucose intake. The *P. falciparum* hexose transporter one or PfHT1 has been genetically confirmed to be crucial for the intraerythrocytic parasite's existence. The parasite may be eradicated by being "starved out" using a chemically modified hexose transporter or active compound that binds to the active site of PfHT1 and inhibit glucose transport [9,10].

The amino acid that forms proteins with the least molecular weight is glycine ($\text{NH}_2\text{-CH}_2\text{-COOH}$; 75.067 g/mol). Glycine may combine hydrophilic and hydrophobic environments inside the polypeptide chain because of the hydrogen in its side chain [27]. Previous studies have shown that glycine can be combined with other molecules to produce new antimalarial drugs [26][28]. The analysis of this study (Figure 1 and Table 1) proved that glycine could bind to PfHT1 through the same active site as the control (ASN311) and other pathways (ASN 435 and TRP 436). Palmitic acid ($\text{C}_{16}\text{H}_{32}\text{O}_2$) is a saturated long-chain fatty acid. It comprises a 16-carbon structure, which can be obtained via diet and endogenous production. A previous study found that Ananas comosus extract, which has the highest concentration of linoleic and palmitic acids, exhibits antimalarial action [29]. In silico analysis showed that this active substance can bind to PfHT1 through the same active sites as the control (ASN 341 and GLN169) but also bind through other sites by hydrogen, hydrophobic and unfavorable bonds.

L-arginine, a semi-essential amino acid, is well established to be essential for regulating immunological function, cell development, and division. It serves as a substrate for the enzymes arginase and nitric oxide synthase (NOS), which are needed to synthesize L-citrulline and nitric oxide [30]. This study showed that arginine could act as antimalarial through PfHT1 binding by the same control site (GLN169, GLN305, and GLY408) and different sites using hydrogen, hydrophobic and unfavorable bonds. Awasthi et al. (2017), using in vitro research, showed that arginine supplementation media could induce malaria parasite growth [30]; this result contradicts this study's outcome. Quinoxaline derivatives ($\text{C}_8\text{H}_6\text{N}_2$) are in organic chemistry and are called benzopyrazine. Quinoxaline has N substitutes for carbon atoms in the ring of naphthalene, a significant class of heterocycle chemicals. It was created by combining two aromatic rings, benzene and pyrazine. Quinoxaline derivatives have several functions, such as treatment for chronic metabolic diseases, anti-inflammation, anticancer, anti microbial, anti fungal, and antiparasitic (antimalaria and antileishmania) [31]. In silico analysis predicts the binding of quinoxaline compounds to PfHT1 through various sites with controls: THR145, HIS168, TRP412, and ILE172 through hydrogen bonds and hydrophobic bonds. The results of prior investigations are similar to this in silico study's findings.

Chondroitin sulfate ($\text{C}_{13}\text{H}_{21}\text{NO}_{15}\text{S}$) is one of the sulfated glycosaminoglycan (GAG); it is composed of N-acetyl galactosamine and glucuronic acid in connective tissue proteoglycans. It has several functions anti thrombus, anti-atherosclerosis, anticoagulation, anti-inflammation, and antioxidant [32]. Another research demonstrates the application of chondroitin sulfate-chitosan nanoparticles in treating malaria as a transdermal medication release mechanism [33]. This study revealed that chondroitin sulfate could bind PfHT1 using the same route as control (ASN311 and GLN305) but also bind with different active sites using hydrogen and hydrophobic bonds. Glutamic acid ($\text{C}_5\text{H}_9\text{NO}_4$) is a non-essential amino acid that functions as a precursor for other amino acids, such as proline and arginine [34]. In this study, in silico prediction revealed that glutamic acid bind PfHT1 protein using the same active site as control (GLN169, GLN305, and ASN311) and distinct areas via hydrogen, hydrophobic, and unfavorable bonds.

Glucosamine ($C_6H_{13}NO_5$) is an amino sugar which is a widely consumed supplement and natural cartilage component that is usually paired with chondroitin sulfate and used to manage osteoarthritis and nonspecific joint pain. Previous research revealed that glucosamine has an antimalarial activity that could selectively suppress GPI production in the erythrocytic cycle of *P. falciparum*. In a novel mode of inhibition, glucosamine inhibits GPI formation by inhibiting the transfer of the fatty acyl moiety to GlcN-PI and decreases inositol-acylating enzyme activity but does not suppress gene expression [35]. This study's outcomes showed that glucosamine could bind PfHT1 with all sites as same as the control's sites (GLN169, GLN305, ASN311, ASN341, and GLY408) by hydrogen and unfavorable bonds.

Arachidonic acid, or AA ($C_{20}H_{32}O_2$), is an essential unsaturated fatty acid. AA has antimalarial activity through enhancing immunological response (both cellular and humoral), modulating macrophage activity (from M1 to M2), and directly inhibiting fatty acid production, which is required for living microorganisms. Polyunsaturated fatty acids or PUFA, consist of linoleic acid, docosahexaenoic acid/DHA, and eicosapentaenoic acid/EPA, have a cytotoxic effect on the parasite of *P.falciparum* and also induce an immune response to combat pathogen agents [36]. This study analysis predicts that AA, EPA, and DHA has antimalarial activity by binding to PfHT1 on the same control's active site (ASN341 and GLN169) and other pathways using hydrogen, hydrophobic, and unfavorable bonds. Carotenoids ($C_{46}H_{68}O_6$) are natural pigments composed of eight isoprene units with a 40-carbon backbone. Carotenoids are found in photosynthetic organisms. A previous in vitro study revealed that the extract of carotenoids from yeast has antimalarial activity[37]. Our research showed that carotenoids could bind with PfHT1 with the same control's active site (GLN169) and different areas with hydrogen, hydrophobic, and unfavorable bonds.

Heparan sulfate ($C_{18}H_{28}N_2O_{27}S_4^{-4}$) is a polysaccharide that belongs to the glycosaminoglycan family and is structurally similar to heparin; it is found in all mammalian tissues. Previous research stated that heparan sulfate could block the invasion of merozoite and reduce erythrocyte cytoadherence to endothelium receptors (ICAM-1 and CSA) [37]. Our analysis predicts that heparan sulfate could bind PfHT1 on the same control's active sites (ASN311, ASN341, GLY408) and other regions using hydrogen, unfavorable, and other bonds. Binding energy is the strength of the interaction between two or more molecules (proteins and ligands). The affinity between receptors and ligands will decrease as binding energy increases. Conversely, higher receptor affinities are associated with lower binding energy values [38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49].

The results of the binding affinity analysis between control against PfHT1 and the active compound against PfHT1 showed that: eicosapentaenoic acid/EPA had the lowest binding energy (-276.4 Kj/mol) compared to control and other active compounds. These results indicate that the binding of EPA with PfHT1 is the strongest. Heparan sulfate has the highest binding energy(-99.8 Kj/mol); these outcomes showed that the bond between heparan sulfate and PfHT1 was the weakest compared to control and other active compounds. The binding energy of ten active compounds (palmitic acid, arginine, chondroitin sulfate, glucosamine, glutamic acid, arachidonic acid, DHA, EPA, linoleic acid, and carotenoids) is lower

than control (-180.4 KJ/mol). The binding energy of three active compounds is higher than control, such as glycine, Quinoxaline, and heparan sulfate. According to the results of the binding energy estimations, our study determined that three active compounds have a lower affinity to bind PfHT1 than the control. In contrast, ten active compounds have a more significant affinity.

Conclusion

S. hermanni's active substances have antimalarial properties. Glycine, arginine, glutamic acids, arachidonic acid, palmitic acid, DHA, EPA, chondroitin sulfate, heparan sulfate, Quinoxaline, glucosamine, and carotenoids are the active ingredients on *S. hermanni*. *S. hermanni* active substances could bind protein PfHT1 on the substrate site with similar control sites and other particular sites via hydrogen, hydrophobic, unfavorable, and other bonds. The binding energies of ten active substances (palmitic acid, arginine, chondroitin sulfate, glucosamine, glutamic acid, arachidonic acid, DHA, EPA, linoleic acid, and carotenoids) are lower than those of the control, which means that the substances have a stronger affinity for the receptor. The remaining substances (glycine, Quinoxaline, and heparan sulfate) have lower affinities than the control because they have larger binding energies.

Conflict of Interest

We have no conflict of interest.

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