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Submission date: 03-Apr-2023 03:36PM (UTC+0800)
Submission ID: 2054417689
File name: Jurnal_terbit_dengan_Prof_Loeki_IJPR_new.pdf (1.43M)
Word count: 9654
Character count: 53950

ISSN 0975-2366
DOI:https://doi.org/10.31838/ijpr/2021.13.01.321

Research Article

Antimalarial Properties of Streptomyces hygroscopicus subsp Hygroscopicus Secondary Metabolite Active Fractions: In Silico and In Vivo Analysis

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Received: 15.10.20, Revised: 07.11.20, Accepted: 09.12.20

ABSTRACT

Background: Crude extract of Streptomyces hygroscopicus has antimalarial effect in vivo and its active fractions showed Plasmodium inhibition in vitro. This study aims to analyze the antimalarial activity of fraction 41 and 44 of S.hygroscopicus in vivo and predict their antimalarial mechanism in silico.

Material and methods: Secondary metabolite of S.hygroscopicus was identified using LC/MS. In silico analysis was done using PfA-M1, Falcipain 2 protease, and PfCRT as protein targets from PDB and active compound ligand from PubChem. The pharmacokinetic analysis was done using SwissADME. In vivo study was conducted using Plasmodium berghei infected mice that divided into 1 positive control group, 1 Artesunate group, and 8 treatment groups that administrated with fraction 41 and 44 each in dose 8,125 µg/kgBW, 32,5 µg/kgBW, 130 µg/kgBW, and 520 µg/kgBW.

Result: Tryptanthrin was identified from S.hygroscopicus. Tryptanthrin has fulfilled drug-likeness criteria and stronger binding affinity to PfA-M1, Falcipain 2 protease, and PfCRT than control ligand. Fraction 41 showed a significant difference in parasite density between positive control group and 130µg/kgBW group (p=0.046) as well as 520µg/kgBW group (p=0.004). Fraction 44 showed a significant difference in parasite density between positive control group and 130µg/kgBW group (p=0.024) as well as 520µg/kgBW group (p=0.004). Correlation test of fraction 41 showed a strong negative relationship between doses and parasite density on day 3 (p=0.001; r=-0.607) and day 4 (p=0.000; r=-0.700) as well as fraction 44 on day 3 (p=0.044; r=-0.589).

Conclusion: Active fractions of Streptomyces hygroscopicus subsp Hygroscopicus that contained Tryptanthrin has a potential antimalarial agent.

Keywords: Streptomyces hygroscopicus, Tryptanthrin, molecular docking, parasite density.

INTRODUCTION

Malaria is a life-threatening infectious disease that can cause various complications. According to the World Health Organization (WHO), in 2017 approximately there were 219 million cases of malaria in 90 countries and 435.000 death caused by malaria (WHO Report, 2017). The development of antimalarial drugs is continuously developed to achieve the global target of eliminating the world from malaria. However, current efforts to develop anti-malarial drugs does not consistent with the increase of Plasmodium resistance (CDC, 2018; World Health Organization, 2010; World Health Organization, 2018).

A previous study using Liquid Chromatography-Mass Spectrometry (LC-MS) revealed one compound identified from the 5 compounds that have the highest peak contained in the crude extract of Streptomyces hygroscopicus subsp. Hygroscopicus, namely the Isoquinoline compound which has been shown to have an antimalarial effect (Nugraha et al., 2020). Then, from a previous in vitro study, the fractionated secondary metabolite extract of Streptomyces hygroscopicus subsp. Hygroscopicus revealed

antimalarial activity through mitochondrial enzyme inhibition which is characterized by decreasing levels of Plasmodium falciparum Lactate Dehydrogenase (PfLDH) (Fitri et al., 2019).

The potential metabolite derived from the extract of the metabolites Streptomyces hygroscopicus subsp. Hygroscopicus needs to be explored and developed further as an effective antimalarial. Research shows in silico analysis and the effectiveness of active fractions from secondary metabolite extract of Streptomyces hygroscopicus subsp. Hygroscopicus in vivo will have a major role in the development of new antimalarial drugs.

MATERIALS AND METHOD

Design and Settings

The design of the study was an explorative method using LC/MS and continued to analyze the antimalarial activity of fraction 41 and fraction 44 of S. hygroscopicus in vivo and predict their antimalarial mechanism in silico. The pharmacokinetic analysis was done using SwissADME. In vivo study was conducted using Plasmodium berghei infected mice that divided into 1 positive control group, 1 Artesunate group, and 8 treatment groups that administrated intraperitoneally with fraction 41 and 44 each in dose 8.125 µg/kgBW, 32.5 µg/kgBW, 130 µg/kgBW, and 520 µg/kgBW. Parasite density was observed daily for 3 days of treatment. Each group consisted of four female Swiss mice with the age-range between 6-8 weeks. Parasite density was observed daily for 3 days of treatment. This research was conducted from April to July 2019.

Culture of Streptomyces hygroscopicus

Streptomyces hygroscopicus was obtained from LIPI Microbial Collection, Cibinong, Indonesia. Then, S. hygroscopicus was subcultured in the Microbiology Laboratory of the Faculty of Medicine, Universitas Brawijaya using ISP4 culture media. The bacteria that grew from the subculture were characterized as microscopic and macroscopic.

Inoculation of Streptomyces hygroscopicus

Inoculation was carried out using ISP4 liquid media 1000 ml with a pH condition of 7.0-7.4 in aseptic conditions. The bacterial colonies in ISP4 were scraped with an inoculum needle (ose) and then homogenized using a shaking incubator.

Secondary Metabolic Fermentation of Streptomyces hygroscopicus

The inoculum was calculated 25.8 $\times 10^6$ bacteria then added to the broth ISP4 and fermented at 28°C with 150 rpm in a shaking incubator for 5 days with the ratio of ISP4 and inoculum 10: 1 (v/v). Then, the media is covered with cotton and aluminum foil, to ensure that there is no air in Erlenmeyer.

Extraction Procedure for Metabolites of Streptomyces hygroscopicus

Before extraction, the media were harvested and centrifuged at 3000 rpm for 10 minutes to separate the cells from the debris. The filtrate was then collected in a container and ready for extraction. The filtrate mixed with a solvent (ethyl acetate) in a ratio of 1: 1 (v / v) and then hand-shaken for 1 hour in a separating funnel. Then it is allowed to stand until it forms 2 layers (solvent phase and water phase). The solvent phase containing secondary metabolites of Streptomyces hygroscopicus bacteria then separated from the water phase and then the solvent phase (ethyl acetate) is evaporated using a rotary evaporator at a temperature of 40-50°C until the liquid turns into a paste.

Secondary Metabolite Extract Fractionation of Streptomyces hygroscopicus

The fraction was produced using the BUCHI Reveleris® PREP Purification System flash column with silica gel 60 eluted by n-hexane and ethyl acetate in ratio 1:1 (v/v). The UV light detector used was 254nm, 365nm, and 366nm, and ELSD (Evaporative Light Scattering Detector). Running process set with a flow rate of 5mL / minute, and gradient mode. The collected samples were evaporated.

Liquid Chromatography-Mass Spectrometry (LCMS) Based Identification of Secondary Metabolite Extract Fractionation

The fractions of secondary metabolite Streptomyces hygroscopicus were carried out by Liquid Chromatography-Mass Spectrometry (Xevo G2-S QTof, Waters, USA) using Ultra Performance Liquid Chromatography (UPLC) system (ACQUITY UPLC®H-Class System) with C_{18} column (ACQUITY UPLC®H-Class, 2.1 mm x 10 mm, 1.8 µm, Waters, USA) at a constant temperature of 50°C (column) and 25°C (room). The flow rate was 0.2 ml/minute, with a running time of 23 minutes, and a sample injection volume of 0.2 mL. For the ionization system detector electrospray ionization (ES) with positive ion mode, mass analysis range of 50-1200 m / z, with the energy used 4 volts for low energy and 25 - 50 volts for high energy. The mobile phase was a mixture of water and 5mM ammonium format (solvent A) and a mixture of 0.05% formic acid and acetonitrile (solvent B). The results were analyzed using the MassLynx application.

Pharmacokinetic Profile

The pharmacokinetic profile of S. hygroscopicus derivative compound was analyzed using Swiss ADME by entering the SMILES formula.

Construction of Protein Target Database

The protein target database was obtained through literature studies. The protein target screening process was done by searching the keywords "Tryptanthrin", "Plasmodium", "malaria", "in silico", "food vacuole", and "protein target". Plasmodium falciparum Chloroquine Resistance Transporter (PfCRT) (PDB ID: 6UKJ), Plasmodium falciparum Aminopeptidase-M1 (PfA-M1) (PDB ID: 4ZW3), and Falcipain 2 protease (PDB ID: 6SSZ) were used as protein targets for this in silico study. The protein targets were selected and downloaded from Protein Data Bank via the http://www.rscb.org.pdb in .pdb file extension. Then, the residual separation and optimization of the protein target were conducted by removing the remaining water, ligands from the crystallization process, and cofactors using the PyMol 2.0 program. The residue needs to be separated from the protein to optimized the protein-ligand bond. The results of this process were saved in the form of .pdb extension.

Preparation of Ligands and Target Structures

The ligand structure was obtained from the identification of the derivative compound of S. hygroscopicus via LC/MS. The derivative compound that had been identified is Tryptanthrin. The active ligand was found in PubChem (CID: 73549). The information on control ligands for each protein target was found in RSCB Protein Data Bank and from the literature review. The 3D structure was downloaded from https://pubchem.ncbi.nlm.nih.gov/ as .sdf file. Control ligand from each protein target was obtained from the literature review. The control ligand for PfCRT in silico analysis was a protonated Chloroquine structure which was drawn using the MarvinSketch program (Antony et al., 2019) and was downloaded as .pdb format.

Reverse Molecular Docking Studies

Reverse molecular docking was done using PyRx 0.9.5, with a specific dimension of grid box based on native ligands of protein targets as control ligand. Reverse molecular docking was performed between ligands of Tryptanthrin compounds, control ligands, and protein targets from the Plasmodium parasite using PyRx with a specific docking approach. For PfCRT docking, a grid box of 30 x 30 x 30 (x, y, z) was set around the active site, Transmembrane Domain 1 (TMD1) which is amino acid ranging from 59 to 79, where the control ligand can interact (Antony et al., 2019). Then, the center of the grid box was fixed at (143, 160, 143) and the exhaustiveness was set as 9. For PfA-M1 docking, a grid box dimension was generated by fixing the number of point x, y, and z-direction to 19 x 19 x 19, the center of the grid box was fixed at (87.813, 120.015, 11.315), and the exhaustiveness was set as 8. While for Falcipain 2 protease docking, a grid box dimension was generated by fixing the number of point x, y, and z-direction to 13 x 13 x 13, the center of the grid box was fixed at (18.004, -34.949, 5.797), and the exhaustiveness was set as 8. The docking process of each protein target with its control ligand and the active compound was performed three to five times for accuracy, then the average binding affinity was calculated. The binding affinities were compared between the ligand from S. hygroscopicus and control ligands to each protein target.

The binding affinities between the derivative compound and protein targets compared to the control ligands were visualized using LigPlot 2.2. The visualization was used to assess whether the residues formed were consistent with the score of binding affinities. Then, further analysis of the bond similarity was compared between the derivative compound bond with the protein target as well as the control ligand with the protein target. The goal was to find out the location of derivative compounds that binds with the protein target.

Experimental study

Healthy Swiss female mice (6-8 weeks) weighing 25-30 grams were purchased from Bandung, Indonesia, and kept in the Parasitology Laboratory of the Faculty of Medicine, Universitas Brawijaya, Malang. The mice were kept in a cage measuring 30x30 cm, each cage contained 4 mice. Mice were adapted for 1 week for environmental adjustments. Mice were fed with standard feed once daily in sufficient quantities per cage to avoid food competition between mice. Drinking boiled water was replaced every day. During the study, mice were treated carefully and paid attention to the ethical feasibility of research with experimental animals. Treatment of mice was started with intraperitoneal inoculation of the parasites with an aseptic procedure. Parasitemia density was checked daily using a capillary blood sample from the mice tail. The treatment was carried out by intraperitoneal injection for 3 days. The study had been approved by the Ethics Commission of the Faculty of Medicine Universitas Brawijaya with Ethic No 272/EC/KEPK/11/2018.

Preparations and inoculation of P. berghei

Parasites were obtained from Biomedical Laboratory, Universitas Brawijaya. Erythrocyte pellet infected with P. berghei stored in liquid nitrogen tank at -135°C was thawed and subjected to centrifugation at 2000 rpm for 5 minutes. Pellets were washed twice in RPMI medium and diluted as needed for inoculation to donor mice. After parasitemia of donor mice reached more than 10%, blood was collected through cardiac puncture. Inoculation was done intraperitoneally to the experimental mice intraperitoneally at 10^7 parasites in 0.2 mL of blood for mice.

Fractions of S. hygroscopicus subsp. Hygroscopicus treatment

Active fraction 41 and 44 of Streptomyces hygroscopicus subsp. Hygroscopicus, weighed to a standard concentration of 5 mg/mL in DMSO by sonicator. The liquid was diluted with RPMI based on dose 8.125 µg/kgBW, dose 32.5 µg/kgBW, dose 130 µg/kgBW, and a dose 520 µg/kgBW. Treatment was given by injecting a total of 200 µl each fraction of S. hygroscopicus subsp. Hygroscopicus in mice intraperitoneally once daily for three days. The positive control group was treated by injecting Phosphate-buffered saline (PBS) while the Artesunate group as another antimalarial control group was treated by injecting Artesunate with mice-converted dose 29.52 mg/kgBW intraperitoneally.

Parasitemia evaluation

Thin blood smear from the mouse tail was made on the object-glass with methanol fixated. The smear was stained with 20% Giemsa in a ratio of 1: 4 for Giemsa and Giemsa buffer for 20 minutes. Then rinsed with water and dried. Observation of parasitemia was done at 1000x magnification with immersion oil. A thin blood smear was made to evaluate parasitemia after each treatment once daily for three days. Parasite density was calculated based on the number of erythrocytes infected with malaria for every 1000 erythrocytes. Parasite density measurements were carried out every day.

Data analysis

Data analysis was performed using the SPSS program with a significance level or a probability value of 0.05 ($p=0.05$) and a confidence level of 95% ($\alpha=0.05$). Statistical significance was stated when the P-value was equal to or less than 0.05 ($p<0.05$).

RESULT AND DISCUSSION

The Fractions and LCMS Identification

The fractions were carried out to simplify the extract samples containing various components. In this process, 49 samples were obtained. From the 49 samples, fraction numbers 15,16, 41, and 44 were selected to identify the compounds contained. The fractions were chosen because previous studies revealed antimalarial activity. Then the fractions were identified using LCMS and analyzed using MassLynx software to determine the molecular weight of the compound obtained. The molecular weight was compared to the data on the mass bank to determine the possible compounds and spectra of the compounds obtained. The Spectra was compared with data in the MoNA and HMDB database. The results of the identification and analysis of the four fractions are listed in **Table** 1.

Table 1: The results of secondary metabolites S. hygroscopicus identification using LCMS

Fraction Number	Compound	Retention Time	Molecular Formula	Molecular Weight (m/z)	Area (%)
15	Erucamide	15.58	$C_{22}H_{43}NO$	337.6	4.83
16	Flavanone	11.98	$C_{15}H_{12}O_2$	224.25	1.04
	Trachelanthine	3.66	$C_{15}H_{27}NO_5$	301.38	0.22
	Erucamide	16.29	$C_{22}H_{43}NO$	337.6	14.45
41	Tryptanthrine	9.91	$C_{15}H_8N_2O_2$	248.24	0.93
	Erucamide	18.21	$C_{22}H_{43}NO$	337.6	4.83
	Farnesol	15.14	$C_{15}H_{26}O$	222.37	0.17
	2-phenylethanol	14.00	$C_8H_{10}O$	122.16	0.93
	Hexadecanamide	15.56	$C_{16}H_{33}NO$	255.44	0.05
44	Tryptanthrine	9.91	$C_{15}H_8N_2O_2$	248.24	0.93
	Erucamide	18.41	$C_{22}H_{43}NO$	337.6	13.97
	2-phenylethanol	13.39	$C_8H_{10}O$	122.16	0.16
	9-octadecanamide (oleamide)	15.86	$C_{18}H_{35}NO$	281.5	0.05
	Hexadecanamide	15.56	$C_{16}H_{33}NO$	255.44	0.25

There was one compound of eight compounds found, that had an antimalarial activity based on literature studies, namely Tryptanthrin. Tryptanthrin (indolo [2,1] quinazoline-6,12-dione) is a weak alkaloid class compound. It has a molecular weight of 248.24 g / mol. It is bright yellow, consisting of a quinazoline ring, which is attached to the indolent part of the carbonyl group at positions 6 and 12. Tryptanthrin name comes from early research where the compound was produced by Candida lipolytica in the Tryptophan medium (Tucker & Grundt, 2012). The antimalarial activity was evaluated through strains of P. falciparum which sensitive to Chloroquine and resistant to Chloroquine using the Malstat test. The concentration of the compounds used ranged from 0.01 μM to 100 μM and the inhibitory concentration (IC50) was measured by measuring the parasite's lactic dehydrogenation activity. The results showed that the IC50 Tryptanthrin value was 288 nM for Chloroquine sensitive P. falciparum strains and 114 nM P. falciparum strains resistant to Chloroquine (Onambele et al., 2015).

Pharmacokinetic Profile Prediction

Pharmacokinetics is defined as a dynamic movement of foreign chemical compounds during their delivery in the body, which represents how the body treats these chemical compounds. In drug development, a drug candidate should have balance pharmacokinetics and pharmacodynamics profile as well as the safety of the drug. Pharmacokinetic profiles that must be considered are Absorption, Distribution, Metabolism, and Excretion (ADME), then toxicity (T) (Fitri et al., 2019; Wang et al., 2015). After obtaining active compounds, a predictive analysis of pharmacokinetic profiles or ADMET was carried out. Pharmacokinetic profiles were obtained using the SwissADME website by entering the compound SMILES formula, showed in **Table 2**.

The pharmacokinetic profile of a compound can be explained through its physicochemical criteria. The partition coefficient (cLogP) shows the membrane permeability, absorption, distribution, and route of clearance of ADME parameters. The hydrogen bond parameter of the physicochemical component is related to the permeability of the drug across the membrane. Topological Polar Surface Area (TPSA) parameters are also related to permeability and absorption. The higher the TPSA value, the lower the absorption and permeability of the compound. Then, Rotatable Bond (ROTB) in Veber's criteria is related to absorption, permeability, and drug distribution in the body where the oral drug has fewer donors and acceptors for Hydrogen and ROTB bonds than drugs with other routes of administration (M. Honorio et al., 2013).

Lipophilicity is fat solubility explained by the value of the partition coefficient (P). Lipophilicity is the complex effect of the molecular interactions between the solute and the solvent as well as the interactions between the solvent molecules in each phase. These substances when dissolved in a solvent can disrupt their structure causing the breakdown of intermolecular bonds. Lipophilicity affects the biological activity of drugs because it plays an important role in drug-receptor interactions (Czyrski, 2019).

Based on the principle of "Rule of Five" from Lipinski (Lipinski, 2004), the results of the physicochemical profile of Tryptanthrin from the pharmacokinetic analysis has a molecular weight of 248.24 g / mol, the number of H-bond acceptors as much as 3, the number of H-bond donors as much as 0, and the lipophilicity shown by the cLogP value of 2.16. All of Lipinski's drug-likeness criteria have been met. In addition to Lipinski's criteria, Tryptanthrin also meets the additional criteria of Veber's Rule (Veber et al., 2002), which were zero Rotatable Bond (ROTB), which should less than 10, and a TPSA value of 51.96 Å2, which should less than 140. Tryptanthrin fulfilled all of the drug-likeness criteria which revealed its good permeability properties and can be administered orally.

Table 2: Tryptanthrin Pharmacokinetic Prediction

Tryptanthrin Physicochemical Properties	
Molecular weight	248.24 g/mol
Rotatable bonds	0
H-bond acceptor	3
H-bond donor	0
TPSA	51.96 $Å^2$
Lipophilicity	
cLogP	2.16

Reverse Molecular Docking Result

LC/MS analysis of fraction 41 revealed 15 compounds, while fraction 44 revealed 14 compounds. Of all the compounds, both fraction 41 and 44 contained Tryptanthrin ($C_{15}H_8N_2O_2$) with a molecular weight of 248.24 g/mol. Tryptanthrin was chosen as a candidate compound for in silico method because it has a good pharmacokinetic profile and antimalarial activity in previous studies. Tryptanthrin has activity against sensitive and resistant Plasmodium to Chloroquine. The ligand structure was prepared by downloading the compound from PubChem in .sdf format **(Figure 1)**. In silico method, the protein target used was the

Plasmodium food vacuole which has a role in hemoglobin degradation and catabolism. Plasmodium food vacuole is related to antimalarial drug resistance. Compound that can bind to these targets could potentially become a new and effective antimalarial therapeutic agent. The method used was reverse molecular docking, which predicts the interaction between one ligand, namely Tryptanthrin, and many protein targets.

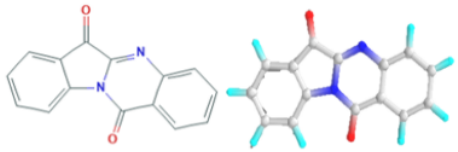

Fig.1: The chemical structure Indolo[2,1-b]quinazoline-6,12-dione (Tryptanthrin)

Protein targets selected were PfCRT, PfA-M1, and Falcipain 2 protease from P. falciparum, each protein target has a different control ligand. The control ligands were used to compare the binding affinity between control ligands and compounds. Reverse molecular docking procedure was done using the PyRx software program to analyze the binding affinity and the bond interactions between the compound and the protein target. Binding affinity is the ability of a ligand to bind with a macromolecular target. A negative value for binding affinity indicates that the ligand is predicted to bind to the macromolecular target. The more negative the binding affinity, the better the predicted bond between a ligand and a macromolecule (Lamarque et al., 2008; Wunderlich et al., 2012). Tryptanthrin compounds and control ligands were docked and analyzed by comparing their binding affinities against protein targets. The binding affinity between Tryptanthrin and the protein targets are presented in **Table 3**.

Table 3: Reverse Molecular Docking Result

ID PDB	Protein target	Control Ligand	Binding Affinity (Kcal/mol)	
			Control	Tryptanthrin
4ZW3	Plasmodium falciparum Aminopeptidase M1 (PfA-M1)	tert-butyl [(1s)-1-(4-bromophenyl)-2-(hydroxyamino)-2-oxoethyl]carbamate	-8.1	-9.4
6SSZ	Falcipain 2 protease	(~{e})-3-(1,3-benzodioxol-5-yl)-1-(3-nitrophenyl)prop-2-en-1-one	-7.3	-7.7
6UKJ	Plasmodium falciparum Chloroquine Resistance Transporter (PfCRT)	Chloroquine^{2+}(*)	-7,13	-8,33
(*) Based on a previous study (Antony et al., 2019)				

The results showed that Tryptanthrin could act on existing protein targets with different binding affinities which stronger than each control ligand indicating the possibility of protein target binding, so they could inhibit the biological activity of the native ligand. The binding affinity of Tryptanthrin and protein target PfA-M1 (-9.4 kcal/mol), Falcipain 2 protease (-7.7 kcal/mol), and PfCRT (-8.33 kcal/mol) were more negative than each control ligand, -8.1 kcal/mol, -7.3 kcal/mol, and -7.13 kcal/mol, respectively. The bond interaction between Tryptanthrin and the protein target was visualized using Ligplot 2.2. **Table 4** shows the presence of ligand-protein interactions in the form of hydrophobic interactions and hydrogen bonds. Hydrogen bond and hydrophobic interactions are weak intermolecular interactions that play an important role in stabilizing the ligands in a conformational environment of the protein target (Azimzadeh et al., 2010). Tryptanthrin compounds act on the active site of the protein target by forming hydrogen bond and hydrophobic interactions with the protein targets PfA-M1, Falcipain 2 protease, and PfCRT.

Table 4: Results of the Interaction Analysis of Tryptanthrin Compounds with Protein Target

ID PDB	Protein target	Ligand	Interaction with the protein target	The bond
4ZW3	Plasmodium falciparum Aminopeptidase M1 (PfA-M1)	Indolo[2,1-b]quinazoline-6,12-dione (Tryptanthrin)	Hydrogen bond: -	0
			Hydrophobic interaction: **Glu497, Ala461, His496, Tyr575, Glu319, Val 459, Tyr 580**	7
		Tert-butyl[(1s)-1-(4-bromophenyl)-2-(hydroxyamino)-2-oxoethyl]carbamate (Control)	Hydrogen bond: Tyr580, Ala461, Glu497, His496, His 500	5
			Hydrophobic interaction: **Glu497, Ala461, His496, Tyr575, Glu319, Val 459, Tyr 580**, His500, Gln317, Ala320, Met462, Gly460, Glu519, Glu 462	14
6SSZ	Falcipain 2 protease	Indolo[2,1-b]quinazoline-6,12-dione (Tryptanthrin)	Hydrogen bond: Trp189	1
			Hydrophobic interaction: **Trp189, Ala140, Trp193, Gln192**	4
		(~{e})-3-(1,3-benzodioxol-5-yl)-1-(3-nitrophenyl)prop-2-en-1-one (Control)	Hydrogen bond: -	0
			Hydrophobic interaction: **Trp189, Ala140, Trp193, Gln 192**, Gln19	5
6UKJ	Plasmodium falciparum Chloroquine Resistance Transporter (PfCRT)	Indolo[2,1-b]quinazoline-6,12-dione (Tryptanthrin)	Hydrogen bond: -	0
			Hydrophobic interaction: **Ile347, Ile351, Arg392, Leu385, Thr344, Val348, Ser65, Ser388, Ile389**	9
		Chloroquine^{2+} (Control)	Hydrogen bond: -	0
			Hydrophobic interaction: Ile61, Tyr62, Tyr391, Tyr384, **Ile347, Ile351, Arg392, Leu385, Thr344, Val348, Ser65, Ser388, Ile389**	13

Note: Bonds in bold indicate similar interactions with control ligands

Tryptanthrin and PfA-M1 do not form hydrogen bonds as well as active compounds and PfCRT which is similar to control ligands. In contrast to both protein targets, the active compounds formed a hydrogen bond with Falcipain 2 protease through Trp189 which makes its interaction more optimal (Azimzadeh et al., 2010). For protein target PfA-M1, Tryptanthrin formed hydrophobic interactions through Glu497, Ala461, His496, Tyr575, Glu319, Val459, and Tyr580. For protein target Falcipain 2 protease, Tryptanthrin formed hydrophobic interactions through Trp189, Ala140, Trp193, Gln192. For protein target PfCRT, Tryptanthrin formed hydrophobic interactions through Ile347, Ile351, Arg392, Leu385, Thr344, Val348, Ser65, Ser388, Ile389.

Tryptanthrin binds to protein target PfA-M1, Falcipain 2 protease, and PfCRT through hydrophobic interactions with similar protein residues to control ligands. Seven protein residues of Tryptanthrin interacted with PfA-M1 protein targets through hydrophobic bonds, while Falcipain 2 protease has four protein interactions, and PfCRT has nine protein interactions. The hydrophobic interaction of Tryptanthrin and PfA-M1 was the same as seven of the fourteen hydrophobic interactions found in the control ligands and PfA-M1 interactions, while Falcipain 2 protease was the same as four of the five hydrophobic interactions, and PfCRT was the same as nine of the thirteen hydrophobic interactions. The interaction between Tryptanthrin and the protein targets are presented in **Figure 2.**

3.1 Role of Protein Target in Plasmodium

Plasmodium parasites may grow and multiply in host erythrocytes, degrade the host cell's hemoglobin, then utilize the released amino acids

to synthesize their proteins. Plasmodium falciparum food vacuole is a lysosome-like organelle where erythrocyte hemoglobin digestion occurs. In this food vacuole, the hemoglobin is degraded, heme polymerizes, free radicals detoxified, amino acids transported, acidification maintained, drugs accumulated, and free iron can be produced. Several proteins are involved in vesicle-mediated trafficking and play a key role in food vacuole biogenesis and food vacuole protein trafficking (Lamarque et al., 2008).

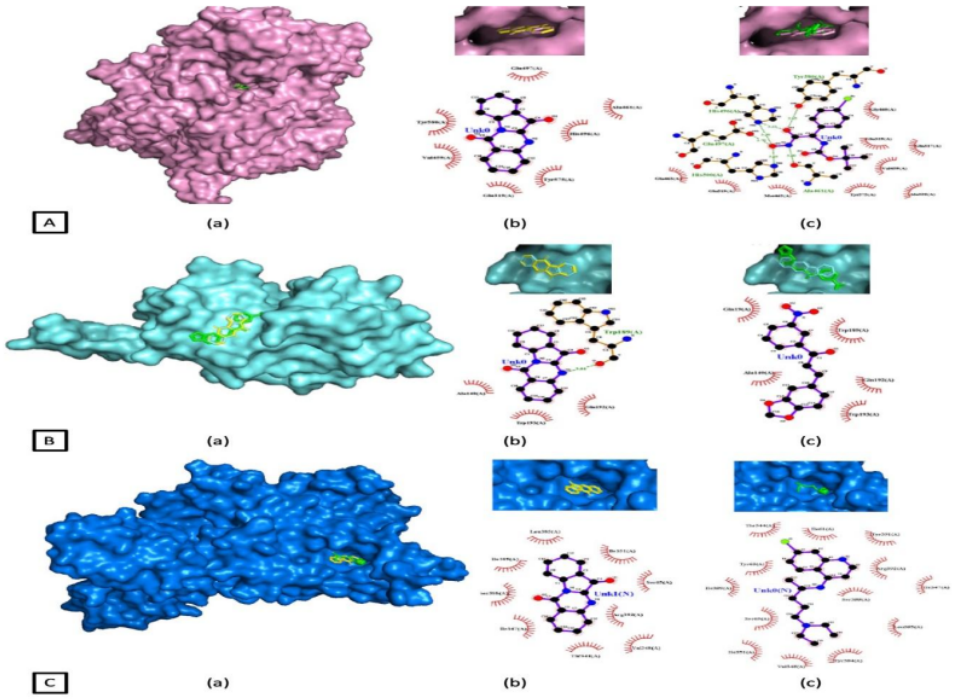

Fig.2: Comparison of the Binding Interaction of Tryptanthrin Compounds and Control Ligands: (A) PfA-M1-Tryptanthrin and PfA-M1-Control Ligands, (B) Falcipain 2 protease-Tryptanthrin and Falcipain 2 protease-Control Ligand, and (C) PfCRT-Tryptanthrin and PfCRT-Control Ligand. Tryptanthrin and all of the control ligands shared similar binding pockets.

During the erythrocytic stage, the digestive vesicles fused and produce a large food vacuole. Approximately 60-80 percent of hemoglobin is processed to provide a collection of essential amino acids for parasite development. Heme is released from hemoglobin during proteolysis as a toxic product and detoxified by biocrystallization. During the breakdown of hemoglobin, proteases arrived via several different transport routes to the active site. Chloroquine, a quinoline antimalarial drugs can bind to heme and prevent hemozoin formation. Artemisinin can cause oxidative damage to macromolecules and food vacuole membranes. The development of parasitic resistance to some antimalarial drugs is related to ion pumps and transporters in the food vacuole membrane which is important in low pH maintenance (Wunderlich et al., 2012).

PfA-M1 plays role in the termination phase of hemoglobin digestion and the formation of amino acids in parasites, which occurs outside the food vacuole. PfA-M1 has a molecular weight of 126 kDa but detected as a 122-kDa protein in the membrane fraction of the parasites, and encoded by a single-copy gene on chromosome 13. PfA-M1 has optimal activity at pH 7.4 with neutral aminopeptidase activity and remains 40% active from pH 5.8 to pH 8.6. Non-acidic pH conditions can activate PfA-M1. PfA-M1 has three different soluble enzymes (p120, p96, and p68). The two original forms of PfA-M1 are the soluble enzymes p68 and p120. The soluble enzyme which is obtained specifically from parasites

without protease inhibitor is the p96 form (Azimzadeh et al., 2010; Jones et al., 2011).

PfA-M1 can be directed into a food vacuole but will stop at the border in the cytosomal vesicles that have a neutral pH. In the vesicular trafficking process, PfA-M1 was directly involved from the parasitophorous vacuole to the food vacuole. Particularly, PfA-M1 can be found in P. falciparum gametocytes and gametes in P. falciparum sporozoites (Azimzadeh et al., 2010; Jones et al., 2011). In this study, the binding affinity of Tryptanthrin with PfA-M1 was better than the control ligand. The binding affinity of Tryptanthrin with PfA-M1 was -9.4 Kcal/mol, while the control ligand was -8.1 Kcal/mol. Tryptanthrin can bind stronger than the control ligand in the protein target PfA-M1 indicating that Tryptanthrin can replace the control ligand in its binding site.

Based on the importance of the process of hemoglobin digestion, insight into effective antimalarial drug targets points to the pathway for hemoglobin degradation at the erythrocytic stage of malaria. Falcipain 2 protease is the key enzyme in this pathway. The process of hemoglobin digestion is regulated by food vacuole. Food vacuole is a 200 kDa protein complex containing Falcipain 2 protease, histo-aspartic proteases, aspartate (plasmepsin II and IV), and special enzymes (heme detoxification protein) to convert toxic heme into an inert crystal form (hemozoin) (Machin et al., 2019).

Cysteine protease is an effective antimalarial target because of its role in parasite development and infection (Dasaradhi et al., 2007). Cysteine protease plays a key role in hemoglobin degradation, so that cysteine protease inhibitors can block hemoglobin hydrolysis. Falcipain 2 proteases are cysteine proteases family which have a role in the erythrocytic stage of P. falciparum to hydrolyze hemoglobin and localized in the Plasmodium falciparum food vacuole (Pandey et al., 2005). Hemoglobin degradation and cleavage of cytoskeletal elements inside the food vacuole involved the Falcipain 2 protease. Falcipain 2 protease is transported to the food vacuole inside cytosomal vesicles (Dasaradhi et al., 2007). Inhibition of the Falcipain 2 protease causes the accumulation of undegraded hemoglobin in trophozoites, thus inhibiting the development of parasites (Pandey et al., 2005). The binding affinity of Tryptanthrin with Falcipain 2 protease was -7.7 Kcal/mol, while the control ligand was -7.3 Kcal/mol. Tryptanthrin can bind stronger than the control ligand in the protein target PfA-M1 indicating that Tryptanthrin can replace the control ligand in its binding site.

Chloroquine resistance has been known to be mediated by the presence of mutations in PfCRT specifically in TMD1. PfCRT mutations mediate the occurrence of quinoline-containing drug efflux such as Chloroquine from Plasmodium digestive vacuole. The TMD1 region in the mutated PfCRT had a stronger binding affinity for protonated Chloroquine than neutral Chloroquine. The presence of mutations in this region leads to target recognition of the protonated Chloroquine molecule. Therefore, protonated Chloroquine which should have been trapped in the digestive vacuole can exit through the PfCRT (Antony et al., 2019). In this study, the binding affinity of Tryptanthrin and PfCRT was better than the control ligand ($Chloroquine^{2+}$) and PfCRT. The binding affinity of Tryptanthrin and PfCRT was -8.33 Kcal/mol, while between $Chloroquine^{2+}$ and PfCRT was -7.13 Kcal/mol. Tryptanthrin can bind stronger than $Chloroquine^{2+}$ in the protein target PfCRT indicating that the replacement of $Chloroquine^{2+}$ in its binding site of PfCRT will occur.

Tryptanthrin bond with PfCRT can prevent Chloroquine efflux from the food vacuole which reveals Chloroquine resistance-reversal properties of Tryptanthrin. The use of Tryptanthrin as a combination therapy with the quinoline class antimalarials has a strong potential ability to reverse Chloroquine resistance condition and sensitize the utilization of Chloroquine itself (Chloroquine chemosensitizer) which is called as dual mechanism antimalarial strategy (Egan & Kuter, 2013).

Experimental Result

Parasite Density

The antimalarial activity of fraction 41 and 44 was determined by measuring the parasite density. The measurement was carried out for four days including the first day before treatment followed by three days of treatment. The examination of the parasite density on day 1 aims to prove that all mice are within the same degree range of parasitemia on the first day of treatment. Parasite density after treatment was measured on days 2, 3, and 4. The measurement results showed that there was an inhibition of parasite density compared to positive controls. The result of parasite density is shown in **Figure 3. A-B.**

One-way ANOVA test analysis of fraction 41 treatment, showed the parasite density on day 1 before treatment was $p = 0.955$, indicating there was no significant difference between groups before therapy. The analysis for parasite density on day 2 (first day of treatment) was $p = 0.491$, indicating there was no significant difference between groups after the first day of treatment. The analysis for parasite density on day 3 (second

day of treatment) was p = 0.018, indicating the data between groups was significantly different. The post-hoc Tukey HSD analysis for day 3 showed a significant difference between the positive control group and the Artesunate group (p = 0.013), but there was no difference between the treatment group and the positive control group. One-way ANOVA test analysis showed the parasite density on day 4 (third day of treatment) was p = 0.002, indicating the data between groups was significantly different.

The post-hoc Tukey HSD analysis for day 4 showed a significant difference between the positive control group and the Artesunate group (p = 0.003), between the positive control group and the treatment group dose 8,125 µg/kgBW (p = 0.022), treatment group dose 130 µg/kgBW (p = 0.046), also the treatment group dose 520 µg/kgBW (p = 0.004). There was no significant difference between the positive control group with the treatment group dose 32,5 µg/kgBW (p = 0.369). Pearson correlation test on day 1 showed no relationship between the doses and the parasite density (p = 0.933, r = -0.17), as well as treatment on day 2 (p = 0.064, r = -0.369). Pearson correlation test on day 3 showed a strong relationship between the increasing doses and decreasing parasite density (p = 0.001 and r = -0.607). Pearson correlation test on day 4 showed a very strong relationship between the increasing doses and decreasing parasite density (p = 0.000, r = -0.700). The negative correlation coefficient shows that the higher treatment dose, followed by the lower parasite density.

One-way ANOVA test of fraction 44 treatment, showed the p-value of parasite density on day 1 before treatment was p = 0.078, indicating there was no significant difference in parasite density in all treatment groups. Then, the One-way ANOVA test for day 2 (first day of treatment), day 3 (second day of treatment), and day 4 (third day of treatment) had a significant p-value of 0.018, 0.018, and 0.002, respectively, indicating a significant difference between groups after therapy. From the Post-hoc test, on the first day of treatment, the results were significantly different between the positive control with the treatment group with a dose of 520 µg/kgBW (p = 0.005) and the Artesunate group (p = 0.001). On the second day of treatment, the results were significantly different between the positive control and the treatment group at the dose of 520 µg/kgBW (p = 0.008) as well as the Artesunate group (p = 0.001) and between the Artesunate control group and the treatment group with the dose of 8.125 µg/kgBW (p = 0.018), 32,5 µg/kgBW (p = 0.020), and 130 µg/kgBW (p = 0.026). On the third day of treatment, the results were significantly different between the positive control group and the treatment group with doses of 32.5 µg/kgBW (p = 0.036), 130 µg/kgBW (p = 0.024), and 520 µg/kgBW (p = 0.004) and between the positive control group and the Artesunate group (p = 0.000).

Pearson's correlation test of parasite density at day 2 and day 4 of fraction 44 treatment showed no significant results with p and r values, p = 0.354; r = -0.294 and p = 0.060; r = -0,558, respectively. These results indicate that there was no significant relationship between increased dose fraction 44 and the parasite density. Pearson correlation test on day 3, showed a significant relationship between increased dose and parasite density, with p = 0.044, r = -0.589. The result of the correlation test shows a negative correlation, which means that the higher the dose was given will further inhibit the parasite density that explains the drug-dose dependent relationship in this study.

3.5.2 Parasite Inhibition

Parasite inhibition is the inhibitory effect caused by the treatment given. Parasite inhibition was obtained by the formula % inhibition = ((% positive control parasitemia - % treated parasitemia) / % positive control parasitemia) x 100. Inhibition analysis was performed on day 2 until day 4 compared to the positive control group (**Figure 3. C-D**). The parasite inhibition on day 1 was seen on day 2 and so on.

Fraction 41 showed the largest percentage of inhibition was due to the Artesunate group, followed by the treatment group dose 520 µg/kgBW. One-way ANOVA test of inhibition of the degree of parasitemia showed a significant difference between treatment groups after therapy with a value of p = 0.000 (p <0.05). Tukey's post hoc test also showed a significant difference between each group, dose 8.125 µg/kgBW (p = 0.000), dose 32.5 µg/kgBW (p = 0.000), dose 130 µg/kgBW (p = 0.000), and dose 520 µg/kgBW (p = 0.000). Pearson correlation test obtained p-value = 0.001 and r = 0.457. These results indicate that there is a significant relationship with a positive correlation, the higher the fraction dose, the higher the percentage of parasite inhibition. Linear regression analysis showed the results of R^2 = 0.209, indicating that 20.9% inhibition of parasitemia was caused by the administration of therapy.

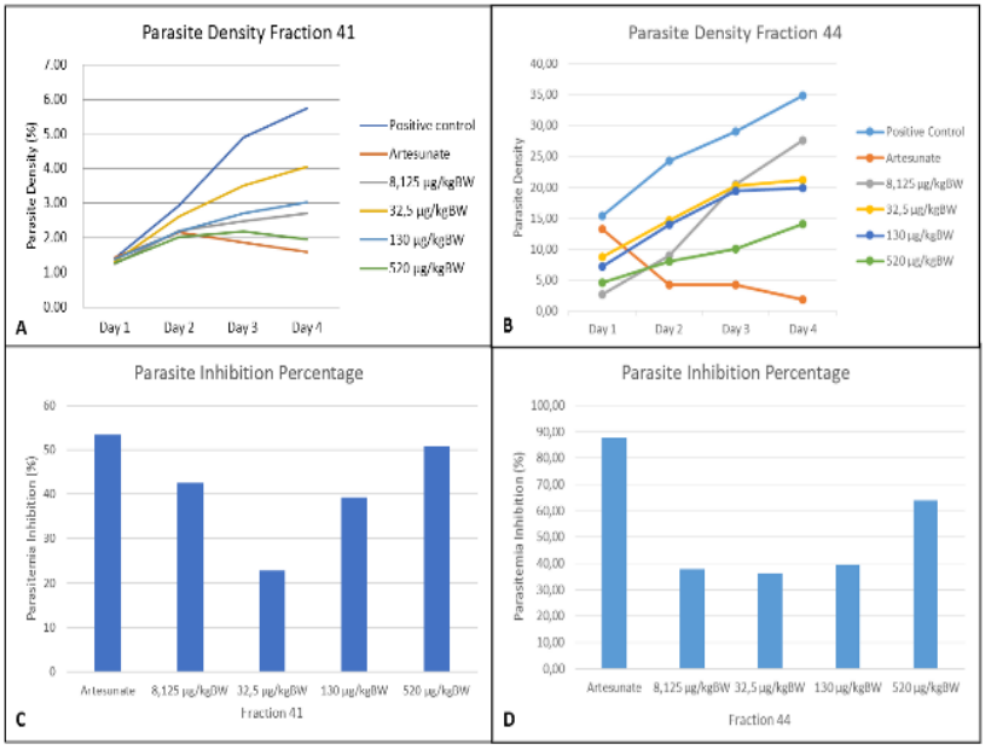

Fig.3: A-B Parasite density of fraction 41 and fraction 44 was measured for four days (day 1 to day 4). The parasite density of the therapy group decreased significantly compared to the control. C-D Parasite inhibition of fraction 41 and fraction 44 showed the Artesunate group gave the highest parasite inhibition, followed by the treatment group dose 520 µg/kgBW.

One way-ANOVA test of parasite inhibition percentage from fraction 44 treatment showed significant differences between groups (p=0.001). The post-hoc test showed a significant difference between the Artesunate control group and the treatment group at the dose of 8.125 µg/kgBW (p = 0.000), 32.5 µg/kgBW (p = 0.000), 130 µg/kgBW (p = 0.000), and 520 µg/kgBW (p = 0.027). Significant differences were also found between the treatment groups, specifically between the dose of 520 µg/kgBW and 8.125 µg/kgBW (p = 0.016), as well as 32.5 µg/kgBW (p = 0.012), and 130 µg/kgBW (p = 0.023). Pearson's correlation test from fraction 44 treatment showed a significant correlation between the increase in dose and the percentage of inhibition of parasitemia degree with p = 0.005 and r = 0.749. These results indicated that the higher the fraction dose, the higher the percentage of inhibition of parasite growth. Then, from the linear regression test, it was found that the value of R^2 = 0.561, indicating 56.1% inhibition of parasitemia was caused by the administration of therapy.

Effective Dose 50 (ED50) of Fraction 41 and 44

The effective dose 50 (ED 50) in this study was determined using a probit analysis of the parasite inhibition, followed by linear regression analysis on SPSS. The result of the probit analysis of fraction 41 using the log dose and probit size obtained the equation Y = 0.25X+0.48 with R^2 = 0.236. While the ED50 of fraction 44 obtained the equation Y=0.55X-1.25 with R^2= 0.942. From this equation, the ED50 for fraction 41 and 44 in mice is 84.988 µg/kgBW and 190.810 µg/kgBW, respectively (**Figure 4**). To obtain the equivalent effective dose in humans, the ED50 dose is multiplied with a conversion coefficient of 12.3. The ED50 of fraction 41 in humans is 1,045.35 µg/kgBW or 1 mg/kgBW and fraction 44 is 2,346.96 µg/kgBW or 2.35 mg/kgBW (Nair & Jacob, 2016). This dosage can be a good candidate for designing potent antimalarial. Further investigation using a modification of Tryptanthrin derivatives and a combination using

other antimalarial groups, especially the Quinoline group is still needed.

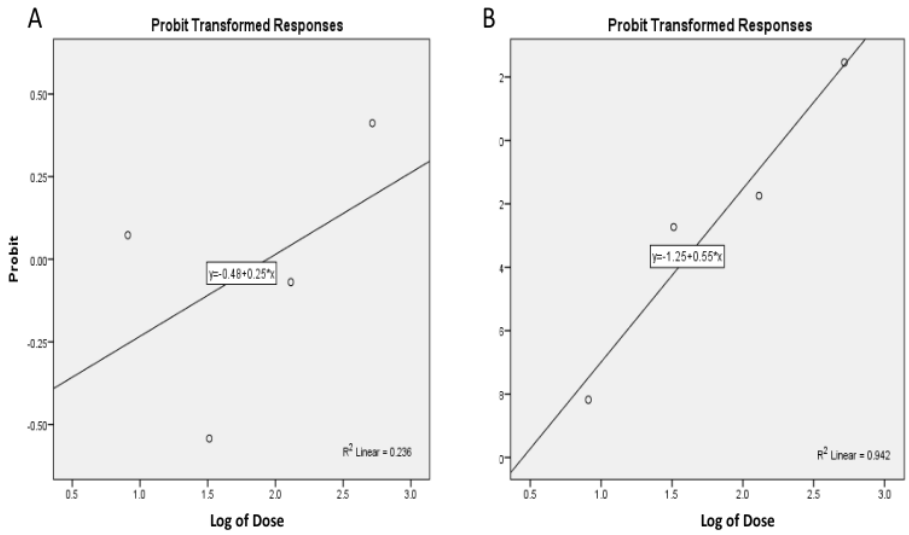

Fig.4: Analysis of the effective dose 50 (ED 50) of fraction 41 (A) and fraction 44 (B) using linear regression analysis of dose logs and probit parasite inhibition. The equation for fraction 41 is Y = 0.25X+0.48 with R^2 = 0.236 and fraction 44 is Y=0.55X-1.25 with R^2= 0.942.

Tryptanthrin Mechanism as Antimalarial Agent

In vivo study showed that parasite density of the highest dose group (520 µg/kgBW) was significantly different from that of the positive control group. It was also found that there was a significant parasite inhibition compared to the positive control group. The highest percentage of inhibition of parasitemia among the treatment groups was at dose 520 µg/kgBW which was significantly different than the other doses.

Tryptanthrin has antimalarial activity against parasites, which are sensitive or resistant to Chloroquine at the sexual and asexual stages of Plasmodium falciparum. Tryptanthrin (Indolo [2,1-b] quinazoline-6,12-dione) is an alkaloid compound isolated from a medicinal plant (Isatis tinctoria) as a source of indigo ink production from ancient times (Lee et al., 2007). Tryptanthrin is also used as herbal medicine in traditional Japanese culture for fungal infections. A previous study showed that Tryptanthrin has several biological activities, including antibacterial activity against Mycobacterium tuberculosis and antiparasitic activity against the protozoan parasites such as Plasmodium falciparum, Toxoplasma gondii, Trypanosoma brucei, and Leishmania donovani (Bhattacharjee et al., 2004; Hwang et al., 2013). Tryptanthrin also has an inhibitory effect at the cellular level against Nitric Oxide Synthase (NOS), 5-lipoxygenase (LOX), cyclooxygenase (COX)-2, and prostaglandin E expression (Jahng, 2013). Tryptanthrin also has activity against tumors and cancer cells (Yang et al., 2013).

The antimalarial activity of the Tryptanthrin compound against a certain protein target is not known yet. However, several studies have been carried out to identify targets and to design potential inhibitors as targets for Tryptanthrin compounds (Bhattacharjee et al., 2004). The inhibitory activity of parasite growth in this study was caused by several mechanisms. The mechanism of Tryptanthrin as antimalaria was through inhibition of the enzyme Plasmodium glycolysis pathway, inhibition of heme detoxification, and crystallization of hematin through interactions with heme and hematin (Hicks et al., 2005; Onambele et al., 2015). Tryptanthrin can be targeted as transmission-blocking interventions because of its exflagellation inhibition activity by adult stage gametocytes which is essential in human to mosquitoes transmission (Delves et al., 2013).

Glucose utilization of infected erythrocytes is almost 100 times greater than uninfected erythrocytes. Adenosine Triphosphate (ATP) production in Plasmodium is regulated by the Lactate Dehydrogenase (LDH) enzyme through the glycolysis process. In the glycolysis process, LDH catalyzes the conversion reaction of pyruvate

compounds to lactate (Singh et al., 2020). The inhibition of parasitic LDH by Tryptanthrin compound contained in fraction 41 and 44 interferes with the energy supply for the Plasmodium life cycle. Based on our in vitro preliminary study in 2019, fraction 16 of the secondary metabolite extract of Streptomyces hygroscopicus subsp Hygroscopicus showed antimalarial activity marked by decreased levels of PfLDH. The fraction 16 used in the preliminary study was known to contain the Tryptanthrin compound (Putri, 2020). Based on this in vivo study, it was found that the treatment using fraction 41 and 44 of the secondary metabolite extract of Streptomyces hygroscopicus subsp Hygroscopicus containing Tryptanthrin provided antimalarial activity, characterized by inhibition of growth of parasitemia for three days of treatment that was significantly different from the positive control group. Tryptanthrin inhibition against the LDH enzyme was also reported in a study conducted by Onambele et al which states that Tryptanthrin compounds are known to have an antiplasmodial activity which is shown through parasite LDH activity. Tryptanthrin is active against both strains of Plasmodium falciparum sensitive and resistant to Chloroquine (Onambele et al., 2015).

Another inhibitory pathway of Tryptanthrin compounds as an antimalarial agent is the inhibition of heme detoxification and hematin crystallization. Tryptanthrin compounds have structural similarities with potent antimalarial compounds such as Chloroquine which can interact with heme and can inhibit hematin crystallization. The similarity of the Chloroquine structure with Tryptanthrin is at A and B rings of Tryptanthrin. Tryptanthrin can interact with heme through π-stacking and cation-stacking interactions. The formation of the heme-Tryptanthrin complex plays a role in the mechanism of killing and inhibiting Plasmodium growth (Hicks et al., 2005). However, from another research related to the mechanism of heme detoxification and hematin crystallization inhibition, Tryptanthrin derivatives with biochemical modifications have higher efficacy as an antimalarial agent compared to the parent compound in the form of Tryptanthrin. Tryptanthrin derivatives that can inhibit hematin crystallization are 8-nitroTryptanthrin (Olson et al., 2018). This study is consistent with previous research which states that Tryptanthrin derivatives, especially NT1, have an antimalarial effect that is five times more potent than Tryptanthrin on the LDH enzyme (Onambele et al., 2015).

CONCLUSION

Based on this study, it can be concluded that Tryptanthrin, the compound of the metabolite extract of Streptomyces hygroscopicus subsp. Hygroscopicus can be identified using LC/MS and had antimalarial activity. The administration of active fraction 41 and 44 in Plasmodium berghei infected mice showed an antimalarial activity by inhibition of parasite density and was marked by an increase in parasite inhibition. Tryptanthrin contained in fraction 41 and 44 has a strong binding affinity with the protein target PfA-M1, Falcipain 2 Protease, and PfCRT. The interaction from in silico analysis showed the Chloroquine-resistance-reversal property of Tryptanthrin, so it can be a potential antimalarial candidate with dual-mechanism strategy when used in combination with Chloroquine.

CONFLICT OF INTEREST

There is no conflict of interest regarding the publication of this paper.

FUNDING STATEMENT

This work was funded by Doctor and Professor Research Grant of Universitas Brawijaya

ACKNOWLEDGEMENTS

The authors would like to thank National Police Criminal Investigation Forensic Laboratory Center, East Jakarta and PT. BUCHI Indonesia, BSD City, Tangerang

REFERENCES

1. Antony, H. A., Topno, N. S., Gummadi, S. N., Siva Sankar, D., Krishna, R., & Parija, S. C. (2019). In silico modeling of Plasmodium falciparum chloroquine resistance transporter protein and biochemical studies suggest its key contribution to chloroquine resistance. Acta Tropica, 189(October 2018), 84–93. https://doi.org/10.1016/j.actatropica.2018.10.001
2. Azimzadeh, O., Sow, C., Gèze, M., Nyalwidhe, J., & Florent, I. (2010). Plasmodium falciparum PfA-M1 aminopeptidase is trafficked via the parasitophorous vacuole and marginally delivered to the food vacuole. Malaria Journal, 9(1), 189. https://doi.org/10.1186/1475-2875-9-189
3. Bhattacharjee, A., Hartell, M., Nichols, D., Hicks, R., Stanton, B., Van Hamont, J., & Milhous, W. (2004). Structure-activity relationship study of antimalarial indolo [2, 1-b] quinazoline-6, 12-diones (tryptanthrins). Three dimensional pharmacophore modeling and identification of new antimalarial candidates. European Journal of Medicinal Chemistry, 39(1), 59–67.
4. CDC. (2018). CDC - Malaria - Malaria Worldwide -How Can Malaria Cases and Deaths Be Reduced? - Vaccines. Global Health - Division

of Parasitic Diseases and Malaria. http://www.cdc.gov/malaria/malaria_worldwide/reduction/vaccine.html

5. Czyrski, A. (2019). Determination of the Lipophilicity of Ibuprofen, Naproxen, Ketoprofen, and Flurbiprofen with Thin-Layer Chromatography. Journal of Chemistry, 2019. https://doi.org/10.1155/2019/3407091
6. Dasaradhi, P. V. N., Korde, R., Thompson, J. K., Tanwar, C., Nag, T. C., Chauhan, V. S., Cowman, A. F., Mohmmed, A., & Malhotra, P. (2007). Food vacuole targeting and trafficking of falcipain-2, an important cysteine protease of human malaria parasite Plasmodium falciparum. Molecular and Biochemical Parasitology, 156(1), 12–23. https://doi.org/10.1016/j.molbiopara.2007.06.008
7. Delves, M. J., Ruecker, A., Straschil, U., Lelièvre, J., Marques, S., López-Barragán, M. J., Herreros, E., & Sinden, R. E. (2013). Male and female Plasmodium falciparum mature gametocytes show different responses to antimalarial drugs. Antimicrobial Agents and Chemotherapy, 57(7), 3268–3274. https://doi.org/10.1128/AAC.00325-13
8. Egan, T., & Kuter, D. (2013). Effect of Metabolite Extract of Streptomyces hygroscopicus subsp. hygroscopicus on Plasmodium falciparum 3D7 in Vitro Dual-functioning antimalarials that inhibit the chloroquine-resistance transporter. Future Microbiology, 8(4), 475–489.
9. Fitri, L. E., Alkarimah, A., Cahyono, A. W., Lady, W. N., Endharti, A. T., & Nugraha, R. Y. B. (2019). Effect of metabolite extract of Streptomyces hygroscopicus subsp. hygroscopicus on Plasmodium falciparum 3D7 in vitro. Iranian Journal of Parasitology, 14(3), 444–452. https://doi.org/10.18502/ijpa.v14i3.1484
10. Hicks, R., Nichols, D., DiTusa, C., Sullivan, D., Hartell, M., Koser, B., & Bhattacharjee, A. (2005). Evaluation of 4–Azaindolo[2,1–b]quinazoline–6,12–diones' Interaction with Hemin and Hemozoin: A Spectroscopic, X–ray Crystallographic and Molecular Modeling Study. Internet Electronic Journal of Molecular Design, 4(2), 751–764.
11. Hwang, J., Oh, T., Kaneko, T., Upton, A., Franzblau, S., Ma, Z., Cho, S., & Kim, P. (2013). Design, synthesis, and structure–activity relationship studies of tryptanthrins as antitubercular agents. Journal of Natural Products, 76(3), 354–367.
12. Jahng, Y. (2013). Progress in the studies on tryptanthrin, an alkaloid of history. Archives of Pharmacal Research, 36(5), 517–535. https://doi.org/10.1007/s12272-013-0091-9
13. Jones, P., Robinson, M., Dalton, J., & George, A. (2011). The Plasmodium falciparum malaria M1 alanyl aminopeptidase (Pf A-M1): Insights of catalytic mechanism and function from MD simulations. PLoS One, 6(12), e28589.
14. Lamarque, M., Tastet, C., Poncet, J., Demettre, E., Jouin, P., Vial, H., & Dubremetz, J. F. (2008). Food vacuole proteome of the malarial parasite Plasmodium falciparum. Proteomics - Clinical Applications, 2(9), 1361–1374. https://doi.org/10.1002/prca.200700112
15. Lee, S., Kim, G., Kim, D., Kim, D., Jahng, Y., & Jeong, T. (2007). Identification of a tryptanthrin metabolite in rat liver microsomes by liquid chromatography/electrospray ionization-tandem mass spectrometry. Biological and Pharmaceutical Bulletin, 30(10), 1991–1995.
16. Lipinski, C. (2004). Lead-and drug-like compounds: the rule-of-five revolution. Drug Discovery Today: Technologies, 1(4), 337–341.
17. M. Honorio, K., L. Moda, T., & D. Andricopulo, A. (2013). Pharmacokinetic Properties and In Silico ADME Modeling in Drug Discovery. Medicinal Chemistry, 9(2), 163–176. https://doi.org/10.2174/157340641130902000 2
18. Machin, J. M., Kantsadi, A. L., & Vakonakis, I. (2019). The complex of Plasmodium falciparum falcipain-2 protease with an (E)-chalcone-based inhibitor highlights a novel, small, molecule-binding site. Malaria Journal, 18(1), 388. https://doi.org/10.1186/s12936-019-3043-0
19. Nair, A., & Jacob, S. (2016). A simple practice guide for dose conversion between animals and human. Journal of Basic and Clinical Pharmacy, 7(2), 27. https://doi.org/10.4103/0976-0105.177703
20. Nugraha, R. Y. B., Faratisha, I. F. D., Mardhiyyah, K., Ariel, D. G., Putri, F. F., Nafisatuzzamrudah, Winarsih, S., Sardjono, T. W., & Fitri, L. E. (2020). Antimalarial Properties of Isoquinoline Derivative from Streptomyces hygroscopicus subsp. Hygroscopicus: An in Silico Approach. BioMed Research International, 2020. https://doi.org/10.1155/2020/6135696
21. Olson, J. A., Terryn, R. J., Stewart, E. L., Baum, J. C., & Novak, M. J. (2018). New insight into the action of tryptanthrins against Plasmodium falciparum: Pharmacophore identification via a novel submolecular QSAR descriptor. Journal of Molecular Graphics and Modelling, 80, 138–146. https://doi.org/10.1016/j.jmgm.2017.12.013
22. Onambele, L. A., Riepl, H., Fischer, R., Pradel, G., Prokop, A., & Aminake, M. N. (2015). Synthesis and evaluation of the antiplasmodial activity of tryptanthrin derivatives. International Journal for Parasitology: Drugs and Drug Resistance, 5(2), 48–57. https://doi.org/10.1016/j.ijpddr.2015.03.002
23. Pandey, K. C., Wang, S. X., Sijwali, P. S., Lau, A. L., McKerrow, J. H., & Rosenthal, P. J. (2005). The Plasmodium falciparum cysteine protease falcipain-2 captures its substrate, hemoglobin, via a unique motif. Proceedings of the National Academy of Sciences of the United States of

America, 102(26), 9138–9143. https://doi.org/10.1073/pnas.0502368102
24. Putri, F. (2020). Profiling of Anti-Malaria Compounds from Secondary Metabolite Fractions of Streptomyces hygroscopicus Bacteria Using Liquid Chromatography Mass Spectrometry (LCMS). Universitas Brawijaya.
25. Singh, R., Bhardwaj, V., & Purohit, R. (2020). Identification of a novel binding mechanism of Quinoline based molecules with lactate dehydrogenase of Plasmodium falciparum. Journal of Biomolecular Structure and Dynamics, 1–9. https://doi.org/10.1080/07391102.2020.1711809
26. Tucker, A. M., & Grundt, P. (2012). The chemistry of tryptanthrin and its derivatives. Arkivoc, 2012(1), 546–569. https://doi.org/10.3998/ark.5550190.0013.113
27. Veber, D. F., Johnson, S. R., Cheng, H. Y., Smith, B. R., Ward, K. W., & Kopple, K. D. (2002). Molecular properties that influence the oral bioavailability of drug candidates. Journal of Medicinal Chemistry, 45(12), 2615–2623. https://doi.org/10.1021/jm020017n
28. Wang, Y., Xing, J., Xu, Y., Zhou, N., Peng, J., Xiong, Z., Liu, X., Luo, X., Luo, C., Chen, K., Zheng, M., & Jiang, H. (2015). In silico ADME/T modelling for rational drug design. Quarterly Reviews of Biophysics, 48(4), 488–515. https://doi.org/10.1017/S0033583515000190
29. WHO Report. (2017). World Malaria Report 2017. In World Health Organization. http://apps.who.int/iris/bitstream/handle/10665/259492/9789241565523-eng.pdf;jsessionid=3B05B30235850B2B29A7FFFDD8A874D8?sequence=1
30. World Health Organization. (2010). Global report on antimalarial drug efficacy and drug resistance. In World Health Organization. http://apps.who.int/iris/bitstream/10665/44449/1/9789241500470_eng.pdf
31. World Health Organization. (2018). Artemisinin resistance and artemisinin-based combination therapy efficacy. In Who (Issue August). https://www.who.int/malaria/publications/atoz/artemisinin-resistance-august2018/en/
32. Wunderlich, J., Rohrbach, P., & Dalton, J. P. (2012). The malaria digestive vacuole. Frontiers in Bioscience - Scholar, 4 S(4), 1424–1448. https://doi.org/10.2741/s344
33. Yang, S., Li, X., Hu, F., Li, Y., Yang, Y., Yan, J., Kuang, C., & Yang, Q. (2013). Discovery of tryptanthrin derivatives as potent inhibitors of indoleamine 2, 3-dioxygenase with therapeutic activity in Lewis lung cancer (LLC) tumor-bearing mice. Journal of Medicinal Chemistry, 56(21), 8321–8331.

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