Journal of Applied Pharmaceutical Science Vol. 12(09), pp 114-120, September, 2022
Available online at http://www.japsonline.com
DOI: 10.7324/JAPS.2022.120913
ISSN 2231-3354

In vitro antiplasmodial activities of the fractions of *Hyrtios reticulatus* sponge extract

Mahfur[1,2], Subagus Wahyuono[3], Indah Purwantini[3], Erna Prawita Setyowati[3*]

[1]Doctoral Program in Pharmaceutical Science, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia
[2]Department of Pharmaceutical Biology, Faculty of Pharmacy, Pekalongan University, Pekalongan, Indonesia
[3]Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

ARTICLE INFO

Received on: 30/02/2022
Accepted on: 13/07/2022
Available Online: 04/09/2022

Key words:
Hyrtios reticulatus, antiplasmodial, malaria, 3D7, FCR3.

ABSTRACT

The discovery of new compounds sourced from nature, which are active against malaria, is very important. Sponge *Hyrtios reticulatus* from Bali, Indonesia, is one of the examples that can be investigated. The sponge samples were extracted using ethanol, followed by trituration fractionation and vacuum liquid chromatography to get the samples. The nine samples obtained, their main extracts, chloroform fraction, residue, and SF 1–9 were tested for activities against *Plasmodium falciparum* variants 3D7 and FCR3. The results showed that all samples had a moderate antiplasmodial activity, where the most active sample was SF 3 with an IC_{50} of 12.98 ± 1.88 µg/ml on 3D7 and 19.81 ± 0.75 µg/ml on FCR3. This study discovered that *H. reticulatus* sponges had antiplasmodial activities and could be further used as a guide to finding a new antiplasmodial compound.

INTRODUCTION

Globally, there are an estimated 229 million cases of malaria, and 409,000 of them died in 2019 in 87 malaria-endemic countries, making it the leading cause of morbidity and mortality in tropical countries. This is further exacerbated by the increase in parasite resistance to various drugs accompanied by the slow development of vaccines and drug discovery (World Health Organization, 2021). Malaria is caused by five species of *Plasmodium* spp., which include *Plasmodium falciparum*, *P. vivax*, *P. malariae*, *P. ovale*, and *P. knowlesi. Plasmodium falciparum* is the variant that is the most common cause of morbidity and mortality in malaria (Hyde, 2007; Kenangalem *et al*., 2019; Kotepui *et al*., 2020), and the variants that are often found in endemic areas are *P. falciparum* 3D7 and FCR3 (Molina-Cruz *et al*., 2012). Therefore, it is necessary to search for new compounds that are active against malaria (Capela *et al*., 2019; Fattorusso and Taglialatela-Scafati, 2009).

Marine resources are great sources for the synthesis of new molecules, and they need to be studied. According to evolutionary history, marine microorganisms are more diverse than land microorganisms (Anjum *et al*., 2016; Setyowati *et al*., 2009). During 2014–2015, numerous articles described several marine extracts as *in vitro* natural product discoveries to predict the activity in extracts of bioactive components (Mayer *et al*., 2020). A marine sponge is one of the invertebrate creatures which is intriguing to be investigated from the standpoint of drug development due to its potency in producing novel chemicals (Hikmawan *et al*., 2018; Kirsch *et al*., 2000; Setyowati *et al*., 2008). It is a significant source of new marine natural products, as this group of species has acquired most of the biomedically or ecologically relevant chemicals (Tajuddeen and Van Heerden, 2019).

In our research for new antiplasmodials of marine invertebrates, sponge *Hyrtios reticulata* was investigated. This sponge was used because, based on the results of previous studies, several species of the genus *Hyrtios* have antiplasmodium activity (Ju *et al*., 2018; Shady *et al*., 2017). Previous studies on *Hyrtios reticulatus* sponge have identified it as an abundant source of

**Corresponding Author*
Erna Prawita Setyowati, Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia.
E-mail: erna_prawita @ ugm.ac.id

uncommon secondary metabolites, such as alkaloid, sesterterpene, and macrolides, and it has an activity as an inhibitor of the ubiquitin-activating enzyme, and anticancer and antimicrobial activities (Inman *et al*., 2010; Mahfur *et al*., 2022; Shady *et al*., 2017; Yamanokuchi *et al*., 2012). In this research, the sponge samples *H. reticulatus* (family of Thorectidae) were collected from Bali, Indonesia. This study aims to determine the antiplasmodial activity of crude ethanol extract and its fraction from the sample. Antiplasmodial activity will be tested against *P. falciparum* 3D7 and FCR3 variants.

MATERIALS AND METHODS

Material and extraction process

Sponge *H. reticulatus* was collected by scuba diving in Bali, Indonesia. The permit for sampling was given by the Head of the West Bali National Park No. S.931/BTNBB/Kons/7/2017. The identity of the material was confirmed at the Marine Natural Product Laboratory at the University of Diponegoro, Indonesia. The sample obtained was washed to remove impurities and then reduced in size to make it easier to be extracted. The result (100 g) was extracted first with ethanol. The crude extract (6.2 g) obtained was then trituration-fractionated with chloroform to produce the chloroform fraction and residue.

Column separation of crude extract

The chloroform fraction (5 g) was partitioned by column chromatography using silica gel (60–120 meshes) and gradient-eluted with various solvents: N-hexane:ethyl acetate (7:3 v/v, 150 ml) to obtain 11.86% yield; N-hexane:ethyl acetate (5:5 v/v, 150 ml) to obtain 2.52% yield; N-hexane:ethyl acetate (3:7 v/v, 150 ml) to obtain 2.14% yield; ethylacetate (150 ml) to obtain 3.41 yield; chloroform:methanol (1:1 v/v, 150 ml) to obtain 23.96% yield; and methanol to obtain 26.7% yield. The fractions were collected and monitored by thin-layer chromatography (TLC), and their activity was tested.

Culture of *P. falciparum*

Parasites were cultured by using the Trager and Jensen method (Jensen and Trager, 1977). *Plasmodium falciparum* variants 3D7 and FCR3 were maintained at 2% hematocrit (human type O-positive red blood cells) in a complete culture medium at 37°C. The complete medium contained RPMI 1640 medium (Gibco-BRL; 24 mM NaHCO3) with the addition of 10% heat-inactivated O-positive human plasma, 20 mM N-2-hydroxyethylpiperazine-N-2-ethane sulfonic acid (HEPES), and 2 mM glutamine (Biological Industries). All of the cultures were maintained in a standard gas mixture consisting of 1% O_2, 5% CO_2, and 94% N_2. When parasitemia exceeded 2%, subcultures were taken; the culture medium was changed every second day. Erythrocytes infected with *P. falciparum* (ring stage, 1% parasitemia) were resuspended in a complete culture medium at a hematocrit of 1.5%. The suspension was distributed in 96-well microtiter plates (200 µl per well). Sample testing was carried out in triplicate. For each assay, parasite cultures were incubated with the samples for 48 hours in 5% CO_2 at 95% relative humidity and frozen until biochemical tests could be run.

Antiplasmodial activity assay

The antiplasmodial activity was studied *in vitro* against *Plasmodium* variants 3D7 and FCR3 by the micro method using the method described by Trager and Jensen. Compounds were dissolved in dimethyl sulfoxide (DMSO), and then they were diluted with a medium to achieve the required concentrations (in all cases, the final concentration contained 1% DMSO, which was found to be nontoxic to the parasite). Samples were placed in 96-well flat-bottomed microplates (Costar 3596) consisting of negative control (DMSO), samples, and positive control (chloroquine). Sample dilutions were made starting at 125 µg/ml for all of the compounds tested, except for chloroquine, the initial concentration of which was 500 ng/ml. Asynchronous cultures with parasitemia of 1%–1.5% and 1% final hematocrit were aliquoted into the plates and incubated for 72 hours at 37°C. Parasitemia was determined by observing the Giemsa-stained thin blood smears using a microscope. The culture of *Plasmodium* without sample was used as a negative control, which was considered a 100% growth of *Plasmodium*. Percentage of inhibition of parasitemia was calculated using the following formula:

$$\text{Inhibition\%} = \frac{\text{parasetemia of negative control} - \text{parasitemia sample}}{\text{parasetemia of negative control}} \times 100$$

Statistical analysis

The potency of antiplasmodial activity was expressed as IC_{50} which was calculated with the logarithm between % inhibition and the test concentration by statistical analysis regression probit using SPSS 16. The differences in IC_{50} of the samples were analyzed using one-way analysis of variance (ANOVA) with a 5% level of significance, i.e., $p \leq 0.05$.

RESULTS AND DISCUSSION

Hyrtios reticulatus is a marine sponge of the genus *Hyrtios* (family of Thorectidae) with specifications of dark brown branches, oscules with a diameter of up to 2 mm, 3–4 mm conules that are 1–2 mm high, and brown to orange tops of conules (De Forges, 2007; De Voogd, 2007). They are known to be a rich source of unusual secondary metabolites, such as alkaloids, sesterterpenes, and macrolides (Imada *et al*., 2013). Many of these metabolites have important biological activities (Shady *et al*., 2017).

In this research, we evaluated the *in vitro* antiplasmodial activity of *H. reticulatus* sponge. The evaluation began from the sponge *H. reticulatus* ethanol extract tested with *P. falciparum* 3D7 and FCR3 variants. The antiplasmodial activity of the sponge *H. reticulatus* extract showed that it had IC_{50} 42.01 ± 3.74 and 41.90 ± 2.32 µg/ml against 3D7 and FCR3, respectively. The activity of the extract sample included moderate activity criteria. The criteria for *in vitro* antiplasmodial activity of the extracts were divided into the following: IC_{50} <1 µg/ml, potent; IC_{50} <10 µg/ml, good; IC_{50} 10–50 µg/ml, moderate; IC_{50} 50–100 µg/ml, low; and IC_{50} >100 µg/ml, inactive (Fattorusso and Taglialatela-Scafati, 2012). The fractionation step continued leading us to the fraction that was active against *Plasmodium*. The chloroform fraction showed that it had IC_{50} 34.15 ± 7.79 µg/ml on 3D7 and 21.23 ± 7.23 µg/ml

on FCR3, while the residue had lower activity with IC_{50} 39.42 ± 7.57 and 39.05 ± 1.80 µg/ml against 3D7 and FCR3 *Plasmodium* variants, respectively.

The chloroform fraction had become a guide to reach the active compound, and from the fraction six subfractions were obtained. TLC was used to detect the characteristic type of the organic compounds in the fraction, whether polar or nonpolar, and groups of the compounds by separating them on a silica gel GF254 plate as the stationary phase using a specific solvent or eluent as the mobile phase. In this study, the eluent used for the six fractions was a mixture of N-hexane and ethyl acetate. The profiles of TLC of subfractions (Fig. 1) were different from each other. This visual detection method was chosen because it only requires simple equipment. Under 254 nm UV light, the substances in the fractions showed aromatic rings, conjugated double bonds, and unsaturated substances. Fluorescence under 366 nm showed that the fractions contained long-chain conjugated double bonds. Analysis continued to chemical detection by a spraying chromatogram with cerium sulfate, resulting in finding the fractions containing terpenes that were indicated by black color spots on the white background. Terpenes, also referred to as terpenoids, such as diterpenes and sesquiterpene, are involved in activities against microbial pathogens.

All of the subfractions were active against *Plasmodium* with different strengths (Table 1). The most active fraction is SF 3 with IC_{50} 12.98 ± 1.88 µg/ml on 3D7 and 19.81 ± 0.75 µg/ml on FCR3. This was followed by SF 2, SF 1, SF 4, and SF 5 with IC_{50} 16.44 ± 0.84, 31.24 ± 0.85, 33.88 ± 1.21, and 42.14 ± 4.50 µg/ml on 3D7 and 24.71 ± 2.84, 35.79 ± 0.72, 35.07 ± 2.60, and 25.99 ± 1.23 µg/ml on FCR3. Meanwhile, the lowest activity was with SF 6 with IC_{50} 43.56 ± 4.54 and 44.77 ± 3.57 µg/ml. Chloroquine as a positive control had IC_{50} 0.018 ± 0.002 on 3D7 and 0.039 ± 0.004 on FCR3. The antiplasmodial activity of the subfraction samples has significant differences than the positive control, i.e., $p < 0.05$. Based on the results of the one-way ANOVA statistical analysis of the IC_{50} data on the 3D7 variant, it is divided into three groups: the first group is SF 3 and SF 2; the second group is SF 1 and SF 4; and the third group is SF 5 and SF 6, where the activities of group members do not have significant differences, i.e., $p > 0.05$, but have significant differences with other group samples, i.e., $p < 0.05$. The results of the one-way ANOVA statistical analysis of the IC_{50} data on the FCR3 variant are divided into three groups: the first group is SF 3, SF 2, and SF 5; the second group is SF 1 and SF 4; and the third group is SF 6. The sample with the strongest activity is very likely to contain active compounds that are potent against *P. falciparum* 3D7 and FCR3 variants even though the subfraction activity is included in the moderate category (Kamaraj *et al*., 2014).

The activity of the samples is shown in Table 2 and specific SF 3 activity in Figure 2. The activity data in Table 2 show the response between concentration samples, %parasitemic growth, and %inhibition growth of parasitemia. The activity of the samples was concentration-dependent, which was seen at a high concentration of very little parasitemic growth and showed strong inhibitory activity. However, the smaller the concentration, the higher the parasitemia growth and the lower the inhibitory activity. The 3D7 and FCR3 parasitemia variants in the SF 3 sample did not grow at a concentration of 125 g/ml, but parasitemia growth increased when the concentration was reduced. The percentage of inhibitory growth of parasitemic 3D7 and FCR3 variants at 125 µg/ml is 100% and decreased with decreasing concentration.

Antiplasmodial activity in extracts, fractions, and subfractions is strongly influenced by the content of secondary metabolites that they have. The results of this antiplasmodial test can be used as a reference in an effort to find antiplasmodial active compounds. Previously, there were no reports on this sample related to antiplasmodial activity because the previous studies only reported that two types of sponges from the genus *Hyrtios*

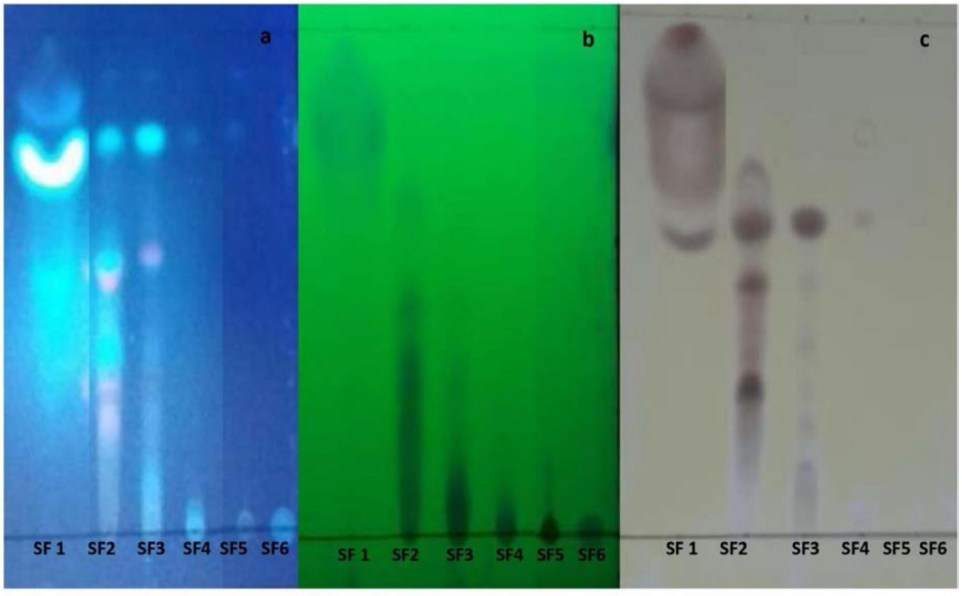

Figure 1. Profile TLC of column separation obtained from chloroform fraction of *H. reticulatus* extract. Stationery phase = silica gel GF254, mobile phase = n-hexane:ethyl acetate (3:1). a) uv 366, b) uv 254, and c) cerium sulfate.

Table 1. IC_{50} values of the samples tested against *P. falciparum* 3D7 and FCR3 variants.

Sample	IC_{50} (µg/ml)	
	3D7	FCR3
Extract	42.01 ± 3.74	41.90 ± 2.32
$CHCl_3$ fraction	34.15 ± 7.79	21.23 ± 7.23
Residue	39.42 ± 7.57	39.05 ± 1.80
SF 1	31.24 ± 0.85	35.79 ± 0.72
SF 2	16.44 ± 0.84	24.71 ± 2.84
SF 3	12.98 ± 1.88	19.81 ± 0.75
SF 4	33.88 ± 1.21	35.07 ± 2.60
SF 5	42.14 ± 4.50	25.99 ± 1.23
SF 6	43.56 ± 4.54	44.77 ± 3.57
Chloroquine	0.018 ± 0.002	0.039 ± 0.004

Data are expressed as mean ± SD.

sponge had antiplasmodial activity. They were *Hyrtios erectus* and *Hyrtios* sp.

Hyrtios erectus has homofascaplysin A and fascaplysin against *P. falciparum* strain NF54 with IC_{50} values of 24 and 34 ng/ml (Shady *et al*., 2017) and smenotronic acid, ilimaquinone, and pelorol with 3.51 ± 0.63, 2.11 ± 0.23, and 0.80 ± 0.19 µM against the *P. falciparum* Dd2 strain, respectively (Ju *et al*., 2018). *Hyrtios* sp. has 15α-methoxypuupehenol against *P. falciparum* strain FcB1 with IC_{50} 1.4 µg/ml (Bourguet-Kondracki *et al*., 1999). Up until recently, there have been seven classes of antiplasmodials that have been shown to own antiplasmodial activity, such as endoperoxides, alkaloids, terpenes, polyphenols, quinones and polyketides, nonpeptide macrocyclic, and β-resorcylic lactone (Nogueira and Lopes, 2011). The mechanism of antiplasmodial activity from the sample above is unknown, but based on the previous research, the action mechanism of the antiplasmodial itself has several target mechanisms, such as the action mechanism in the cytosol, on

Table 2. Activity of extract, chloroform fraction, and SF 1–6 against *P. falciparum* 3D7 and FCR3 variants.

Sample	Concentration (µg/ml)	3D7		FCR3	
		% parasetimia	% inhibition	% parasetimia	% inhibition
Extract	CN	7.02 ± 0.22	0 ± 0	7.56 ± 0.18	0 ± 0
	125.00	2.25 ± 0.10	67.89 ± 1.37	2.87 ± 0.39	62.05 ± 5.11
	62.50	3.25 ± 0.10	53.67 ± 1.42	3.32 ± 0.2	56.08 ± 2.62
	31.25	3.84 ± 0.14	45.29 ± 2.05	4.52 ± 0.23	40.16 ± 3.03
	15.63	4.44 ± 0.38	36.76 ± 5.45	5.05 ± 0.45	33.21 ± 5.99
	7.81	5.42 ± 0.49	22.81 ± 7	6.17 ± 0.58	18.38 ± 7.69
	3.91	6.59 ± 0.28	6.15 ± 3.92	7.47 ± 0.04	1.21 ± 0.49
Chloroform fraction	CN	7.02 ± 0.22	0.00 ± 0.00	7.56 ± 0.18	0.00 ± 0.00
	125.00	1.26 ± 0.13	82.08 ± 1.87	0.00 ± 0.00	100 ± 0.00
	62.50	2.05 ± 0.08	70.81 ± 1.09	2.11 ± 0.17	72.07 ± 2.28
	31.25	2.39 ± 0.17	65.91 ± 2.41	3.02 ± 0.17	60.05 ± 2.31
	15.63	3.48 ± 0.02	50.41 ± 0.35	3.92 ± 0.2	48.12 ± 2.59
	7.81	4.23 ± 0.26	39.7 ± 3.7	4.48 ± 0.14	40.76 ± 1.82
	3.91	5.03 ± 0.32	28.34 ± 4.62	5.46 ± 0.06	27.82 ± 0.73
Residue	CN	7.02 ± 0.22	0.00 ± 0.00	7.56 ± 0.18	0.00 ± 0.00
	125.00	2.01 ± 0.06	71.36 ± 0.81	1.72 ± 0.22	77.26 ± 2.87
	62.50	2.96 ± 0.4	57.82 ± 5.72	2.35 ± 0.18	68.93 ± 2.33
	31.25	3.72 ± 0.15	47.05 ± 2.16	4.33 ± 0.14	42.73 ± 1.84
	15.63	4.35 ± 0.18	38.02 ± 2.5	5.23 ± 0.52	30.77 ± 6.87
	7.81	5.69 ± 0.24	19.00 ± 3.46	5.82 ± 0.23	23.04 ± 3.01
	3.91	6.42 ± 0.26	8.52 ± 3.67	6.96 ± 0.42	7.92 ± 5.53
SF 1	CN	10.17 ± 0.62	0.00 ± 0.00	8.13 ± 0.23	0.00 ± 0.00
	125.00	0.29 ± 0.01	97.15 ± 0.11	0.07 ± 0.06	99.18 ± 0.71
	62.50	2.51 ± 0.05	75.36 ± 0.47	2.74 ± 0.11	66.3 ± 1.31
	31.25	5.34 ± 0.24	47.48 ± 2.37	4.21 ± 0.14	48.23 ± 1.7
	15.63	8.02 ± 0.10	21.12 ± 0.94	6.36 ± 0.18	21.78 ± 2.18
	7.81	8.84 ± 0.61	13.08 ± 5.97	7.51 ± 0.2	7.62 ± 2.46
	3.91	10.05 ± 0.10	1.19 ± 1.01	7.76 ± 0.17	4.55 ± 2.07

Continued

Sample	Concentration (µg/ml)	3D7		FCR3	
		% parasetimia	% inhibition	% parasetimia	% inhibition
	CN	10.17 ± 0.62	0.00 ± 0.00	8.13 ± 0.23	0.00 ± 0.00
	125.00	0.03 ± 0.06	99.67 ± 0.57	0.03 ± 0.06	99.59 ± 0.71
	62.50	2.52 ± 0.08	75.25 ± 0.77	2.39 ± 0.15	70.66 ± 1.82
SF 2	31.25	4.38 ± 0.10	56.96 ± 0.95	3.11 ± 0.11	61.7 ± 1.39
	15.63	4.66 ± 0.16	54.17 ± 1.54	5.08 ± 0.07	37.55 ± 0.89
	7.81	6.34 ± 0.13	37.67 ± 1.28	6.59 ± 0.09	18.9 ± 1.06
	3.91	7.98 ± 0.39	21.55 ± 3.82	7.74 ± 0.34	4.77 ± 4.24
	CN	10.17 ± 0.62	0.00 ± 0.00	8.13 ± 0.23	0.00 ± 0.00
	125.00	0.00 ± 0.00	100 ± 0.00	0.00 ± 0.00	100 ± 0.00
	62.50	0.43 ± 0.16	95.74 ± 1.6	0.29 ± 0.09	96.4 ± 1.11
SF 3	31.25	1.11 ± 0.29	89.06 ± 2.9	2.49 ± 0.24	69.39 ± 2.92
	15.63	2.89 ± 0.21	71.57 ± 2.05	4.04 ± 0.07	50.37 ± 0.88
	7.81	4.67 ± 0.09	54.12 ± 0.86	6.1 ± 0.36	24.92 ± 4.38
	3.91	5.83 ± 0.19	42.63 ± 1.87	7.0 ± 0.13	13.85 ± 1.64
	CN	7.02 ± 0.22	0.00 ± 0.00	7.56 ± 0.18	0.00 ± 0.00
	125.00	0.63 ± 0.15	91.04 ± 2.12	0.89 ± 0.09	88.24 ± 1.21
	62.50	2.02 ± 0.16	71.27 ± 2.24	2.82 ± 0.14	62.71 ± 1.89
SF 4	31.25	3.59 ± 0.05	48.88 ± 0.78	3.68 ± 0.29	51.38 ± 3.89
	15.63	4.04 ± 0.10	42.42 ± 1.46	5.47 ± 0.60	27.60 ± 7.95
	7.81	5.52 ± 0.20	21.43 ± 2.88	6.75 ± 0.12	10.78 ± 1.57
	3.91	6.06 ± 0.11	13.64 ± 1.53	7.18 ± 0.08	5.03 ± 1.07
	CN	10.17 ± 0.62	0.00 ± 0.00	8.13 ± 0.23	0.00 ± 0.00
	125.00	0.46 ± 0.06	95.47 ± 0.56	0.07 ± 0.12	99.18 ± 1.42
	62.50	2.08 ± 0.08	79.53 ± 0.74	1.26 ± 0.10	84.56 ± 1.28
SF 5	31.25	5.52 ± 0.32	45.77 ± 3.16	4.18 ± 0.19	48.64 ± 2.34
	15.63	6.12 ± 0.06	39.84 ± 0.59	5.74 ± 0.19	29.41 ± 2.32
	7.81	6.63 ± 0.12	34.82 ± 1.22	7.10 ± 0.23	12.61 ± 2.81
	3.91	8.45 ± 0.12	16.88 ± 1.19	8.12 ± 0.01	0.17 ± 0.11
	CN	10.17 ± 0.62	0.00 ± 0.00	8.13 ± 0.23	0.00 ± 0.00
	125.00	0.9 ± 0.10	91.17 ± 0.97	2.03 ± 0.23	75.07 ± 2.86
	62.50	4.51 ± 0.06	55.67 ± 0.60	3.53 ± 0.11	56.54 ± 1.38
SF 6	31.25	6.34 ± 0.35	37.61 ± 3.40	4.31 ± 0.11	47.01 ± 1.35
	15.63	7.45 ± 0.26	26.77 ± 2.59	6.20 ± 0.29	23.77 ± 3.51
	7.81	8.37 ± 0.23	17.74 ± 2.29	7.70 ± 0.40	5.24 ± 4.88
	3.91	9.68 ± 0.23	4.79 ± 2.23	8.79 ± 0.56	-8.17 ± 6.93
	CN	10.94 ± 0.49	0.00 ± 0.00	8.13 ± 0.23	0.00 ± 0.00
	0.50	0.00 ± 0.00	100 ± 0.00	0.00 ± 0.00	100 ± 0.00
	0.25	0.00 ± 0.00	100 ± 0.00	0.00 ± 0.00	100 ± 0.00
Chloroquine	0.12	0.00 ± 0.00	100 ± 0.00	0.00 ± 0.00	100 ± 0.00
	0.062	0.00 ± 0.00	100 ± 0.00	0.97 ± 0.05	88.12 ± 0.67
	0.031	1.30 ± 0.30	88.13 ± 2.76	2.49 ± 0.35	69.41 ± 4.25
	0.012	5.31 ± 0.31	51.43 ± 2.88	4.02 ± 0.04	50.58 ± 0.52

Data are expressed as mean ± SD, CN is control negative.

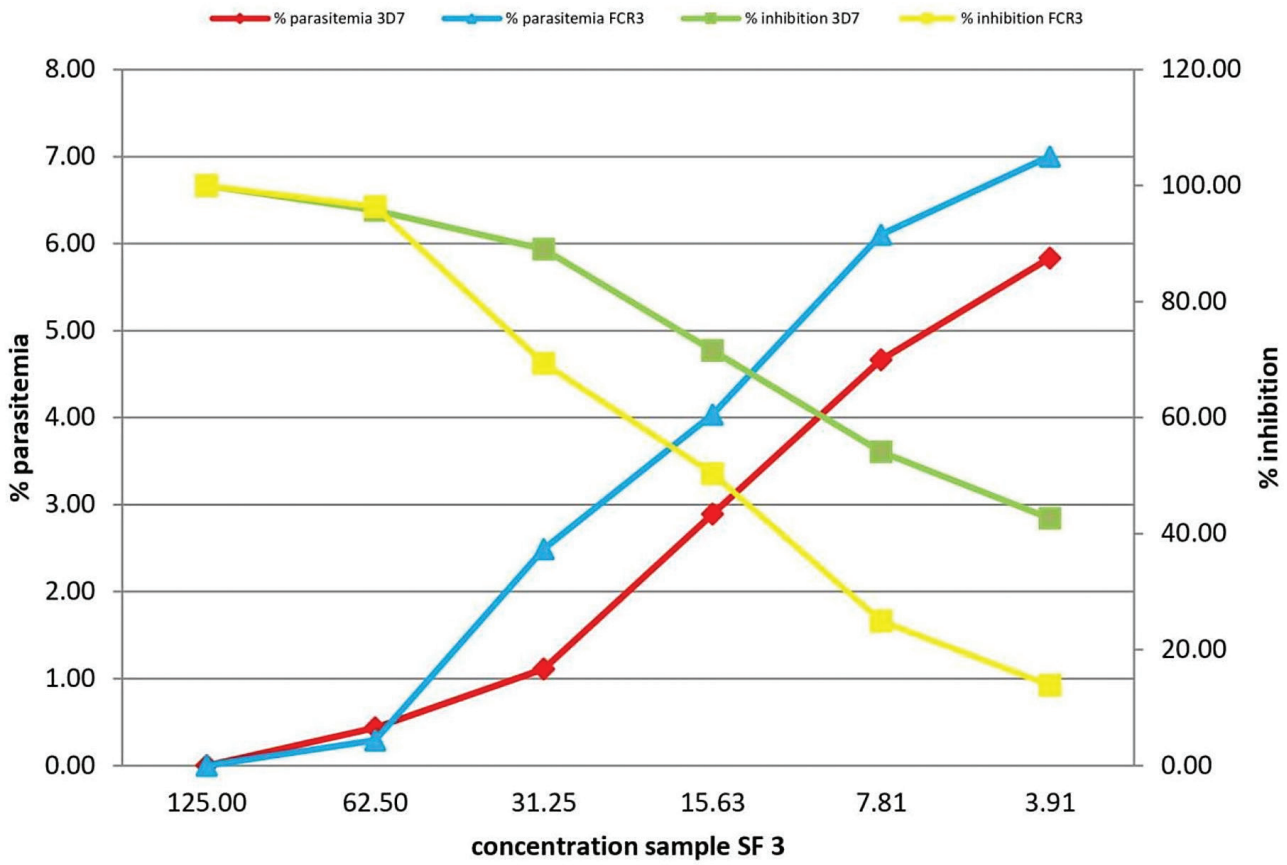

Figure 2. Antiplasmodial activity of SF 3 against 3D7 and FCR3.

parasitic membranes, on food vacuoles, on mitochondria, and on apicoplasts (Rosenthal, 2003).

CONCLUSION

The fractions of *H. reticulatus* sponge extract have an antiplasmodial activity, especially SF 3 with IC_{50} 12.98 ± 1.88 µg/ml against *P. falciparum* 3D7 variant and 19.81 ± 0.75 µg/ml against *P. falciparum* FCR3, and have the potency to be researched about the new compound with potent antiplasmodial activity.

ACKNOWLEDGMENT

The authors would like to acknowledge the funding support from the Indonesian Endowment Fund for Education/ *Lembaga Pengelola Dana Pendidikan* (LPDP).

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included within this research article.

PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

REFERENCES

Anjum K, Abbas SQ, Shah SAA, Akhter N, Batool S, Hassan SSU. Marine sponges as a drug treasure. Biomol Therap, 2016; 24:347–62.

Bourguet-Kondracki ML, Lacombe F, Guyot M. Methanol adduct of puupehenone, a biologically active derivative from the marine sponge Hyrtios species. J Nat Prod, 1999; 62:1304–5.

Capela R, Moreira R, Lopes F. An overview of drug resistance in protozoal diseases. Int J Mol Sci, 2019; 20(22):5748.

De Forges R. Compendium of marine species from New Caledonia, 2nd edition, Nouvelle-Caledonia, 2007.

De Voogd NJ. An assessment of sponge mariculture potential in the Spermonde Archipelago, Indonesia. J Mar Biol Assoc UK, 2007; 87:1777–84.

Fattorusso E, Taglialatela-Scafati O. Marine antimalarials. Mar Drugs, 2009; 7:130–52.

Fattorusso E, Taglialatela-Scafati O. The contribution of marine chemistry in the field of antimalarial research. Royal Soc Chem, 2012; 374–90.

Hikmawan BD, Wahyuono S, Setyowati EP, Marine sponge compounds with antiplasmodial properties: focus on *in vitro* study against *Plasmodium falciparum*. J Appl Pharm Sci, 2018; 8:001–11.

Hyde JE. Drug-resistant malaria - an insight. FEBS J, 2007; 274:4688–98.

Imada K, Sakai E, Kato H, Kawabata T, Yoshinaga S. Reticulatins A and B and hyrtioreticulin F from the marine sponge *Hyrtios reticulatus*. Tetrahedron, 2013; 69:7051–5.

Inman WD, Bray WM, Gassner NC, Lokey RS, Tenney K, Shen YY. A β-carboline alkaloid from the Papua New Guinea marine sponge *Hyrtios reticulatus*. J Nat Prod, 2010; 73:255–7.

Jensen JB, Trager W. *Plasmodium falciparum* in culture: use of outdated erythrocytes and description of the candle jar method. J Parasitol, 1977; 63:883–6.

Ju E, Latif A, Kong CS, Seo Y, Lee YJ, Dalal SR. Antimalarial activity of the isolates from the marine sponge *Hyrtios erectus* against the chloroquine-resistant Dd2 strain of *Plasmodium falciparum*. Z Naturforsch C J Biosci, 2018; 73:397–400.

Kamaraj C, Rahuman AA, Roopan SM, Bagavan A, Elango G, Zahir AA. Bioassay-guided isolation and characterization of active antiplasmodial compounds from Murraya koenigii extracts against *Plasmodium falciparum and Plasmodium berghei*. Parasitol Res, 2014; 113:1657–72.

Kenangalem E, Poespoprodjo JR, Douglas NM, Burdam FH, Gdeumana K, Chalfein F, Prayoga, Thio F, Devine A, Marfurt J, Waramori G, Yeung S, Noviyanti R, Penttinen P, Bangs MJ, Sugiarto P, Simpson JA, Soenarto Y, Anstey NM, Price RN. Malaria morbidity and mortality following introduction of a universal policy of artemisinin-based treatment for malaria in Papua, Indonesia : a longitudinal surveillance study. Plos Med, 2019; 16(5):e1002815.

Kirsch G, Köng GM, Wright AD, Kaminsky R. A new bioactive sesterterpene and antiplasmodial alkaloids from the marine sponge *Hyrtios cf. erecta*. J Nat Prod, 2000; 63:825–9.

Kotepui M, Kotepui KU, De Jesus Milanez G, Masangkay FR. *Plasmodium* spp. mixed infection leading to severe malaria: a systematic review and meta-analysis. Sci Rep, 2020; 10:1–12.

Mahfur M, Setyowati EP, Wahyuono S, Purwantini I. Sponge *Hyrtios reticulatus*: phytochemicals and bioactivities. Res J Pharm Technol, 2022: 15(6): 2022

Mayer AMS, Rodr AD, Taglialatela-Scafati O, Fusetani N. Marine pharmacology in 2014–2015: marine compounds with antibacterial, antidiabetic, antifungal, anti-inflammatory, antiprotozoal, antituberculosis, antiviral, and anthelmintic activities; affecting the immune and nervous systems, and other miscellaneous. Mar Drugs, 2020; 18:1–61.

Molina-Cruz A, DeJong RJ, Ortega C, Haile A, Abban E, Rodrigues J, Barillas-Mury C. Some strains of *Plasmodium falciparum*, a human malaria parasite, evade the complement-like system of *Anopheles gambiae* mosquitoes. Proc Natl Acad Sci U S A, 2012; 109(28):1–6.

Nogueira CR, Lopes LMX. Antiplasmodial natural products. Molecules, 2011; 16:2146–90.

Rosenthal PJ. Antimalarial drug discovery: old and new approaches. J Exper Biol, 2003; 206:3735–44.

Setyowati EP, Jenie UA, Sudarsono, Kardono LBS, Rahmat R. Identification of cytotoxic constituent of Indonesian sponge *Kaliapsis* sp. (Bowerbank). J Pak Biol Sci, 2008; 11(22);2560–6.

Setyowati EP, Jenie UA, Sudarsono, Kardono LBS, Rahmat R. Theonellapeptolide Id: structure identification of cytotoxic constituent from *Kaliapsis* sp. Sponge (Bowerbank) collected from West Bali Sea Indonesia. J Biol Sci, 2009; 9(1):29–36.

Shady NH, El-Hossary EM, Fouad MA, Gulder TAM, Kamel MS, Abdelmohsen UR. Bioactive natural products of marine sponges from the genus *Hyrtios*. Molecules, 2017; 22:781–802.

Tajuddeen N, Van Heerden FR. Antiplasmodial natural products: an update. Malar J, 2019; 18:1–62.

World Health Organization. Global Technical Strategy For Malaria 2016–2030. World Health Organization, Geneva, Switzerland, 2021.

Yamanokuchi R, Imada K, Miyazaki M, Kato H, Watanabe T, Watanabe M. Hyrtioreticulins A–E, indole alkaloids inhibiting the ubiquitin-activating enzyme, from the marine sponge *Hyrtios reticulatus*. Bioorg Med Chem, 2012; 20:4437–42.=

How to cite this article:
Mahfur M, Purwantini I, Wahyuono S, Setyowati EP. *In vitro* antiplasmodial activities of the fractions of *Hyrtios reticulatus* sponge extract. J Appl Pharm Sci, 2022; 12(09):114–120.