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

Mahfur1,2, Subagus Wahyuono1, Indah Purwantini, Erna Prawita Setyowati1*

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

**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 IC50 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 antimalarial 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; Kotepeui 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 Taglialetela-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 antimalarials 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 antimalarial activity (Ju et al., 2018; Shady et al., 2017). Previous studies on *Hyrtios reticulatus* sponge have identified it as an abundant source of...
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 antimalarial activity of crude ethanol extract and its fraction from the sample. Antimalarial 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 with parasitemia of 0.1%–1% 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:

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

**Statistical analysis**

The potency of antimalarial 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* antimalarial activity of *H. reticulatus* sponge. The evaluation began from the sponge *H. reticulatus* ethanol extract tested with *P. falciparum* 3D7 and FCR3 variants. The antimalarial 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* antimalarial 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 (Fattoruso and Tagliabue-Scafat, 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 parasitic 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.
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 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 1. IC$_{50}$ values of the samples tested against *P. falciparum* 3D7 and FCR3 variants.

| Sample   | 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 ± 0.84 | 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.

### 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 |
| 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           | 61.5 ± 3.92  | 7.47 ± 0.04   | 12.1 ± 0.49  |
| 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  | 3.48 ± 0.02           | 50.41 ± 0.35 | 3.92 ± 0.2    | 48.12 ± 2.59 |
| 15.63  | 4.42 ± 0.26           | 39.7 ± 3.7   | 4.48 ± 0.14   | 40.76 ± 1.82 |
| 7.81   | 5.03 ± 0.32           | 28.34 ± 4.62 | 5.46 ± 0.06   | 27.82 ± 0.73 |
| 3.91   | 7.02 ± 0.22           | 0.00 ± 0.00  | 7.56 ± 0.18   | 0.00 ± 0.00  |
| CN     | 2.01 ± 0.06           | 71.36 ± 0.81 | 1.72 ± 0.22   | 77.26 ± 2.87 |
| 125.00 | 2.96 ± 0.4            | 57.82 ± 5.72 | 2.35 ± 0.18   | 68.93 ± 2.33 |
| 62.50  | 3.72 ± 0.15           | 47.05 ± 2.16 | 4.33 ± 0.14   | 42.73 ± 1.84 |
| 31.25  | 4.35 ± 0.18           | 38.02 ± 2.5  | 5.23 ± 0.52   | 30.77 ± 6.87 |
| 15.63  | 5.69 ± 0.24           | 19.00 ± 3.46 | 5.82 ± 0.23   | 23.04 ± 3.01 |
| 7.81   | 6.42 ± 0.26           | 8.52 ± 3.67  | 6.96 ± 0.42   | 7.92 ± 5.53  |
| 3.91   | 10.17 ± 0.62          | 0.00 ± 0.00  | 8.13 ± 0.23   | 0.00 ± 0.00  |
| CN     | 0.29 ± 0.01           | 97.15 ± 0.11 | 0.07 ± 0.06   | 99.18 ± 0.71 |
| 125.00 | 2.51 ± 0.05           | 75.36 ± 0.47 | 2.74 ± 0.11   | 66.3 ± 1.31  |
| 62.50  | 5.34 ± 0.24           | 47.48 ± 2.37 | 4.21 ± 0.14   | 48.23 ± 1.7  |
| SF 1   | 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 |
|        | 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.
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.

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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.

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