

# High Antiplasmodial Activity of Golden Gamat (*S.hermanni*) Extract Through In Vitro Study

Prawesty Diah Utami and Varidianto Yudho

## ABSTRACT

*S.hermanni* is a marine biota that contains active components that are anti-fungal, anti-bacterial, antioxidant, anti-inflammatory, and anti-cancer. The research focused on examining the antiplasmodial activity of an ethanol extract of *S. hermanni* on the proliferation of *P. falciparum* in vitro. The total sample size was 30 mediums, which included: (G1). No extract or chloroquine administration in the *P.falciparum* medium (G2). Chloroquine treatment administration on *P. falciparum* medium (G3). *P. falciparum* medium with *S. hermanni* extract. Parasitaemia, growth percentage, inhibitory rate, and IC<sub>50</sub> were among the parameters evaluated. Administration of extract serial doses succeeds in reducing the percentage of parasite growth and parasitemia levels. G3 demonstrated an inhibitory rate of 88.51 % with a dose of 100 µg/ml and an IC<sub>50</sub> of 2.86 µg/ml, indicating high antimalarial activity, although chloroquine had greater antimalarial activity than *S. hermanni* extract. In vitro studies on *S hermanni*, ethanol extract indicated that it contains bioactive components and can be an effective antiplasmodial agent.

**Keywords:** Antiplasmodial activity, in vitro, *P.falciparum*, *S.hermanni*.

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## I. INTRODUCTION

Malaria is a fatal illness induced by blood protozoan parasites transmitted through female Anopheles species [1]. Malaria remains a global health issue; malaria cases worldwide totaled 228 million, with Africa and Southeast Asia accounting for most cases. In 2018, the global death rate was 405 thousand fatalities[2]. Antimalarial drug resistance has led to an increase in malaria mortality rates over the past 20 years[3]. One potential source of new antimalarial drugs is compounds derived from marine biota. The world's marine, the pharmaceutical agency confirms the great potential of marine species as a source for developing new pharmaceutical components and drugs. Land biota has much lower phylogenetic diversity than marine and freshwater biota. A variety of environmental factors influences the ability of marine biota to adapt [4], [5].

*Sticophus hermanni*, or golden gamat, includes various bioactive substances such as chondroitin sulfate, polysaccharides, glycosaminoglycans, saponins, peptides, sterols, phenolics, and cerebroside [4]–[6]. Numerous research has revealed that golden gamat contains biological activities such as wound healing, pain relief, antibacterial, antifungal, antioxidant, cytotoxic, and anticancer effects. [4], [6], [7]. *S.hermanni*'s antiplasmodial effect has never been investigated. The purpose of this research was to evaluate the antiplasmodial activity of golden gamat (*S. hermanni*) ethanol extraction *Plasmodium falciparum* proliferation. The research approach used in this study was an in vitro investigation with *P.falciparum*-containing media. The study

outcomes are expected to provide scientific knowledge regarding the antiplasmodial activity of golden gamat (*S. hermanni*) extraction the growth of *P. falciparum* in vitro, which may be used as a conceptual framework for in vivo research.

## II. METHODS

### A. Study Design and Setting

The research was conducted using an experimental study with in vitro methods. The study was performed using a post-test-only control group design because we tested the parameters in all groups during the end of treatments. The researcher was conducted in vitro study at the Institute of Tropical Diseases, Airlangga University, Surabaya, Indonesia, from March to July 2021. The ethics committee of Faculty Medicine, Universitas Hang Tuah conducted a research ethics review (I/208/UHT.KEPK.03/III/2021).

### B. Experimental Unit

This study's experimental unit consists of 30 *P. falciparum* culture mediums. The experimental unit will be divided into the following three groups:

- 1). G1 consists of ten *P. falciparum* culture mediums without any antimalaria drugs (chloroquine).and extract.
- 2). G2 consists of ten *P.falciparum* culture mediums with antimalarial drugs (chloroquine).
- 3). G3 consists of ten *P.falciparum* culture mediums with the administration of *S. hermanni* ethanol extract.

Study groups G2 and G3, administration of chloroquine and *S. hermanni* extract will be separated into five dose groups: 100, 10, 1, 0.1, and 0.01 µg/ml [8]. Each dose will be repeated twice, resulting in 10 mediums with five different doses in each group. This study obtained *P. falciparum* culture mediums from the Tropical Disease Center at Universitas Airlangga, Surabaya, Indonesia.

### C. *S. hermanni* Material

The researchers performed this study using fresh golden gamat *S. hermanni* from the waters around the island of Sapeken, Madura-East Java, Indonesia, during the rainy season, and taxonomic testing at the Laboratory of Plant Bioscience and Technology, Sepuluh November Institute of Technology Surabaya, Indonesia (No. 002/IT.1/Biosains dan Teknologi Tumbuhan/2021). *S. hermanni* was extracted at the Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia.

### D. Phytochemical Assay

Phytochemical analysis was carried out to evaluate several bioactive components found in the crude extract of *S. hermanni*. Phytochemical testing can detect bioactive components in primary metabolites that induce functional biological activity[9]. The phytochemical test in this study used a qualitative method to assess the presence of an active component. We used three methods to assess the alkaloid content: Dragendroff/test Kraut's (using Dragendroff reagent – potassium, bismuth, and iodide solution and marked by the development of a red-orange precipitate); Meyer test (with Meyer's reagent – potassium mercuric iodide solution and characterized by the formation of a white precipitate). And the Wagner test (distinguished by the formation of brown precipitate from Wagner's reagent, which is an iodine solution in potassium iodide) [9]. Flavonoid test (using lead acetate – lead acetate solution and characterized by the production of an orange precipitate); tannin concentration is determined using the Braymer test, which yields a greenish-blue liquid. Saponin content test using froth test-dilution with distilled water for 15 minutes, followed by froth formation on the surface. The steroid content was evaluated using concentrated H<sub>2</sub>SO<sub>4</sub> and chloroform, and we noticed a red color change under the chloroform layer. As previously stated, conventional procedures and methods are used in other fields [9]–[11].

### E. Ethanol Extraction

The golden gamat *S. hermanni* was cleaned, sliced into small pieces, and dried. After drying, gold sea cucumber pieces are mashed to make 900 g of gold sea cucumber powder. The powder is extracted using a maceration process with 96 % ethanol (3000 ml). The resultant macerate was collected and evaporated using a rotary evaporator under vacuum, yielding a viscous extract of 45 grams of golden sea cucumber ethanol extract, which was deposited in an airtight container and refrigerated until use [12].

### F. *Plasmodium falciparum* Cultivation In Vitro

The cultivation process requires the use of two primary ingredients, such as *P. falciparum* strain 3D7 (chloroquine-sensitive strain) provided by Malaria Laboratory - Institute of Tropical Disease, Airlangga University, Surabaya, and also

Surabaya red cross provided human blood (packed red cell and plasma) from the Red Cross in Surabaya, Indonesia. In vitro study was conducted to evaluate the antimalarial effect applying a modified Trager and Jensen technique. The parasites were cultivated in fresh human erythrocytes (with blood type O positive, male) up to 5% hematocrit and planted in RPMI 1640 media. We added hypoxanthine to the RPMI 1640 medium at a concentration of 50 micrograms per milliliter, together with 2 mg/mL NaHCO<sub>3</sub>, 25 mM HEPES buffer, and ten micrograms/milliliter gentamycin. Then we put the culture inside a modified candle jar and incubated it at 37 °C. [3], [13], [14]. A thin blood smear stained with Giemsa is evaluated to confirm *P. falciparum* growth. A microscope with a magnification of 1000 times was used to observe the infected erythrocytes. The parasite utilized in the test is synchronized (ring form stage) with an approximated parasitemia level of 1%. (hematocrit 5 %) [13].

### G. In Vitro Antiplasmodial Activity Assay

The extract (10 mg) and chloroquine were diluted in 1000 µl of DMSO (to form a 10,000 µg/ml). From the stock solution, serial dilutions were made, providing a homogeneous solution of 0.01; 0.1; 1; 10; 100 micrograms per milliliter[3]. Each microwell (well 96) received 2 µl of extract solution (for G3 groups) and chloroquine (for G2 groups) at varying doses (dosage gradation 0.01; 0.1; 1; 10; and 100 micrograms per milliliter), followed by 198 µl of parasite cell suspension (we made each concentration in duplicate). The parasite suspension was the only component in the negative control well (G1), with no extract or chloroquine added. The well containing the extract liquid and parasites was placed into a mixture of five percent oxygen, five percent carbon dioxide, and ninety percent of nitrogen and maintained around 37 °C for 48 hours. We checked parasite cell cultures for thin blood smears to indicate parasitemia before being injected into the microwell (parasitemia levels at -0 hours). Following culture harvesting, a thin blood film was produced using 10% Giemsa staining [3], [15]. The data was analyzed using light microscopy, and we calculated the number of infected red blood cells per one thousand red blood cells [16] (data on parasitemia levels after treatment/data at -48 hours). The researcher will use this information to calculate parasitemia growth and inhibition percentages. The percentage of growth and the inhibitory rate was calculated using the following [3]:

$$\% \text{ growth} = \% \text{ parasite after treatment} - \% \text{ parasite before treatment}$$

$$\% \text{ Inhibitory} = \frac{100\% - \% \text{ growth on experimental group}}{\% \text{ growth on negative control group}} \times 100\%$$

The final stage in assessing the antiplasmodial activity of *S. hermanni* extract was to estimate the IC<sub>50</sub>. A statistical analysis based on the percent inhibitory rate was performed using probit analysis and the SPSS version 20 software. IC<sub>50</sub> is a measurement used in pharmacological research to determine the dose of drug/active ingredient necessary to inhibit biological processes by 50%, and it serves as a measure of the drug/active ingredient's potency [17], [18]. Probit analysis is a subset of regression analysis in which the concentration-response curve is converted into a straight line

and it serves as a measure of the drug/active ingredient's potency [17], [18]. Probit analysis is a subset of regression analysis in which the concentration-response curve is converted into a straight line that can then be examined using least squares or maximum likelihood regression [19].

#### H. *In vitro* Antiplasmodial Activity Criteria

In this study, we analyzed the IC<sub>50</sub> results to WHO standard requirements for antimalarial drug development projects, and the extract materials were grouped into different groups based on their IC<sub>50</sub>:

- 1) High potency if IC<sub>50</sub> value less than or equal to 5 µg/ml.
- 2) promising potency if IC<sub>50</sub> value greater than 5 µg/ml or less than or equal to 5 µg/ml.
- 3) moderate potency if < IC<sub>50</sub> value greater than 15 µg/ml, or less than or equal to 50 µg/ml.
- 4) If the IC<sub>50</sub> value is larger than 50 micrograms per milliliter, the potency is poor.

A pure material is deemed very active when its IC<sub>50</sub> value is less or equal 1 µg/ml. [3].

#### I. Statistical Analysis

Data from parasitaemia level measurements were processed in excel software to calculate percent parasitaemia growth and percent inhibitory rate. IC<sub>50</sub> analysis using Probit analysis in SPSS version 20 software.

### III. RESULT

#### A. Phytochemical Assay

Qualitative phytochemical assays to establish active compounds in ethanol extract of *S.hermani*. According to the phytochemical test, the ethanol extract of *S. hermanni* includes various active compounds, including flavonoids, saponins, steroids, and alkaloids, but not tannins.

#### B. *In Vitro* Antimalarial Activity Assay

The parasitemia level was determined twice: before treatment (at 0 hours) and after treatment and incubation (at 48 hours). Data on parasitemia levels at 0 hours revealed homogenous findings in each treatment group, 1.03 %. After 48 hours of incubation and treatment, parasitemia levels were measured and found to be varied. The following table shows the results of measuring the parasitemia level in *S hermanni* extract with ethanol solvent:

TABLE I: PARASITEMIA LEVEL ASSAY

Groups	0 hours	% Parasitemia at 48 Hours				
		100*	10*	1*	0.1*	0.01*
control (-)	1,03	6,73	6,73	6,73	6,73	6,73
control (+)	1,03	-	1,46	2,09	2,79	3,67
Ethanol - <i>S.hermani</i>	1,03	1,685	3,23	4,64	5,8	6,475

\*: all doses in micrograms per milliliter.

According to the table above, the level of parasitemia in the treatment group (control positive and ethanol extract of *S hermanni*) decreased with increasing doses of extract and chloroquine. According to the table above, the level of parasitemia in the treatment group (control positive and ethanol extract of *S hermanni*) decreased with increasing doses of extract and chloroquine. We did not measure parasitemia level on the G2 group/control positive group at a

concentration of 100 µg/ml because the parasitemia level was very low at ten micrograms/milliliter.

The following figure describes the parasitemia increase percentage obtained from the difference between parasitemia levels at 0 and 48 hours:

TABLE II: PARASITE GROWTH ASSAY

Groups	% Parasite Growth				
	100*	10*	1*	0.1*	0.01*
control (-)	5,7	5,7	5,7	5,7	5,7
control (+)	-	0,43	1,06	1,76	2,64
Ethanol - <i>S.hermani</i>	0,65	2,2	3,61	4,77	5,44

\*: all doses in micrograms per milliliter.

The data from parasite growth measurements revealed that the higher the amount of chloroquine and *S.hermani* extract administered, the slower the parasite grew. We did not assess the parasite growth rate in the positive control group at 100 µg/ml because it was nearly zero at 10 µg/ml. Among the other groups, the lowest dose of chloroquine (0.01 µg/ml) up to 10 µg/ml shown the least amount of parasite development

The following table summarizes the findings of the inhibitory rate measurement and probit analysis of the positive control and *S.hermani* extract with ethanol:

TABLE III: INHIBITORY RATE & IC<sub>50</sub> ASSAY RESULT

Groups	IC <sub>50</sub> (µg/ml)	% Inhibitory Rate				
		100*	10*	1*	0.1*	0.01*
control (+)	0,005	-	92,46	81,40	69,12	53,68
Ethanol - <i>S.hermani</i>	2,86	88,51	61,40	36,67	16,32	4,47

\*: all doses in micrograms per milliliter.

According to the table above, increasing doses of chloroquine and *S.hermani* extract enhance the inhibitory rate. Because the negative control group only contained *P.falciparum* without any treatment, we did not measure the inhibitory rate. The inhibitory rate in the positive control group at a concentration of 10 µg/ml was about 100%, the highest compared to the other groups. Chloroquine has a lower IC<sub>50</sub> than *S.hermani*'s ethanol extract; these results suggest that chloroquine has a higher potency (IC<sub>50</sub> ≤ 5 µg/ml) than *S.hermani*'s ethanol extract. However, compared to WHO guidelines, ethanol extract of *S.hermani* showed high antimalarial activity (high potency if IC<sub>50</sub> ≤ 5 µg/ml).

### IV. DISCUSSION

The phytochemical assay in this study differed from the previous study by Pringgenies et al. (2018), who discovered that *S hermanni* contains flavonoids, saponins, steroids, tannins, and terpenoids but no alkaloids [18]. The different result is most likely due to the sampling location of *S.hermani*, which differs in location. Location dissimilarities may result in different environmental exposures and adaptive mechanisms, resulting in variable types and amounts of active compounds. Other parameters that significantly impact extraction efficiency include extraction method, temperature, duration of the extraction process, and solvent applied. If the extraction procedure is the same, the solvent selected will significantly impact the bioactive content [20]. because each solvent has a different



polarity (polar, semipolar, and nonpolar)[21]. The solvent type and polarity also influence extraction quality, amount, extraction velocity, inhibition chemicals, toxicology, other bioactivities, and biosecurity [21], [22].

This research suggests that an ethanol extract of *S. hermanni* could inhibit the growth of *P. falciparum* in culture media. These findings are consistent with the findings of Sarhadizadeh *et al.* [6] who found that *S. hermanni* extract demonstrated antifungal action, with suppression of fungal growth activity rising with increasing extract dose, but no antibacterial activity. Another study found that *S. hermanni* extract has antibacterial properties against *S. aureus*, *Pseudomonas* sp, *E.coli*, and other bacteria. Another study found that *S. hermanni* extract had an antibacterial effect against *S. aureus*, *Pseudomonas* sp, *E.coli*, and *V. vulnificus* [18]. There has been no research on the antimalarial activity of *S. hermanni* extract; thus, no data can be compared.

Based on the inhibitory rate and IC<sub>50</sub> values, *S. hermanni* extract had a high potential antimalarial activity but was less potent than chloroquine. The antimalarial activity of *S. hermanni* extract is attributed to the presence of several active components. Through phytochemical assay on this research, the antimalarial action of *S. hermanni* extract was linked to different active compounds, including flavonoids, saponins, steroids, and alkaloids. Flavonoids have been found to have antimalarial effects via reducing hemoglobin breakdown by blocking the action of the cysteine protease enzyme falcipain-2 [3]. The saponins also have an antimalarial impact by damaging the cell membranes of infected erythrocytes, causing erythrocyte cells to lyse [23], [24]. Alkaloids have been proven to reduce parasitemia levels by suppressing parasite DNA and RNA production<sup>25</sup>. *S. hermanni* extract contains a steroid as the final product. Several investigations have found that steroids and their derivatives have antimalarial properties; however, the mechanism is unknown [26], [27]. Krieg *et al.* [28] have shown that arylmethylamino steroids are particularly effective antiplasmodial components due to their capacity to impede transmission, oral availability, and minimal toxicity. The inclusion of lipophilic steroid carriers to this antiparasitic aids in mediating cellular uptake and intracellular transport pathways, improving the antiparasitic's potency. Estratriene, a steroid derivative, works as an antiplasmodial agent by boosting cellular oxidation [28]. *S. hermanni* extract's mechanisms of action against malaria parasites require further study.

## V. CONCLUSION

*S. hermanni* extract with ethanol solvent had the highest inhibitory rate of 88.51% with a dose of 100 µg/ml and an IC<sub>50</sub> of 2.86 µg/ml (referring to the WHO guidelines, this figure indicates a high potential for antimalarial activity), although its activity is lower than chloroquine. *S. hermanni* ethanol extract's antimalarial activity was associated with a phytochemical assay result, which indicated diverse bioactive components such as alkaloid, flavonoid, saponin, and steroid. Previous studies have shown that each of these bioactive components has its function and mechanism for suppressing the growth of *P. falciparum* through in vitro and in vivo

studies. Based on the results of this study, further studies are needed to explore the antimalarial activity of *S. hermanni*.

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