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Original Article

S.hermanni's Capability as an Antimalarial and Antioxidant in a Mice Model of *P.berghei* ANKA Infection

Prawesty Diah Utami[1]*, Wachjudi Kurnia[2], Irma Andriani Pasaribu[3]

[1]*Parasitology Department, Medicine Faculty, Universitas Hang Tuah, Surabaya, Indonesia*
[2]*Biology Department, Medicine Faculty, Universitas Hang Tuah, Surabaya, Indonesia*
[3]*Department of Ophthalmology, Medicine Faculty, Universitas Hang Tuah, Surabaya, Indonesia*

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ABSTRACT

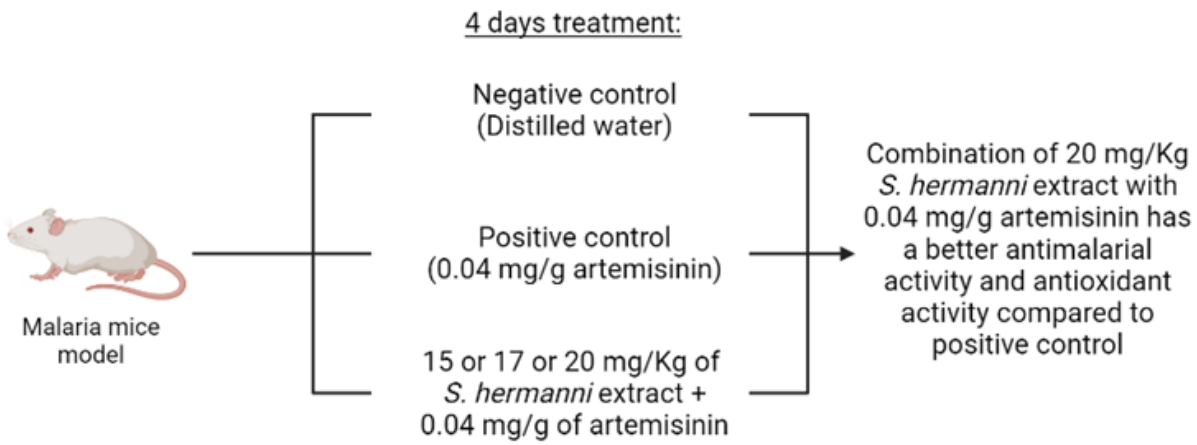
Background: Malaria poses a substantial threat to healthcare systems across the globe. The escalating resistance of malaria parasites to the absence of affordable and accessible antimalarial medications has exacerbated the challenges associated with malaria treatment. This research aims to evaluate the antimalarial and antioxidant properties of *Stichopus hermanni* in combination with artemisinin, employing murine malaria models.

Method: This study employed the 4-day suppression test procedure and conducted an experimental investigation with a post-test-only control group design. The study involved 35 male BALB/c mice, aged 7-10 weeks, each infected with *Plasmodium berghei* ANKA/PbA. The sample was divided into five groups: C1 (negative control), C2 (positive control), T1 (administered 15 mg/Kg BW/day of *Stichopus hermanni* ethanol extract and 0.04 mg/g BW/day of artemisinin), T2 (administered 17 mg/Kg BW/day of *Stichopus hermanni* ethanol extract and 0.04 mg/g BW/day of artemisinin), and T3 (administered 20 mg/Kg BW/day of *Stichopus hermanni* ethanol extract and 0.04 mg/g BW/day of artemisinin). The parameters measured included % parasitemia level, % growth inhibition, and malondialdehyde (MDA) levels.

Results: The T3 group exhibited the lowest parasitemia level and the highest degree of growth inhibition compared to the other groups (p-value < 0.05). However, statistically, it did not differ significantly from C2 and T1. Meanwhile, the T3 group demonstrated the lowest MDA levels and the most significant antioxidant capacity compared to the other groups (p-value < 0.05).

Conclusion: Based on both descriptive and statistical analyses, the combination of *Stichopus hermanni* ethanol extract and artemisinin exhibits antimalarial and antioxidant activities.

GRAPHICAL ABSTRACT



Introduction

The COVID-19 pandemic has had a profound impact on malaria elimination programs and healthcare services, leading to an increase in morbidity and mortality due to malaria. According to data from the World Health Organization (WHO), malaria incidence in 84 malaria-endemic countries is projected to reach 247 million cases in 2021, representing a two-million-case increase compared to 2020. The majority of this increase is expected to occur in countries within the African region, which accounted for approximately 234 million (95%) of global cases in 2021. The Southeast Asia region contributes approximately 2% of global malaria cases, with Indonesia ranking as the second-largest contributor to the malaria burden in the region, following India, and reporting an estimated 811,636 cases in 2021, according to the WHO data. Notably, malaria-related deaths have shifted towards early childhood, with a significant increase compared to the preceding year. Malaria fatalities in 2020 reached 627,000, up from 558,000 in 2019, but exhibited a slight decrease to 619,000 in 2021 [1, 2].

As one of the malaria-endemic countries, Indonesia faces its own set of challenges in malaria elimination, further exacerbated by the COVID-19 pandemic. Routine malaria reports reveal a roughly 30% increase in malaria cases in Indonesia, rising from 304,607 cases in 2021 to 400,253 cases in 2022. Of these, the highest number of cases occurred in Papua Province, accounting for 356,889 cases (90%) of the national total [3-5].

Despite reports of partial resistance to Artemisinin Combination Therapy (ACT) in Southeast Asia [6-8] and East Africa [9, 10], artemisinin-based therapies have remained the foundation of malaria management for over two decades. The rapid elimination of *Plasmodium* sp. parasites has been correlated with higher immune responses in international research on artemisinin resistance in Southeast Asia, indicating that individuals with lower immunity are more susceptible to producing, retaining, and transmitting mutated parasites. Conversely, studies have shown that robust immunity developed through high malaria exposure results in slowed rates of parasite evolution. Artemisinin resistance must be controlled, which requires clinical research and epidemiologic observation. There is an urgent need to evaluate the efficacy of currently existing drugs and drug combos and develop novel antimalarial medications with novel mechanisms of action [11].

Sea cucumbers, specifically *S.hermanni (Stichopus hermanni)*, are elongated tubular invertebrates of a pale yellow color, belonging to the echinoderm family [12]. They resemble worms and feature small, dark-brown papillae on their lateral and posterior segments. Previous research has revealed the presence of various bioactive substances in *S.hermanni*.

Through HPLC analysis, collagen, glycine, glutamic acid, arginine, and glucosamine hydrochloride were identified in *S.hermanni* extract [13]. In accordance with other studies [14], *S.hermanni* has been shown to contain various fatty acids, including arachidonic acid (AA), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), linoleic acid (LA), and palmitic acid (PA). Notably, the integument of *S.hermanni* contains several sulfated glycosaminoglycans (GAGs), including heparan sulfate and chondroitin sulfate [15, 16]. Another literature review [16, 17] underscores the presence of quinoxaline derivatives in this sea cucumber, which are associated with neurologically beneficial properties. The identified active components in *S.hermanni* suggest its potential as an antimalarial agent. Previous *in vitro* studies have demonstrated substantial antimalarial activity associated with *S.hermanni* [18-20].

The mechanism of action of an active substance as an antimalarial agent often involves the inhibition of key metabolic processes in *P. falciparum*, preventing the escalation of parasitemia levels [21]. Malaria is an infectious disease that causes severe inflammation and oxidative stress [22]. The host's immune response to *Plasmodium* infection relies on the production of reactive oxygen species (ROS) by phagocytes. While increased ROS production aids in parasite elimination, elevated levels of reactive oxidants/free radicals can lead to inflammation and severe damage to host cells and tissues, potentially exacerbating severe diseases. Therefore, malaria treatments that restore and maintain oxidative balance may prove effective in mitigating the lethal consequences of the disease [22].

The development of novel antimalarial drugs seeks to limit parasite growth while minimizing oxidative damage. This study aims to assess the antimalarial potential of *S.hermanni* by evaluating parasitemia levels, growth inhibition rates, and its capacity to alleviate oxidative stress, measured through malondialdehyde (MDA) levels.

Materials and Methods

This study received ethical approval from the Research Ethics Committee of the Faculty of Medicine of Universitas Hang Tuah, Surabaya. The experimental design employed in this study involved the utilization of the 4-day suppression test procedure with a post-test-only control group [23]. Male BALB/C mice were used as the animal model for malaria infection, and they were infected with rodent malaria parasites, specifically *Plasmodium berghei* ANKA (PbA) [24]. These mice were obtained from the Laboratory of AIRC Research Indonesia and met the necessary criteria for pathogen-free mice. *Plasmodium* species suitable for rodent experimentation, *Plasmodium berghei* ANKA (PbA), were obtained from the Parasitology Laboratory of the Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia.

The experimental animals in this study comprised male BALB/C mice (*Mus musculus* L.) that conformed to the following inclusion criteria: (1) Mice with an average body weight ranging from 15 to 25 grams, (2) Mice aged between 7 and 9 weeks, (3) Healthy mice, ascertained during a one-week adaptation period, based on the presence of smooth and sleek fur and normal movement, and (4) Mice with parasitemia levels (+) measured following PbA infection. Criteria for dropout included: (1) Mortality during the study phase and (2) Occurrence of unrelated disorders in the mice models.

Sample size

To determine the sample size for each experimental group, the Federer formula [25, 26] was employed:

$$(k-1)\,(n-1) \geq 15$$

Where,

k: number of groups,

n: sample size/minimum number of samples.

Using this method, it was determined that the minimum number of samples per group would be 5, with a total replication per group of 7 BALB/c mice, incorporating a correction factor of 30%. Consequently, for this investigation, a total of 35

male BALB/c mice were required. All of the mice models were infected with PbA and distributed into five groups as follows:

1. C1: This group received distilled water and a standardized diet.
2. C2: This group received 0.04 mg/g body weight/day of artemisinin.
3. T1: This group received a dose of 15 mg/Kg body weight/day of *S.hermanni* extract and 0.04 mg/g body weight/day of artemisinin for four consecutive days.
4. T2: This group received 17 mg/Kg body weight/day of *S.hermanni* extract and 0.04 mg/g body weight/day of artemisinin for four consecutive days.
5. T3: This group received 20 mg/Kg body weight/day of *S.hermanni* extract and 0.04 mg/g body weight/day of artemisinin for four consecutive days.

S.hermanni extraction methods

S.hermanni specimens, weighing 100 to 250 grams, were sourced from the Sumenep Sea region of Madura, Indonesia. To reduce the water content, the *S.hermanni* specimens were initially washed, segmented into pieces measuring 3 to 10 cm in length, and their wet weight was recorded after sun-drying for a period of 3 to 4 days. Following this drying process, the dried sea cucumbers were further prepared by chopping them into one-centimeter parts. The subsequent extraction procedure was carried out using the maceration method, which entailed mixing 500 mL of pure ethanol solvent with 250 grams of the dried ingredients until complete dissolution was achieved.

The specimen was then stored at ambient humidity for a full day. Afterward, the mixture was subjected to filtration, separating the filtrates from the residue using filter paper. Subsequently, the sample was immersed in 500 mL of ethanol for an additional twenty-four hours.

The filtrates were obtained using a sample-to-solvent ratio of 250 grams per 1000 milliliters of solvent (1:4 w/v).

The final extract was produced by concentrating the filtrate from the solvent through the use of a rotary evaporator [6, 19].

Acclimatisation and Generation of PbA infected mice models

Prior to the initiation of the study, the mice underwent a one-week acclimatization period, during which they were housed in individual cages. Each mouse was placed in a cage under controlled conditions, with an ambient temperature and a 12-hour light-dark cycle, along with a humidity range of 40% to 70% [27].

In this research, the generation of PbA-infected mice models involved transferring malaria parasites from donor animals to healthy mice. The infection procedure commenced using donor mice, from which blood samples were collected once the parasitemia level reached 20.37%. When the donor mice displayed signs of illness, they were promptly removed from the study area and transferred to separate cages for medical attention. The infection process was initiated with the collection of blood specimens from the donor mice when their parasitemia level reached 20.37%. The initial dilution was achieved by mixing 990 microliters of phosphate-buffered saline (PBS) solution with ten microliters of the blood sample. Subsequently, a second dilution was performed by adding 990 microliters of phosphate-buffered saline solution to the previous dilution. Prior to injection, the blood specimens were further diluted with normal saline. Finally, 0.2 mL of a 1×10^6 PbA-infected erythrocyte suspension was administered intraperitoneally to all the mice [28].

Serial observation of parasitemia levels and growth inhibition

Parasitemia levels were determined through microscopic examination of thin blood smears stained with Giemsa. Blood specimens were collected from the mice's tails for this purpose. Giemsa Fluka, in a 1:9 ratio with buffer, was employed to stain the blood smears, with the staining process lasting for twenty minutes [29]. This study assessed parasitemia levels both before treatment (daily until parasitemia levels reached approximately 5%-10%, usually within five days post PbA infection) and during treatment (on days 0, 5, and 8 after PbA infection).

The growth inhibition percentage was calculated solely on days 0, 5, and 8 following PbA infection. The percentage of parasitemia levels and parasite growth were determined using the following equation [6, 19]:

$$\% \text{ Parasitemia} = \frac{\text{Infected erythrocytes}}{1000 \ erythrocytes} \times 100\%$$

$$\% \ Growth \ inhibition = 100\% - \left(\frac{\% \text{ average parasite groth rate}}{\% \text{ average parasite groth rate in negative control group}}\right) \times 100\%$$

Administration of S.hermanni extract and artemisinin

Artemisinin therapy was administered to the C2 or positive control group, with a dosage of 0.04 mg/g body weight (BW) per day administered orally [30] for four days. The reference dosage of *S.hermanni* ethanol extract was determined based on the research conducted by Varidianto *et al.* (2022). The T2 group received a daily dosage of 17 mg/Kg body weight (as a reference for the middle value of the extract dose) [31]. The T1 group received a lower dosage than T2, specifically 15 mg/Kg BW/day, while the T3 group received a higher dosage than T2, which was 20 mg/Kg BW/day. The administration of both the extracts and artemisinin drugs was performed orally using a gastric tube.

Oxidative stress assay

Malondialdehyde (MDA), one of the byproducts of lipid peroxidation metabolism, serves as a biomarker of oxidative stress. This technique allows for the evaluation of oxidative stress levels in blood specimens from malaria patients. MDA analysis was conducted using the ELISA method, following the procedure outlined in the mouse ADP/Acrp30 (Adiponectin) ELISA kit (Catalogue no. EM0001).

The examination of MDA levels was carried out at the conclusion of the research on day four after the administration of treatment.

Statistical analysis of the research variables

The research variables, encompassing MDA levels, parasitemia percentage, and parasite growth inhibition levels, were recorded in an Excel spreadsheet and subsequently analyzed using SPSS with a significance level (p-value) of 0.05. Given that normality (as assessed using the Shapiro-Wilk test) and homogeneity (as assessed through Levene's test) indicated that the data did not conform to a normal distribution and were not uniform, non-parametric statistical analyses, such as Kruskal-Wallis and Mann-Whitney U tests, were employed to evaluate the study parameters.

Results and Discussion

Serial observation of parasitemia levels and growth inhibition

Parasitemia levels were assessed on three occasions: day 0, day 2, and day 4 after commencing treatment. The outcomes of these parasitemia measurements are presented in Table 1. The data regarding the mean parasitemia level on day five, as presented in Table 1, indicate that C2 exhibited the highest mean parasitemia level, while T3 displayed the lowest mean parasitemia level. Conversely, the mean parasitemia level on day eight showed that C1 (the negative control group) had the highest mean parasitemia level, while T2 had the lowest mean parasitemia level. The results of the Kruskal-Wallis test conducted on day five yielded a p-value of 0.701 (p-value > 0.05), indicating no significant variation in the mean parasitemia levels among the study groups on that day. However, the Kruskal-Wallis test conducted on day eight resulted in a p-value of 0.0001 (p-value < 0.05), signifying a significant difference in the mean parasitemia levels among the study groups on that specific day. To further analyze these findings, a statistical assessment using the Mann-Whitney U test was employed to compare the mean parasitemia levels between two specific groups on day eight. The results of this analysis are presented in Table 2.

Table 1: Serial measurement results of Parasitemia levels

Group Research	Parasitemia Level Mean Value (%)		
	Day 0	Day 5	Day 8
C1, Control (-)	0	7.43	24.00
C2, Control (+), artemisinin	0	9.53	3.29
T1, a combination of extract of 15 mg/Kg BW dan artemisinin	0	9.71	1.53
T2, a combination of extract of 17 mg/Kg BW dan artemisinin	0	6.36	1.47
T3, a combination of extract of 20 mg/Kg BW dan artemisinin	0	5.87	3.84
Kruskal Wallis Test		p-value = 0.701	p-value = 0.0001
Interpretation of Analysis Results	There is no significant difference	There is no significant difference	*There is a significant difference*

Table 2: Results of statistical analysis of Parasitemia levels on Day 8

Group	P-value	Interpretation
C1 with C2	0.002	There is a significant difference
C1 with T1	0.002	There is a significant difference
C1 with T2	0.002	There is a significant difference
C1 with T3	0.002	There is a significant difference
C2 with T1	0.063	There is no difference
C2 with T2	0.055	There is no difference
C2 with T3	0.406	There is no difference
T1 with T2	0.608	There is no difference
T1 with T3	0.01	There is a significant difference
T2 with T3	0.007	There is a significant difference

C1 (negative control), C2 (positive control, receiving artemisinin), T1 (receiving a combination of 15 mg/KgBW extract with artemisinin), T2 (receiving a combination of 17 mg/KgBW extract with artemisinin), and T3 (receiving a combination of 20 mg/KgBW extract with artemisinin)

Table 3: Percentage mean values of parasite growth inhibition

Group	Parasitemia Level		% Parasite Growth	% Growth Inhibition
	Day 5	Day 8		
C1	7.43	24.00	16.57	30.95
C2	9.53	3.29	3.29	86.31
T1	9.71	1.53	1.53	93.63
T2	6.36	1.47	1.47	93.87
T3	5.87	3.84	3.84	83.99

C1 (negative control), C2 (positive control, receiving artemisinin), T1 (receiving a combination of 15 mg/KgBW extract with artemisinin), T2 (receiving a combination of 17 mg/KgBW extract with artemisinin), and T3 (receiving a combination of 20 mg/KgBW extract with artemisinin).

The Mann-Whitney U statistical test results, presented in Table 2, indicate that the mean parasitemia level on the eighth day in the C1 (negative control) group was significantly higher than the levels observed in the other four groups (p-values < 0.05). Furthermore, the mean parasitemia on day 8 was significantly higher in T1 than in T3, and T3 exhibited a higher mean parasitemia level than T2.

According to the calculated percentage of growth inhibition in Table 3, C1 exhibited the lowest percentage of parasite growth suppression. In contrast, group T2 demonstrated the highest percentage of parasite growth inhibition,

followed by T1 in the second place, C2 in the third place, and T3 in the fourth place.

C1 (negative control), C2 (positive control, receiving artemisinin), T1 (receiving a combination of 15 mg/KgBW extract with artemisinin), T2 (receiving a combination of 17 mg/KgBW extract with artemisinin), and T3 (receiving a combination of 20 mg/KgBW extract with artemisinin). The MDA test results, depicted in Figure 1, reveal that C1, the group infected with PbA without receiving artemisinin or *S.hermanni* extract, exhibited the highest MDA levels. In contrast, Group T3, which received a combination of *S.hermanni* extract at a dose of 20 mg/KgBW with artemisinin, displayed the lowest MDA level. An analysis using the Kruskal-Wallis test yielded a p-value of 0.0001, indicating a significant difference in MDA levels among the study groups.

According to the Mann-Whitney U statistical analysis results in Table 4, the mean MDA levels in the C1 (control -) group were significantly higher than those in the other four study groups (p-value < 0.05). Furthermore, the mean MDA levels in Group T3 were significantly lower than those in C2, T1, and T2. The primary objective of this research was to identify the antimalarial activity of *S.hermanni* through an *in vivo* experiment using mice models infected with PbA. The evaluation was based on parasitemia levels and the ability to inhibit parasite growth, providing quantifiable measures of *S.hermanni*'s antimalarial potential. At the outset of the study, on day 0, the parasitemia levels were consistent across all groups (Table 1).

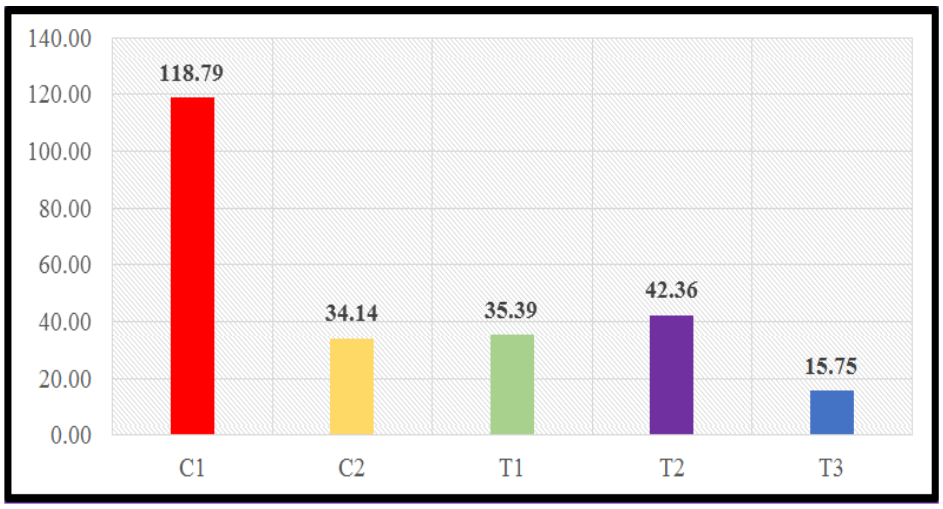


Figure 1: Measurement of mean MDA levels

Table 4: Results of Mann-Whitney U analysis on MDA mean levels

Group	P-value	Interpretation
C1 with C2	0.002	There is a significant difference
C1 with T1	0.002	There is a significant difference
C1 with T2	0.002	There is a significant difference
C1 with T3	0.002	There is a significant difference
C2 with T1	0.749	There is no difference
C2 with T2	0.072	There is no difference
C2 with T3	0.013	There is a significant difference
T1 with T2	0.084	There is no difference
T1 with T3	0.007	There is a significant difference
T2 with T3	0.018	There is a significant difference

C1 (negative control), C2 (positive control, receiving artemisinin), T1 (receiving a combination of 15 mg/KgBW extract with artemisinin), T2 (receiving a combination of 17 mg/KgBW extract with artemisinin), and T3 (receiving a combination of 20 mg/KgBW extract with artemisinin).

Since the mice were infected with PbA on day 0, there was no detectable parasite growth in their blood, explaining the uniform parasitemia levels (parasitemia was 0). As presented in Table 1, by day five following PbA infection, parasitemia levels ranged from 5% to 10%. Statistical analysis (Table 1) revealed that on day 5, there were no significant differences in parasitemia levels between the groups. This indicates that the groups had statistically homogeneous parasitemia levels on day 5, meeting the prerequisites for initiating treatment, which involved the administration of *S.hermanni* extract and artemisinin.

The examination of parasitemia levels on day 8 demonstrated significant differences among the study groups, attributed to the varied treatments they received. The data in Table 2 reveals that the C1 (negative control) group had the highest parasite load, while T2 had the lowest parasite load. Statistical analysis in Table 2 further confirms that the mean parasitemia level on the eighth day in the C1 (control -) group was significantly higher than that in the other four research groups (p-values < 0.05). The high parasitemia level in C1 can be attributed to the absence of both *S.hermanni* extract and artemisinin administration. Statistical analysis on day eight also indicated that the parasitemia level in T1 was higher than that in T3, and T3 exhibited a higher parasitemia level than T2. While T3 had lower parasitemia levels than C2, no substantial distinction was observed. These findings suggest that the combination of *S.hermanni* extract at a dosage of 17 mg/Kg body weight with artemisinin can more effectively reduce parasitemia levels compared to the other treatment groups.

In line with the measurement of parasitemia levels, the assessment of parasite growth inhibition revealed that C1 had the lowest percentage of parasite growth suppression, as indicated in Table 3. T2 exhibited the highest percentage of parasite growth inhibition, with T1 following closely, C2 in the third position, and T3 in the fourth position. The degree of growth inhibition reflects the capacity of an active substance to impede parasite growth, a capability absent in the control group (-), which did not receive *S.hermanni* extract or artemisinin. T2 displayed the most potent capacity to suppress parasite growth compared to all other groups, which could be attributed to the combination of an effective dosage of 17 mg/Kg body weight of *S.hermanni* extract with artemisinin. Both of these components are known to possess anti-malarial properties. The artemisinin used in this study was dihydroartemisinin-piperaquine (DHA-PPQ), a combination of two types of artemisinin derivatives: dihydroartemisinin/DHA as a rapid-acting element and piperaquine/PPQ as a slow-acting element. The widely accepted theory explaining DHA's mechanism of action is that it generates reactive oxygen species (ROS) when activated by heme, which can damage parasite cells through oxidation. *In vitro* studies suggest that PPQ may reduce hemoglobin's metabolic rate, leading to parasite starvation and death. Combining DHA with PPQ aims to eliminate any remaining parasite material after DHA's rapid action. Compared to C2 (the positive control), T3 exhibited decreased parasitemia levels according to descriptive analysis, although the difference was not statistically significant. This might be attributed to the relatively low extract dosage, which resulted in lower levels of bioactive compounds that were less effective in inhibiting parasite growth.

Alternatively, the extract may not have been administered for a sufficient duration to exert a therapeutic effect. Nonetheless, the current research findings corroborate previous *in vitro* studies that demonstrated *S.hermanni* extract's ability to inhibit *P. falciparum* proliferation [32].

Despite the precise way of action of *S.hermanni* not being thoroughly investigated, its potential mode of action can be inferred from several previous studies. A previous study revealed that phytochemical assay of *S.hermanni* extracts had been attributed to various chemical substances, including saponins, steroids, flavonoids, and alkaloids [19, 33]. Flavonoids have been shown to inhibit Falcipain-2, a cysteine protease enzyme of *P. falciparum*, leading to a reduction in hemoglobin breakdown [34]. Saponins, on the other hand, possess antimalarial properties by causing erythrocytes to lyse through the destruction of their cell membranes

[35, 36]. Alkaloids have demonstrated the ability to decrease parasitemia levels by inhibiting the synthesis of parasite DNA and RNA [37]. While steroids and their derivatives have also been found to have antimalarial effects in various studies, the specific mechanism remains unclear. The addition of lipophilic steroid carriers has been shown to enhance antiparasitic efficacy, facilitating intracellular transport pathways and cellular uptake. A steroid derivative known as estratriene has been shown to increase cellular oxidation, acting as an antiplasmodial therapy [38]. In line with the outcomes of previous *in silico* research, one of the potential pathways by which active substances in *S.hermanni* act is by impairing the activity of the *P. falciparum* hexose transporter (PfHT1) enzyme, responsible for glucose transport in the absorption process [39].

Malaria is associated with two related conditions: inflammation and oxidative stress. Upon *Plasmodium* infection, the host initiates the production of ROS (Reactive Oxygen Species), which are radical oxidant molecules. To counteract the increased ROS levels, the host also deploys antioxidant enzymes that catalyze oxidative processes [40]. Increased ROS production is beneficial for parasite elimination, but excessive ROS can lead to extensive damage to the host's cells and tissues, potentially causing severe systemic inflammation. The breakdown of hemoglobin in parasite-infected red blood cells releases free heme, particularly at the end of the replication cycle. This place an additional burden of oxidative stress on the host and contributes to the development of severe and life-threatening malaria complications. In addition, the heightened inflammatory response associated with malaria infection exacerbates oxidative stress [22].

Malondialdehyde (MDA), one of the numerous metabolic by-products of lipid peroxidation, serves as a biomarker for the degree of lipid peroxidation in the body. The MDA generation increases with elevated levels of free radicals. MDA levels provide insight into the level of oxidative stress and antioxidant status in an individual's body. By measuring MDA concentration during the erythrocytic stage, the level of oxidative stress in plasma can be determined [41]. New antimalarial drugs should ideally possess a dual effect: suppressing parasite growth to a minimum level while also regulating the oxidative stress load triggered by the infection process and drug administration. Such drugs can prevent severe complications of malaria by effectively controlling parasite load and reducing oxidative stress [40, 42].

As seen in Figure 1, the C1 group, which was infected with PbA but did not receive artemisinin or *S.hermanni* extract, exhibited the highest MDA levels. In contrast, Group T3, which received a combination of *S.hermanni* extract at a dose of 20 mg/Kg BB with artemisinin, had the lowest MDA levels. These MDA levels exhibited significant variation between groups. Notably, the mean MDA level in the C1/control (-) group was significantly higher than in the other four research groups (p-value < 0.05). This observation is attributed to C1 being a negative control group that did not receive any treatment, resulting in the highest oxidative stress levels characterized by an increase in MDA. Conversely, the positive control group (C2) receiving artemisinin and the groups receiving a combination of artemisinin with *S.hermanni* extract (T1-T3) exhibited lower MDA levels compared to C1. Among these groups, T3 showed the least significant oxidative stress, as evidenced by its substantially lower mean MDA level compared to C2, T1, and T2. These findings demonstrate that the combination of *S.hermanni* extracts at a daily dosage of 20 mg/Kg body weight with artemisinin can greatly decrease oxidative stress. The findings of this study reinforce the notion that the ethanol extract of *S.hermanni* possesses antioxidant capabilities. This aligns with the results of a study by Mogharabi *et al.* (2017), which reported that the methanol extract of *S.hermanni* demonstrated antioxidant activity with an IC_{50} (half-maximal inhibitory concentration) of 369 ± 1.5 µg/mL [43]. Another study also noted weak to moderate antioxidant activity of *S.hermanni* [18].

This study have several limitations. First, the mice model does not fully replicate the complexities of human malaria infection. Translating the findings to human clinical trials may require further investigation to confirm

efficacy and safety in humans. Second, this study employed fixed dosage levels of *S.hermanni* extract and artemisinin for all treated groups. Investigating a broader range of dosage levels and their dose-response relationships could provide valuable information on the optimal dosing regimen for maximal efficacy.

Conclusion

In conclusion, the findings of this study based on the descriptive and statistical analysis suggest that the combination of *S.hermanni* extract with artemisinin has the potential to inhibit parasite growth and exhibit antioxidant properties. Notably, the combination of *S.hermanni* extract at a daily dosage of 17 mg/Kg body weight with artemisinin demonstrated the lowest parasitemia levels and the highest parasite growth inhibition, although statistically comparable to the positive control group (C2) and T1 (receiving *S.hermanni* extract at 15 mg/Kg body weight combined with artemisinin). Furthermore, the T3 group, which received *S.hermanni* extract at a dosage of 20 mg/Kg body weight combined with artemisinin, exhibited strong antioxidant activity which significantly reduced oxidative stress. However, further research is necessary to delve into the precise mechanisms of action of *S.hermanni* and its antimalarial and antioxidant properties, as well as to determine the optimal and effective dosage of *S.hermanni extract*.

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Authors' Contributions

All authors contributed to data analysis, drafting, and revising of the paper and agreed to be responsible for all the aspects of this work.

ORCID

Prawesty Diah Utami
https://orcid.org/0000-0002-8527-4953
Wachjudi Kurnia
https://orcid.org/0009-0000-5431-3991
Irma Andriani Pasaribu
https://orcid.org/0009-0002-7596-1538

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