JPPIPA 11(7) (2025)

Jurnal Penelitian Pendidikan IPA

http://jppipa.unram.ac.id/index.php/jppipa/index

Infusion of *Strychnos lucida* as an Alternative Therapy for Malaria Treatment: An In Vivo Study in *Plasmodium berghei*-Infected Mice (*Mus musculus*)

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Received: April 17, 2025
Revised: May 6, 2025
Accepted: July 25, 2025
Published: July 31, 2025

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DOI: 10.29303/jppipa.v11i7.11270

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Abstract: Malaria drug resistance results in severe symptoms and treatment failure. In an effort to develop new medications, screening of several plants with potential antimalarial properties has been conducted. *Strychnos lucida* is known to contain various active compounds such as alkaloids and tannins, which may exhibit antimalarial properties. This study aims to determine the potential of *Strychnos lucida* infusion as an alternative therapy for malaria treatment. This research was experimental, employing a completely randomized design. A total of 20 mice were divided into 5 groups: a negative control group (mice infected with *Plasmodium berghei* without treatment), a positive control group (mice infected with *Plasmodium berghei* and treated with quinine tablet malaria drug), and three experimental groups of mice infected with *Plasmodium berghei* and treated with *Strychnos lucida* infusions at doses of 1.5g/200 ml, 3g/200 ml, and 6g/200 ml, administered at a volume of 0.52 ml per mouse per day. The treatments were given for 4 consecutive days with observations continuing until the 6th day. The results were analyzed using two-way ANOVA. The findings indicate that the *Strychnos lucida* infusion inhibited the growth of *Plasmodium berghei* in vivo, suggesting antimalarial activity of the plant in the biological system of infected mice.

Keywords: Malaria; *Plasmodium berghei*; *Strychnos lucida*

Introduction

Malaria is a potentially fatal disease transmitted through the bite of a female Anopheles mosquito and affects more than 106 countries worldwide. The disease has become one of the most significant global health concerns, causing approximately million deaths annually, particularly in sub-Saharan regions and across the African continent (Kolawole et al., 2023; Alum et al., 2024). According to Stafford (2024), malaria is caused by an infection of protozoa from the *Plasmodium* genus, which invade human red blood cells and are transmitted through the bite of *Anopheles* mosquitoes. *Plasmodium* parasites reproduce within red blood cells and have a complex life cycle involving both sexual and asexual phases, as well as alternating hosts (Sinden & Gilles, 2017; Stafford, 2024). In humans, four *Plasmodium* species are known to cause malaria: *Plasmodium vivax*, *Plasmodium malaria*, *Plasmodium falciparum*, and *Plasmodium ovale* (Larson, 2019; Sato, 2021). These protozoan parasites are unicellular organisms that can cause anemia by destroying a significant number of red blood cells (Anwar, 2024).

According to the World Health Organization (WHO) report in 2021, the number of malaria cases globally reached 247 million, a slight increase from 245 million cases in 2020. Malaria-related deaths also remained high, with approximately 619,000 deaths reported in 2021, compared to 625,000 in 2020 (WHO, 2020). In Indonesia, malaria cases reported in 2021 totaled 94,610, a substantial decrease from 226,364 cases in 2020 (Anwar & Liberty, 2024). Based on recent

How to Cite:
Mainassy, M. C., & Ukratalo, A. M. (2025). Infusion of Strychnos lucida as an Alternative Therapy for Malaria Treatment: An In Vivo Study in Plasmodium berghei-Infected Mice (Mus musculus). *Jurnal Penelitian Pendidikan IPA*, *11*(7), 1027–1038. https://doi.org/10.29303/jppipa.v11i7.11270

trends, malaria incidence in Indonesia declined in 2018 but rose again in 2019, peaking at 250,628 cases before decreasing steadily through 2020 and 2021. The eastern regions of Indonesia, particularly Papua Province, continue to report the highest case numbers, with 86,022 cases accounting for approximately 90.9% of the national total in 2021 (Tutukansa & Tuffahati, 2022). This is followed by East Nusa Tenggara, with 2,393 cases (2.5%), and West Papua, with 1,841 cases (1.94%) (Hatta & Tanlain, 2022). Hatta & Tanlain (2022) also noted that the high incidence of malaria in Indonesia is strongly associated with several factors, including environmental changes that expand mosquito breeding sites, high population mobility, and climate change resulting in prolonged rainy seasons.

Malaria elimination is defined as efforts undertaken in a specific region to interrupt local transmission, along with preventive strategies to avoid reintroduction (Shretta et al., 2017; WHO, 2022; Lattu et al., 2025). A district, province, or island is considered malaria-free if no new cases are detected for three consecutive years and an effective surveillance system is in place (Haryanty et al., 2025). Poor environmental conditions, such as substandard housing and inadequate sanitation, contribute to increased malaria risk by creating favorable habitats for *Anopheles* mosquitoes to breed and rest (Tatontos et al., 2024). Community behavior also plays a critical role in determining the level of contact between humans and vector mosquitoes, thereby influencing the risk of transmission. The presence of dense vegetation and open water channels near residential areas further elevates the environmental risk of malaria outbreaks Rahayu et al., 2023).

Numerous strategies have been implemented to control malaria, including vector control using insecticides and the eradication of the parasite through synthetic or natural compounds (Tampubolon et al., 2023). However, the use of chemical agents is often associated with side effects and the emergence of drug resistance (Shiff, 2002; Hemingway, 2014; Choi, 2019). The increasing prevalence of resistance to antimalarial drugs has prompted the search for alternative therapies (Rasmussen et al., 2022), including the development of treatments based on traditional medicinal practices.

Strychnos lucida R. Br., commonly known as "kayu ular," is a plant species belonging to the Loganiaceae family and is recognized as an endemic plant of the West Nusa Tenggara (NTB) region in Indonesia (Setyayudi et al., 2019). Although originally native to NTB, the plant has also been found in various other areas, including Rote, Kalimantan, Timor, Bali, Pasuruan, Banyuwangi, and within the Meru Betiri National Park area. It can thrive in a wide range of altitudes, from lowlands to mountainous regions, at elevations ranging from 1 to 1,500 meters above sea level. In addition to "kayu ular," the plant is known by various local names depending on the region, such as *bidara laut*, *bidara pahit*, and *bidara putih* in Sumatra; *dara laut*, *dara putih*, and *bidara gunung* in Java; and *lapai* and *bidara mapai* in Sulawesi.

Traditionally, local communities have utilized *Strychnos lucida as an* herbal remedy for a variety of ailments, with the wood of the plant being the most commonly used part. Based on community experiences, this plant has been increasingly traded outside of NTB as a raw material for herbal medicine since the early 2000s. It is widely believed to be effective in treating diseases such as diabetes, hypertension, malaria, cancer, and others.

Phytochemical studies of *Strychnos lucida* have revealed the presence of several bioactive compounds, including alkaloids (such as brucine and strychnine), tannins in concentrations below 1%, as well as steroid or triterpenoid groups like saponins (Gusmailina & Komarayati, 2015). Alkaloids are well known for their antimalarial potential, as exemplified by quinine, an alkaloid derived from the *Cinchona* plant. Several classes of alkaloids have been identified with antimalarial activity, including terpenoids, bisindole, indole, quinoline, and isoquinoline types (Osorio et al., 2008; Uzor, 2020). Furthermore, tannins are also known to inhibit the invasion of red blood cells by *Plasmodium* species during the asexual blood stage of infection (Pradniwa, 2024). Their mechanism of action involves inhibiting protease enzymes that are critical to the life cycle of the parasite (Min & Hart, 2003; Lutgen, 2018).

Several previous studies have demonstrated that plant extracts containing secondary metabolites can inhibit the growth of Plasmodium. For instance, an extract of *Azadirachta indica* has been shown to inhibit *P. falciparum* growth due to its content of diterpenoids, triterpenoids, proteins, carbohydrates, polyphenols, flavonoids, dihydrochalcones, coumarins, and tannins (Biswas & Caliendo, 2002). Additionally, *Sampare* leaves, which contain alkaloids, quinones, flavonoids, saponins, and tannins, exhibited an inhibitory effect against *Plasmodium falciparum* with an IC_{50} value of 0.125 µg/mL (Umar et al., 2023).

Strychnos lucida shows excellent potential as a herbal antimalarial agent due to its bioactive compounds, which are suspected to effectively inhibit the growth of malaria-causing parasites. However, its utilization remains very limited because in-depth studies on its efficacy and safety have not been conducted extensively. Consequently, the lack of available scientific data has hindered the optimal

exploitation of this plant's therapeutic potential in developing plant-based antimalarial drugs.

Method

Type of Research

This study was conducted as a laboratory experimental research.

Research Design

A completely randomized design (CRD) comprised five treatment groups with three replications each. The experimental groups were divided as follows: a negative control group (mice infected with *Plasmodium berghei* without treatment), a positive control group (mice infected with *Plasmodium berghei* and treated with quinine tablets), and three treatment groups of mice infected with *Plasmodium berghei* and administered aqueous infusions of *Strychnos lucida* at doses of 1.5 g/200 ml, 3 g/200 ml, and 6 g/200 ml, respectively.

Tools and Materials

The equipment used in this study included a digital scale, hot plate, microscope, measuring cylinder, pipette, syringe, oral gavage (sonde), animal cages, glass slides, digital camera, scissors, forceps, and a coconut grater (for pulverizing materials).

The materials used included *Plasmodium berghei*, 15 male mice (*Mus musculus*), methanol, 70% ethanol, Giemsa solution, formalin, *Strychnos lucida* powder, quinine tablets, distilled water (aquadest), aluminum foil, Whatman filter paper No. 0.2, tissue paper, cotton, and standard food and water for laboratory mice.

Experimental Procedure

Acclimatization of Experimental Animals

For one week, acclimatization was conducted at the Zoology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Pattimura University, Ambon. Test animals were excluded from the study if they became ill, died, or experienced a body weight loss exceeding 10% (Ukratalo et al., 2023b; Kaihena et al., 2024).

Preparation of Test Material

The infusion of *Strychnos lucida* was prepared through several steps. First, the stem of *Strychnos lucida* was ground into powder. Each dosage—1.5 grams, 3 grams, and 6 grams—was accurately weighed and transferred into separate measuring cylinders. 600 ml of water was brought to a boil and divided into three portions of 200 ml. Each portion was poured over the corresponding amount of *Strychnos lucida* powder. The mixtures were stirred thoroughly and allowed to cool. After cooling, each solution was filtered using Whatman filter paper No. 0.2 to obtain a pure infusion. The final product was measured at 0.52 ml per dose for use in the bioactivity assay.

Preparation of Donor Mice Infected with Plasmodium berghei

The donor mice were infected with frozen *P. berghei* stock via intraperitoneal (IP) injection of 200 µL of *P. berghei* suspension. Parasitemia was observed daily until it exceeded 20%. Blood was then collected from the heart of the infected mouse for further processing. The blood was diluted with Alceiver to achieve a parasitemia of 5%. Each test mouse was subsequently injected intraperitoneally with 0.1 mL of this infected blood solution (Kakisina & Ukratalo, 2011; Ukratalo, 2022; Kaihena et al., 2023).

Infection of Experimental Mice with Plasmodium berghei

Test and control mice were infected with *Plasmodium berghei* by intraperitoneal injection of 200 µL of the prepared suspension. Parasitemia was monitored by preparing thin blood smears. Blood was collected from the tail tip of the mice after disinfection, and a smear was made at a 30-45° angle on a glass slide using another glass slide. After drying, the preparation was fixed with absolute methanol for 3 minutes, followed by staining with 10% Giemsa solution for 30 minutes. The smear was then rinsed with running water, dried, and observed under a microscope with 1000x magnification after applying immersion oil. The malaria parasites were identified in infected erythrocytes, allowing parasitemia to be quantified by counting the number of infected erythrocytes in 1000 total erythrocytes (Kakisina & Ukratalo, 2011).

Evaluation of Antimalarial Potential

Once parasitemia reached 1% in the test mice, the antimalarial activity of *Strychnos lucida* infusion was evaluated. The injection was administered for four consecutive days, and parasitemia was monitored until day 6 to assess the post-treatment parasitemia profile. Blood was taken from the tail of each mouse every day after treatment (D0 to D6) (Kakisina & Ukratalo, 2011; Ukratalo et al., 2023a; Maatita et al., 2024).

Calculation of Parasitemia Percentage and Parasite Growth Inhibition Percentage

Parasitemia was assessed from the thin blood smear by counting the number of infected erythrocytes in a total of 5000 erythrocytes (% parasitemia). The growth inhibition percentage was calculated based on the following formulas.

Parasitemia percentage (%P):

$$\% P = \frac{\text{Number of Infected Erythrocytes}}{\text{Total erythrocytes}} \times 100\% \quad (1)$$

Average Parasite Growth Percentage (%APG):

$$\% APG = \frac{\text{Number of Infected Erythrocytes on Day X}}{\text{Number of Infected Erythocytes on D}} \times 100\% \quad (2)$$

Inhibition of Parasite Growth (Percentage):

$$\% \text{ Inhibition} = 100\% - \frac{Xe}{Xk} \times 100\% \quad (3)$$

Where:

Xe : average parasite growth percentage of the experimental group.

Xk : average parasite growth percentage of the negative control group.

Data Analysis

In this in vivo study, Analysis of Variance (ANOVA) was used. If the results of the ANOVA test indicate a significant effect, a Least Significant Difference (LSD) test will be conducted to determine the differences between each treatment. Additionally, probit analysis will be used to determine the Effective Dose (ED_{50}).

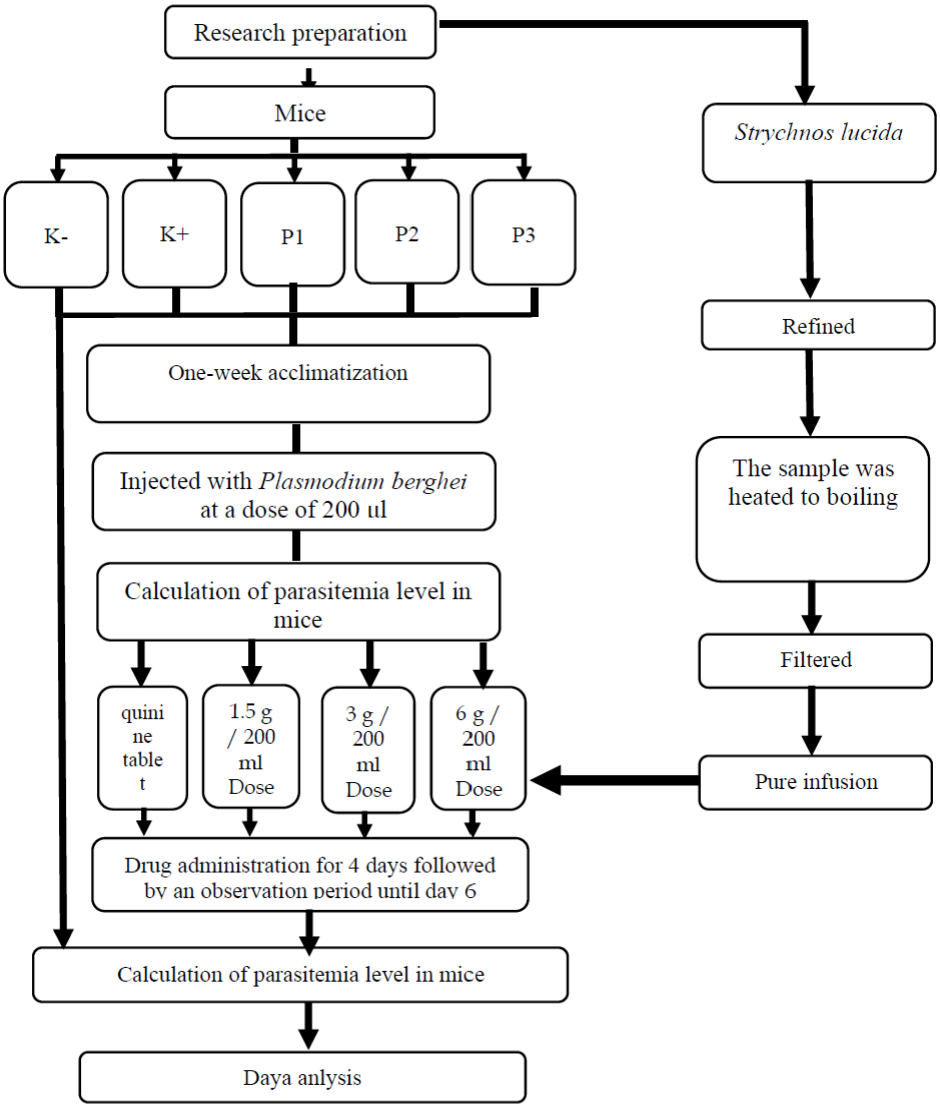

Figure 1. Research workflow diagram

Result and Discussion

Parasitemia Percentage in Mice Infected with Plasmodium berghei

The average parasitemia percentages in the experimental animals from Day 0 (D0) to Day 4 (D4) during the administration of the extract and from Day 5 (D5) to Day 6 (D6) after the administration of the extract were stopped are shown in Table 1.

In Table 1, it can be observed that the average percentage of parasitemia in the experimental animals treated with *Strychnos lucida* infusion from day 0 (D0) to day 4 (D4) showed variations depending on the administered dose. On day 0, before the administration of the extract, the negative control group (Control (-)) had an average parasitemia value of 1.07%, while the positive control group (Control (+)) showed 0.90%. The groups receiving doses of 1.5 g/200 ml, 3 g/200 ml, and 6 g/200 ml had parasitemia values of 0.97, 1.03, and 1.03%, respectively, with an overall average of 1.00 ± 0.16%. Following the administration of the treatment, on day 1, all groups exhibited an increase in parasitemia. The groups treated with doses of 1.5 g/200 ml, 3 g/200 ml, and 6 g/200 ml showed average parasitemia values of 1.50, 1.63, and 1.42%, respectively, with a mean ± SD of 1.47 ± 0.18%. On days 2 through 4, the groups receiving *Strychnos lucida* infusion showed a more significant increase in parasitemia, with the 6 g/200 ml dose group on day 4 reaching an average of 3.45%, although still lower compared to the positive control group, which reached 9.60%. On days 5 and 6, after the treatment was discontinued, parasitemia continued to increase in all groups treated with *Strychnos lucida* infusion. On day 5, parasitemia in the positive control group reached 11.33%, while the group of mice treated with the 1.5 g/200 ml dose had parasitemia of 9.07%. On day 6, parasitemia in the positive control group increased to 14.40%, whereas the 1.5 g/200 ml dose group reached 10.66%. The results shown in Table 1 are further illustrated in Figure 2.

Table 1. Average percentage of Parasitemia in *Strychnos lucida* infusion from day 0 to day 6

Day	Control (-)	Control (+)	1.5 g/200 ml Dose	3 g/200 ml Dose	6 g/200 ml Dose	Average ± SD
D0	1.07	0.90	0.97	1.03	1.03	1.00 ± 0.16^{a}
D1	1.50	1.31	1.50	1.63	1.42	1.47 ± 0.18^{b}
D2	5.77	1.48	3.99	3.72	2.34	3.45 ± 1.67^{c}
D3	8.13	1.66	5.72	4.61	3.02	4.63 ± 2.41^{d}
D4	9.60	1.75	7.69	5.18	3.45	5.54 ± 3.03^{e}
D5	11.33	1.83	9.07	6.08	4.14	6.49 ± 3.59^{f}
D6	14.40	1.92	10.66	6.67	4.63	7.65 ± 4.66^{g}
Average ± SD	7.40 ± 4.75^{h}	1.55 ± 0.39^{i}	5.66 ± 3.72^{j}	4.13 ± 2.06^{k}	2.86 ± 1.30^{l}	

Note: Superscripts with the same letter are not significantly different (P > 0.05).

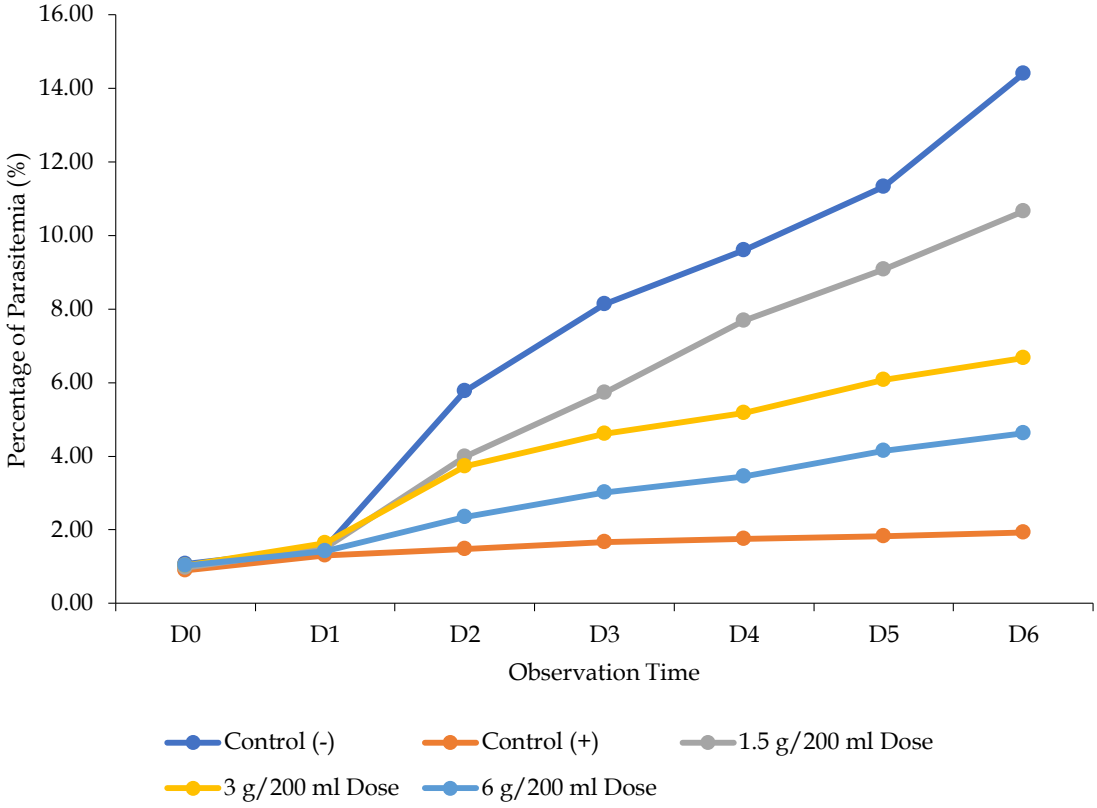

Figure 2. Graph of parasitemia percentage in mice throughout the study

Table 2. Results of two-way analysis of variance (ANOVA)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1251.939a	34	36.822	61.898	.000
Intercept	1959.466	1	1959.466	3293.910	.000
Treatment	443.642	4	110.911	186.443	.000
Time	559.140	6	93.190	156.655	.000
Treatment * Observation Time	249.157	24	10.382	17.452	.000
Error	41.641	70	0.595		
Total	3253.046	105			
Corrected Total	1293.581	104			

a. R Squared = .968 (Adjusted R Squared = .952)

Based on the results of the Two-Way Analysis of Variance (ANOVA) (Table 3), it can be observed that the calculated F value is greater than the critical F value, which indicates that the administration of *Strychnos lucida* infusion significantly affects the percentage of parasitemia in *Mus musculus* infected with *Plasmodium berghei*. The results of the Least Significant Difference (LSD) test also reveal significant differences between the negative control group, the positive control group, and the groups of infected mice that were administered *Strychnos lucida* infusion at doses of 1.5 g/200 ml, 3 g/200 ml, and 6 g/200 ml with a volume of 0.52 ml/mouse/day.

The results of the two-way Analysis of Variance (ANOVA) conducted on the observation time (Table 3) showed that the calculated F-value exceeded the critical F-value, indicating that observation time had a significant effect on the percentage of parasitemia in mice infected with *Plasmodium berghei*. This finding suggests that variations in observation time contributed to changes in the observed parasitemia levels. Moreover, the results of the Least Significant Difference (LSD) test revealed that significant differences existed among the negative control group, the positive control group, the group of mice infected with *Plasmodium berghei*, and the groups of mice treated with *Strychnos lucida* infusion at doses of 1.5 g/200 ml, 3 g/200 ml, and 6 g/200 ml, with an administration volume of 0.52 ml/mouse/day.

Parasitemia level indicates the number of *Plasmodium berghei* parasites present in the mouse's blood. The higher the parasitemia level, the worse the condition of the infected mouse. Based on the study results (Table 2), it can be observed that the *Plasmodium berghei* infection in mice increased parasitemia percentages across all treatment groups. The minimum period from the entry of parasites into the body to the appearance of parasites in the erythrocytes is called the prepatent period (White, 2017; Sinden & Gilles, 2017). The virulence of the parasite influences the prepatent period and parasitemia percentages. A prepatent period of up to 2 days is considered significant in illustrating the virulence of the parasite (Day, 2001; Izhar & Ben-Ami, 2015).

The *P. berghei* erythrocytic stage parasites reproduce inside erythrocytes, so the increase in parasite growth affects the state of the erythrocytes, resulting in an increase in parasitemia, which is typically followed by a decrease in erythrocyte levels in the host (Thakre et al., 2018; Caldelari et al., 2019; Djokic et al., 2021). The immune system responds to parasites entering the blood circulation in a nonspecific manner and then specifically. The nonspecific immune response is the first effector in combating the infection. Leukocytes play a crucial role in the immune system, with lymphocytes and monocytes being the primary leukocyte types involved in the immune response (Darlina et al., 2012). Monocytes are phagocytic cells that destroy antigens by engulfing them, while lymphocytes play a significant role in the body's immune system (Akhand & Ahsan, 2023). After being released from the bone marrow, lymphocytes undergo further differentiation into immunocompetent cells (Monroe & Dorshkind, 2007). Weakening the parasite can trigger an immune response that inhibits the growth and development of *Plasmodium* in erythrocytes, leading to a partial reduction in parasitemia (Belachew, 2018; Antonelli et al., 2020).

In the positive control group (mice infected with *Plasmodium berghei* and treated with quinine tablets), the parasitemia percentage was lower compared to the negative control or mice infected with *Plasmodium berghei* and treated with *Strychnos lucida* decoction. Quinine tablets contain 200 mg of the active compound chloroquine. Chloroquine is an antimalarial drug from the 4-aminoquinoline group, the primary and most widely used antimalarial, which is cost-effective and proven safe (Deshpande & Kuppast, 2016; Kucharski et al., 2022). The mechanism of action of chloroquine, like other quinoline-based drugs, involves inhibiting the activity of heme polymerase, leading to the accumulation of free heme in red blood cells. This accumulation becomes toxic to the parasite, as the *Plasmodium* parasite inside red blood cells alters hemoglobin and creates essential amino acids for

protein formation and energy needs (Chaijaroenkul, 2005; Shone, 2007).

In this study, the parasitemia percentage in the group of mice infected with *Plasmodium berghei* and treated with *Strychnos lucida* decoction at doses of 1.5 g/200 ml, 3 g/200 ml, and 6 g/200 ml still showed an increase. Still, it remained lower compared to the parasitemia percentage in the negative control group. The relatively low parasitemia percentage after treatment with *Strychnos lucida* decoction can be attributed to the secondary metabolite compounds found in *Strychnos lucida*, such as alkaloids and tannins.

Most of the mechanisms of action of alkaloid compounds in combating *Plasmodium* parasites are based on their ability to inhibit the growth and development of the parasites. Alkaloid compounds bind directly to the parasite's DNA by forming specific bonds that disrupt nucleic acid replication and transcription processes. This interaction causes damage to the genetic structure of *Plasmodium*, thereby inhibiting cell division and protein synthesis essential for parasite survival. Additionally, some alkaloids can interfere with enzyme functions and parasite metabolic processes, which enhances the antiplasmodial effects and leads to parasite death or reduced viability. Beyond their physical effects on DNA, alkaloid binding can also trigger oxidative stress responses within the parasite, diminishing its survival capacity. These mechanisms position alkaloid compounds as prime candidates for antimalarial drug development due to their effectiveness in directly disrupting the *Plasmodium* life cycle at the molecular level (Sriwilaijareon et al., 2002).

Tannins significantly inhibit Plasmodium spp.'s infection of red blood cells, particularly during the asexual stage within the bloodstream. At this stage, *Plasmodium* utilizes protease enzymes to degrade proteins inside the host cells, which is crucial for obtaining nutrients and continuing its life cycle. Tannins act by inhibiting the activity of these protease enzymes, thereby disrupting the protein degradation process essential for the parasite. As a result, the parasite cannot reproduce optimally within the red blood cells, allowing the infection to be controlled or minimized. The mechanism of protease enzyme inhibition by tannins demonstrates the great potential of this natural compound as an antimalarial agent. In addition to inhibiting parasite growth, tannins also affect the integrity of red blood cell membranes, further enhancing their protective effect against *Plasmodium* infection (George et al., 2016). Since protease enzymes play a vital role in the parasite's metabolism and life cycle, tannins' ability to target these enzymes opens opportunities for developing new, effective therapies, potentially offering a better safety profile than synthetic drugs.

Generally, antimalarial drugs work by inhibiting the process of heme or Ferriprotoporphyrin IX (Fe (III) PPIX) degradation, disrupting the metabolism of folate in *Plasmodium*, and interfering with other metabolic pathways, such as inhibiting fatty acid metabolism and isoprenoid biosynthesis. 2-C-methyl-d-erythritol-4-phosphate (MEP) is one of the products synthesized by *Plasmodium* during its replication process. Isoprenoid synthesis is essential for the development of *Plasmodium* in the intraerythrocytic phase and the sexual phase.

The 2-C-methyl-d-erythritol-4-phosphate (MEP) synthesis pathway to produce DMAPP (Dimethylallyl Pyrophosphate Isomer) and IPP (Isopentenyl Pyrophosphate) begins when pyruvic acid, along with the precursor GAP (glyceraldehyde 3-phosphate), is converted into DOXP with the help of the enzyme 1-deoxy-d-xylulose-5-phosphate synthase (DXS) and then converted into MEP by the enzyme DXR (1-deoxy-d-xylulose-5-phosphate reductoisomerase). Subsequently, with the assistance of the enzymes CMK, MCS, geranylhydroxybutenyl pyrophosphate synthase (GcPE), and hydromethylbutenyl pyrophosphate reductase (LytB), the MEP compound is converted into DMAPP and IPP. DMAPP (Dimethylallyl Pyrophosphate Isomer) and IPP (Isopentenyl Pyrophosphate) are examples of end products. During isoprenoid synthesis, a polymerization of isoprene chains is formed by the prenyltransferase enzyme, which is produced by the condensation of GGPPS (Geranylgeranyl Pyrophosphate Synthase) and FPPS (Farnesyl Pyrophosphate Synthase) into prenyltransferases. FPPS then catalyzes the condensation of IPP with DMAPP and Geranyl Pyrophosphate (GPP) to form a 15-carbon isoprenoid and Farnesyl Pyrophosphate (FPP), which catalyze the early stages of dolichol biosynthesis, ubiquinone, prenylation, and carotenoid biosynthesis as the final products of isoprenoid biosynthesis by *Plasmodium falciparum* (Veronica & Chrismayanti, 2020).

Inhibition Percentage of Parasitemia Growth

Next, based on the average parasite growth from day 0 (D0) to day 4 (D4), the percentage inhibition of *Strychnos lucida* decoction against malaria parasite growth was calculated. The results of this calculation can be seen in Table 3.

Table 3. Percentage inhibition of *Strychnos lucida* decoction against *P. berghei* growth

Treatment	Average Growth %	Inhibition %
Control (-)	2.22	-
Control (+)	0.17	92.34
1.5 g / 200 ml	1.62	27.33
3 g / 200 ml	0.94	57.33
6 g / 200 ml	0.60	73.05

The results in Table 3 show that the average percentage growth of *Plasmodium berghei* in the negative control group was 2.22%, in the positive control group was 0.17%, and in the groups of infected mice treated with *Strychnos lucida* decoction at doses of 1.5 g/200 ml, 3 g/200 ml, and 6 g/200 ml were 1.62%, 0.94%, and 0.60%, respectively. Additionally, the percentage inhibition of *P. berghei* growth in the positive control group was 92.34%. In contrast, the inhibition rates for the *Strychnos lucida* decoction doses of 1.5 g/200 ml, 3 g/200 ml, and 6 g/200 ml were 27.33, 57.33, and 73.05%, respectively (Figure 3).

The percentage inhibition of parasitemia growth represents the ability to inhibit the growth of parasites (Ziegler et al., 2002; Wezena et al., 2017). The higher the percentage of parasitemia growth inhibition, the better the malaria infection condition, as the growth of the parasite is inhibited. In this study, the negative control group had no percentage of parasitemia growth inhibition since there was no inhibition of parasite growth in this group.

In the positive control group, the percentage of *Plasmodium berghei* growth inhibition was 92.34%. In the group treated with *Strychnos lucida* decoction, the inhibition of parasite growth increased with the dosage. The 1.5 g/200 ml dose showed 27.33% inhibition, indicating that at this lower dose, the decoction has a limited ability to inhibit parasite growth. However, when the dose was increased to 3 g/200 ml, the inhibition of *Plasmodium berghei* growth increased to 57.33%, suggesting that the inhibitory effect became more significant at the medium dose. Further increasing the dose to 6 g/200 ml resulted in 73.05% inhibition, demonstrating the more substantial potential of *Strychnos lucida* decoction to inhibit parasites at higher doses.

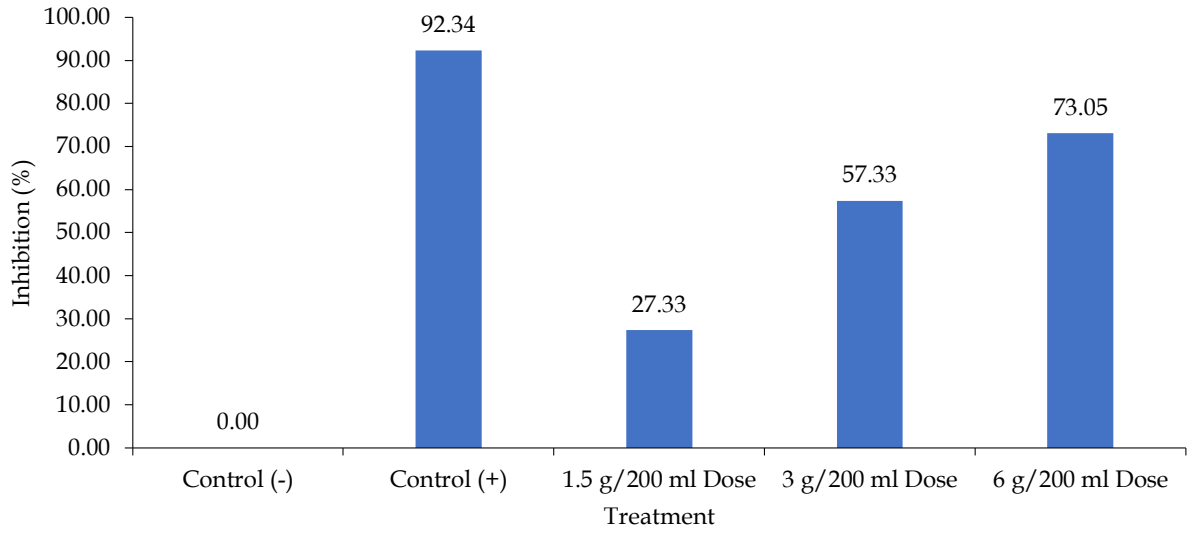

Figure 3. Inhibition percentage of parasitemia growth

Determination of ED50 for Strychnos lucida Decoction

The probit value was used to analyze the estimation of an effective dose by determining the concentration that causes parasite death or inhibits parasite growth. This method allows for the determination of the dose required to produce a specific effect, such as inhibiting malaria parasite growth. In this case, a probit analysis was performed to calculate the Effective Dose 50 (ED_{50}), the dose that inhibits 50% of parasite growth. Based on the results of analysis using the probit method, an ED_{50} (Effective Dose 50) value of 2.22 was obtained. This value indicates that at this dose, *Strychnos lucida* decoction is capable of inhibiting the growth of *Plasmodium berghei* by 50%. In other words, a dose of 2.22 g/200 ml represents the midpoint of effectiveness, where half of the parasite population experiences growth inhibition due to treatment with the extract.

Conclusion

Based on the study's results, the infusion of *Strychnos lucida* inhibited the growth of malaria parasites. The percentage of *Plasmodium berghei* growth

inhibition in the positive control group was 92.34%. In contrast, in the groups treated with *Strychnos lucida* infusion at doses of 1.5 g/200 ml, 3 g/200 ml, and 6 g/200 ml, the inhibition percentages were 27.33, 57.33, and 73.05%, respectively. Although the rate of *Plasmodium* growth inhibition in mice treated with *Strychnos lucida* infusion remained lower compared to the positive control, *Strychnos lucida* still demonstrates potential as a source for developing antimalarial drugs. For future research, it is recommended that the active compounds in *Strychnos lucida* be isolated and characterized to identify the bioactive components responsible for inhibiting the growth of malaria parasites. Furthermore, it is essential to conduct more in-depth studies on the mechanism of action of these compounds on the biochemical activity of the parasite to gain a detailed understanding of how the active compounds affect the life cycle and metabolism of *Plasmodium berghei*.

Acknowledgments
The author expresses his deepest gratitude to the Laboratory of Zoology, Faculty of Science and Technology, Pattimura University, Ambon, which has provided the necessary facilities for this research.

Author Contributions
M.C.M. and A.M.U. contributed to designing the study, preparing samples and test materials, conducting the study, analyzing data, writing the manuscript and reviewing the manuscript content.

Funding
This research did not receive funding from other external sources.

Conflicts of Interest
The authors declare no conflict of interest.

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