The Effects of Water Extract of *Psidium guajava L.* Leaves Against the Percentage of Parasitemia in male *Mus musculus L. Swiss Webster* mice

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Abstract. Due to increasing resistance, the accessibility of antimalarial medications is diminishing, necessitating the need for the development of novel drugs. Nevertheless, there is a dearth of knowledge regarding the examination of the malaria properties present in red guava leaves. This study aimed to investigate the impact of an water extract derived from red guava leaves (P. guajava L.) on the percentage of parasitemia in male mice (Mus musculus L. Swiss Webster). Additionally, the study sought to analyze the composition of phytochemical compounds in water extract obtained from red guava leaves. The findings demonstrated that the water extract of guava leaves (ADJB) and ART-ADJB (combination of artemisinin and ADJB), administered at doses of 100, 300, and 500 mg/kg, effectively decreased the percentage of parasitemia. ED50 value of red guava leaf water extract is 0.7741 mg/Kg BB. In combination with artemisinin, ED50 decreases to 0.7357 mg/Kg BB. Artemisinin alone has an ED50 of 0.43 mg/Kg BB. The measured ED50 value indicates that the utilization of a combination of artemisinin with red guava leaf water extract has greater potential compared to the use of red guava leaf water extract alone. The ADJB extract contains phytochemical compounds including flavonoids, alkaloids, and terpenoids.

1 Introduction

Malaria, a disease that is transmitted via the bite of female anopheles mosquitoes, is initiated by obligate intracellular protozoa belonging to the genus *Plasmodium*. Four species, specifically *Plasmodium vivax*, *Plasmodium falciparum, Plasmodium ovale*, and *Plasmodium malariae*, have the ability to cause malaria in humans. Indonesia is among the nations that exert influence on the prevalence of malaria.

According to the World Health Organization (WHO), the number of malaria cases globally in 2020 amounted to 241 million, compared to 227 million cases in 2019. The projected mortality rate for malaria in 2020 is expected to reach 627,000, representing a

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69,000 increase over the previous year. The World Health Organization (WHO) has implemented a comprehensive strategy aimed at achieving a 90% reduction in malaria infections and deaths by the year 2030. The Indonesian Ministry of Health reported a total of 1,346,715 malaria cases in Indonesia in 2021. Malaria affects individuals between the ages of 15 and 64, with a prevalence of 150,193 cases. *Plasmodium falciparum* is the most prevalent species, with 139,717 reported cases. *Plasmodium vivax* follows with 85,339 cases, while *Plasmodium knowlesi* is the least frequently encountered, with only 31 instances [1].

The development of medication resistance in *Plasmodium* can result in inadequate therapy and management of malaria. Antimalarial drugs, including chloroquine, primaquine, sulfadoxine + pyrimethamine, amodiquine, artemisinin, and mefloquine, induce resistance in certain strains of *Plasmodium* parasites [2]. Due to this resistance, there is a decline in the accessibility of antimalarial medications, necessitating the exploration of novel pharmacological options. Indonesian individuals employ a range of natural resources as alternative treatments, alongside synthetic pharmaceuticals. These include the utilization of plants and natural chemicals, which generally exhibit fewer negative effects in comparison to synthetic drugs. The following plants have been identified as potential sources of antimalarial compounds due to their phytochemical content: mangosteen rind (*Garcinia mangostana*), brotowali stems (*Tinospora crispa*), bitter melon (*Momordica charantia*), guava leaves (*Psidium guajava*), papaya leaves (*Carica papaya*), Pulai sari bark (*Alstonia scholaris*), and soursop leaves (*Annona muricata*) [3].

Guava leaf (*P. guajava L.*) is a plant that possesses antimalarial properties. Guava leaves have widespread usage in traditional medicine for treating many ailments such as diarrhea, canker sores, wounds, menstruation issues, colds, ulcers, constipation, coughs, dengue fever, flu, and malaria [4]. Guava leaves (*P. guajava L.*) contain a variety of chemicals such as flavonoids (quercetin, kaempferol, guaijaverin, avicularin, myricetin, hyperin, and apigenin), triterpenoids, sesquiterpenes, glycosides, alkaloids, saponins, and other phenolic compounds [5]. Alkaloids, terpenes, flavonoids, quinones, peptides, phenols, coumarins, and lignans have been scientifically demonstrated to possess antimalarial properties [6]. The research conducted by Yadav *et al.,* (2022) demonstrated that the aqueous extract derived from guava leaves in India has antimalarial properties, with an IC_{50} value of 2.011 µM. Furthermore, the extract had superior efficacy against *Plasmodium* strains when compared to commercially available antimalarial medicines such as chloroquine and quinine [7].

Each species of guava leaf possesses distinct secondary metabolite chemicals, which can be affected by environmental factors such as temperature, humidity, light intensity, water availability, mineral content, and levels of carbon dioxide [8],[9]. The red guava leaf, a variety native to Indonesia, is commonly utilized as a herbal component in traditional medicine[10]. Currently, there is a significant dearth of knowledge regarding the examination of the malaria properties present in red guava leaves. Previous research has established that various types of guava leaves exhibit notable anti-malarial properties [7],[11].

From the aforementioned explanation, it is evident that the extract derived from the leaves of red guava (*P. guajava L.*) possesses the potential to be utilized as a raw material for the development of antimalarial drugs. Hence, it is crucial to investigate the antimalarial properties and phytochemical composition of red guava leaves that are native to Indonesia. This study was to investigate the effects of a water extract derived from red guava (*P. guajava L.*) leaves on the percentage of parasitemia in male mice (*Mus musculus L. swiss webster*). Additionally, the study aimed to analyze the phytochemical composition of possible extracts obtained from red guava (*P. guajava L.*) leaves.

2 Experimental Details

2.1 Preparation of *P. guajava L.* Simplicia.

5 kg of *P. guajava L.* leaves were collected from Mulyoagung Village, Malang Regency then washed using water and in the oven for ± 40ºC to dry. After drying, the leaves were pulverized using a grinder into powder.

2.2 Extraction of *P. guajava L.* Simplicia.

The powder of *P. guajava L.* leaves was extracted using water as a solvent over a period of 1 to 3 days. The simplicia-to-solvent ratio was 1:4. The extract was dried through evaporation using a rotary evaporator and freeze drying techniques in order to obtain the crude extract (ADJB).

2.3 *In Vivo* Antimalarial Activity Method.

For the experiment, 15 *M. musculus L. Swiss Webster* mice weighing between 20 and 25 grammes and aged 8 weeks were utilised as test subjects. The subjects were acclimated for a duration of 1 week to standardise their feeding and drinking patterns, which were provided without restriction. Additionally, *M. musculus L. Swiss Webster* mice underwent health examinations conducted by a veterinarian[12]. The test materials employed consisted of a 0.5% suspension of CMC-Na, suspensions of ADJB extract at doses of 100, 300, and 500 mg/kg BW, and a suspension of artemisinin at a dose of 0.43 mg/kg BW. The test mice were categorised into four groups: (1) negative control group; (2) positive control group; (3) ADJB extract treatment group (with dosages of 100, 300, and 500 mg/kg BW); (4) ART-ADJB combination group (with extract doses of 100, 300, and 500 mg/kg BW; artemisinin dose of 0.43 mg/kg BW). The *P. berghei* sample was defrosted in a liquid nitrogen tank at ambient temperature. Subsequently, the sample was subjected to centrifugation at a speed of 2000 revolutions per minute for a duration of 5 minutes. The resulting solid mass was then rinsed with 10 millilitres of foetal bovine serum (FBS). The pellet was suspended in 1 mL of RPMI medium and introduced into the donor mice. Each donor mice was administered 0,20 mL of the sample intraperitoneally. The *P. berghei* strain utilised for infecting the test mice was obtained through selective breeding in donor mice. Mice that were blood donors and had *P. berghei* parasites in their blood at levels of 4-5% were selected. These mice were then subjected to a heart puncture procedure to collect their blood. The blood was separated using Ficolle and washed with fetal bovine serum (FBS). The test materials (ADJB and artemisinin) were administered to the mice orally once their parasitemia levels reached 2-3%. The treatment followed the four-day suppressive test procedure, which involved administering the mice with varying doses of the extract for four consecutive days. Throughout the treatment process, daily blood samples were collected from the tails of the mice to monitor the level of parasitemia. After being preserved with methanol, the blood smears were dyed using a 10% Giemsa solution. The parasitemia level was examined using a binocular microscope with a magnification of 100x. The parasitemia percentage and inhibition percentage were determined by calculating the findings using Equations 1 and 2.

Parasitemia Percentage:

$$\frac{(\Sigma\ the\ amount\ of\ infected\ erythrocyte)}{(\pm 1000\ erythrocyte)} x\ 100\% \quad (1)$$

Inhibition Percentage:

$$\frac{(\Sigma\ induced\ parasite - \Sigma\ observed\ parasite)}{(\Sigma\ inducted\ parasite)} x\ 100\% \quad (2)$$

2.4 Qualitative Phytochemical Test of *P. guajava L.* Extract.

Phytochemical analysis was carried out using each of the test samples, namely, 1 mg of an ADJB extract dissolved in 1 mL of water solvent.

2.5 Alkaloid Test.

The alkaloid test was conducted using the Dragendorff method. A 1 mL sample was obtained and dissolved with 1 mL of 2 M HCl. Additionally, one drop of Dragendroff reagent is added to the test tube. Once a yellow precipitate is observed upon adding the aforementioned reagent, it is identified as an alkaloid compound [13].

2.6 Flavonoid Test.

The flavonoid test was conducted by adding 1 mL of the sample to 1 mL of 2% NaOH, followed by the addition of a few drops of weak HCl. Subsequently, monitor the alteration in color, specifically from a concentrated yellow hue to a transparent (colorless) state, since it indicates the existence of flavonoids.

2.7 Steroid and Terpenoid Test.

The steroid and terpenoid assays were conducted by combining 1 mL of the extract with an equal volume of chloroform. Next, add 1 mL of sulfuric acid into the tube by passing it through the tube wall. A brownish or violet colored ring forming on the solution's boundary suggests the presence of terpenoid chemicals [3]. A positive indication for steroids is confirmed if a ring with a greenish blue coloration is observed in the sample [14].

3 Results and Discussion

Fig. 1 illustrates a decline in the percent parasitemia value in the ADJB, ART-ADJB, and positive control groups from the day of injection (S.D0) to the third day of therapy (D3). The increase in parasitemia value seen in the negative control group can be attributed to the absence of therapy administered to this group. The ART-ADJB group administered with a dose of 500mg/kg BW had the most significant reduction in the percent parasitemia value, which amounted to 0.73%. The interaction between red guava leaf extract and artemisinin is the reason for this. The study conducted by Anna *et al.,* (2019) demonstrated that the concurrent administration of carica papaya and artemisinin leads to a substantial reduction in parasitemia [15]. Penelitian Endang et al., (2016) juga membuktikan bahwa kombinasi antara artemisinin dengan ekstrak daun kelor (*Moringa oleifera*) menghasilkan penurunan persen parasitemia hingga 69.4% terhadap *Plasmodium berghei*.

Fig. 2 demonstrates a positive correlation between the dosage administered and the efficacy in inhibiting parasite growth. The optimal dose for parasite inhibition is 500mg/kg Bw, which yields the highest efficiency. The percent inhibition analysis indicates that the highest value of 76.667% is achieved when artemisinin is combined with the aqueous extract of red guava leaves (ART-ADJB) at a dose of 500 mg/kg BW. The positive control group has the lowest figure of percent inhibition, which is 31.140%. Table 1 shows that the red guava leaf water extract has an ED_{50} value of 0.7741 mg/Kg BB for percent inhibition. When combined with artemisinin, the ED_{50} value decreases to 0.7357 mg/Kg BB. The ED_{50} value for artemisinin alone is 0.43 mg/Kg BB. A compound's antimalarial activity is considered good if its ED_{50} value is below 10 mg/kg human [16]. The measured ED_{50} value indicates that the combination of artemisinin with red guava leaf water extract (ART-ADJB) has greater potential compared to using red guava leaf water extract (ADJB) alone.

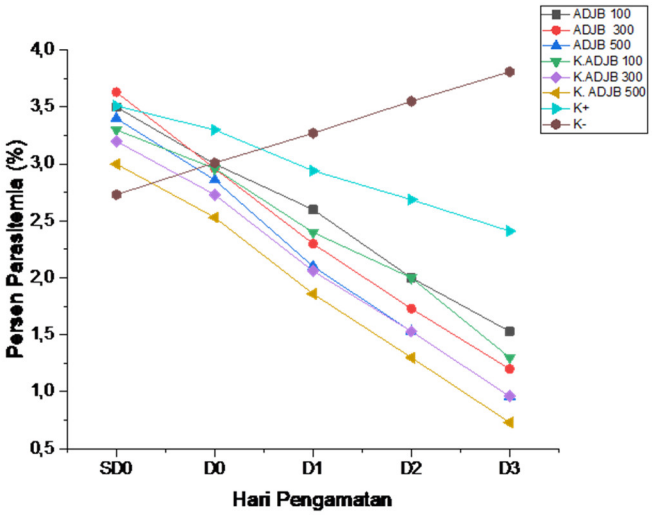

Fig. 1. Percentage of parasitemi

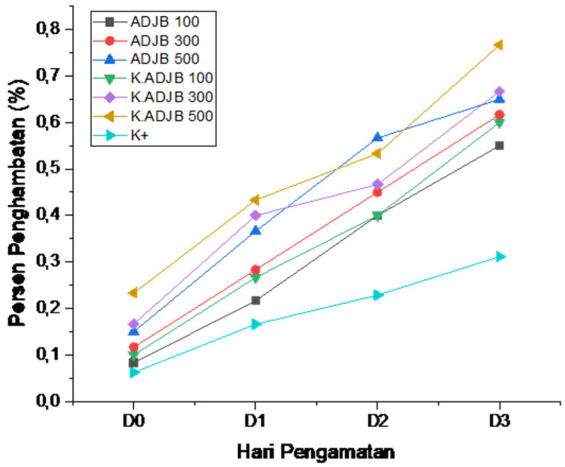

Fig. 2. Percentage of inhibition

Table 1. ED_{50} Values of ADJB Extract, ART-ADJB Combination, and Artemisinin

Treatment	ED_{50} Value (mg/kg BW)
ADJB Extract	0.7741
ART-ADJB Combination	0.7357
Artemisinin	0.43

According to the results obtained from the Tukey Post Hoc test presented in Table 2 below, the ADJB treatment exhibits a significant difference compared to the ART-ADJB, (+) Control and (-) Control treatments. The data shown in the table indicates that the ART-ADJB group exerts a more pronounced impact on the percentage of parasitemia in comparison to the ADJB, Control +, and Control - groups. Artemisinin-based combination therapy is recommended by WHO (2001) for the treatment of malaria. This therapy is preferred because it can enhance the efficacy of extracts and has the ability to delay the development of resistance [17].

Table 2. Results of Post Hoc Tukey Test on Percent Parasitemia Treatment

Treatment	Mean and Standard Deviation of Parasitemia Percentage
ART-ADJB Combination	2.12 ± 0.83^{a}
ADJB Extract	2.34 ± 0.82^{b}
(+) Control	2.97 ± 0.42^{c}
(-) Control	3.27 ± 0.46^{d}

Table 3. Qualitative Phytochemical Testing Results of P. guajava L. Leaves Water Extract

Phytochemical Compounds	Before Test	After Test	Description
Alkaloid	Brownish yellow	Intense yellow	Positive
Flavonoid	Brownish yellow	Bright yellow	Positive
Terpenoid	Brownish yellow	Brown	Positive
Steroid	Brownish yellow	Yellow color with oil-like precipitate	Negative

Table 3 demonstrates that the examination of phytochemical content reveals the presence of flavonoids, alkaloids, and terpenoids in the extract of red guava leaves. Phytochemical compounds contained in red guava leaves are responsible for antimalarial activity. Voravuth *et al.,* (2016) have shown that substances such as alkaloids, terpenes, flavonoids, quinones, peptides, phenols, coumarins, and lignans have antimalarial properties [6]. The presence of flavonoid chemicals, specifically morin and quercetin, has been demonstrated in aqueous extracts of red guava leaves by Pongsak and Parichat (2010) [18]. Arumugam *et al.,* (2020) discovered the presence of alkaloids in guava leaves, specifically zeatin riboside [19]. Guava leaves were shown to contain triterpenoids such as guajanoic acid, β-sitosterol, and ursolic acid [20].

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