

**Antimalarial potential of *Pithecellobium jiringa* (Dogfruit) skin extract against *Plasmodium falciparum***<sup>1,\*</sup>Surya, A., <sup>1</sup>Irawan, M.P., <sup>1</sup>Juariah, S., <sup>2</sup>Endrini, S., <sup>3</sup>Yesti, Y., <sup>4</sup>Zaiyar and <sup>5</sup>Ahmad Rusmili, M.R.A.<sup>1</sup>Faculty of Pharmacy and Health Science, Abdurrah University, Jl. Riau Ujung No. 73, Tampan, Air Hitam, Payung Sekaki, Pekanbaru, Riau 28291, Indonesia<sup>2</sup>Faculty of Medicine, Abdurrah University, Jl. Riau Ujung No. 73, Tampan, Air Hitam, Payung Sekaki, Pekanbaru, Riau 28291, Indonesia<sup>3</sup>Department of Pharmacy, Fort De Kock University, Jl. Soekarno Hatta No.11, Manggis Ganting, Kec. Mandiangin Koto Selayan, Kota Bukittinggi, Sumatera Barat 26117, Indonesia<sup>4</sup>Department of Civil Engineering, Technology High School Pekanbaru, Jl. Dirgantara No.4, Kel. Sidomulyo Tim., Kec. Marpoyan Damai, Kota Pekanbaru, Riau 28125, Indonesia<sup>5</sup>Kulliyyah of Pharmacy, International Islamic University Malaysia, Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200 Kuantan, Pahang, Malaysia**Article history:**

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*Pithecellobium jiringa*,*Plasmodium falciparum***DOI:**[https://doi.org/10.26656/fr.2017.8\(S5\).12](https://doi.org/10.26656/fr.2017.8(S5).12)**Abstract**

Malaria is a high health concern in endemic regions. Effective anti-malarial agents against *Plasmodium falciparum* infection are often studied for novel effective treatment. This study aimed to examine the anti-malarial properties of *Pithecellobium jiringa* (Dogfruit) skin extracts. Ethanol and ethyl acetate extracts of *P. jiringa* skin were assessed in vitro on the growth of *P. falciparum*. The IC<sub>50</sub> values were 16.59 µg/mL and 4.897 µg/mL for the ethyl acetate and ethanol extracts, respectively. The results indicated that the *P. jiringa* skin ethanol extract demonstrated the highest potent inhibitory effect which is a promising agent against malaria. More studies are recommended to identify and characterize the active compounds in *P. jiringa* skin extract.

**1. Introduction**

Malaria is one of the world's important parasitic diseases, especially in tropical and subtropical regions. It is caused by *Plasmodium* spp. and most of the cases were reported to be caused by *Plasmodium falciparum*. Despite the development and utilization of multidrug anti-malarial agent regimens, the possibility of the emergence of malaria parasite resistance to these conventional treatments will be a serious threat to disease management (Dodean *et al.*, 2019).

Bioprospecting potential novel anti-malarial agents from natural sources could provide a solution for the emergence of resistant organism (Chen *et al.*, 2019). *Pithecellobium jiringa* (Dogfruit) is locally available in Indonesia, Malaysia, Singapore and Brunei known as 'jering' or 'jengkol'. It has been used as a traditional medicine to cure a wide range of ailments. According to Tambun *et al.* (2022), *P. jiringa* contain high amounts of protective compounds, i.e., flavonoids, alkaloids and saponins. Previous works on *P. jiringa* have demonstrated its antibacterial and antioxidant activities

(Surya *et al.*, 2021), but few on anti-malarial activity. Hence, this study aimed to examine the potential of *P. jiringa* skin extract as a novel anti-malarial agent. The skins of *P. jiringa* will be extracted using ethanol and ethyl acetate and evaluate in vitro against the growth of *P. falciparum*.

**2. Materials and methods****2.1 Materials**

Donated human red blood cells and human serum, DMSO (Sigma-Aldrich, USA), Hepes buffer (Sigma-Aldrich, USA), Giemsa-stained erythrocytes (Sigma-Aldrich, USA), hypoxanthine (Sigma-Aldrich, USA), Sodium carbonate, Na<sub>2</sub>CO<sub>3</sub> (Sigma-Aldrich, USA), ethanol (Merck, USA), ethyl acetate (Merck, USA), parasite culture 500 µL (1% parasitemia, 5% hematocrit), and gentamicin (Thermo Fisher Scientific, USA). *Plasmodium falciparum* 3D7 strain was originally isolated from patients infected with malaria.

Ethyl acetate and ethanol extract of *Pithecellobium*

\*Corresponding author.

Email: [alfin.surya@univrab.ac.id](mailto:alfin.surya@univrab.ac.id)

*jiringa* skin

## 2.2 Sample collection

Samples were taken from mature fresh plants and identified with the help of botanists to ensure the authenticity of the specimen at the botanical laboratory of the University of Riau, Indonesia. The skin was separated from the fruit and washed thoroughly under running water. It was then rinsed with distilled water and dried using an oven at 45°C. Once the skin is completely dry, the skin is ground to form a puree using a blender. The puree was stored in an airtight container until further analysis.

## 2.3 Extraction process

A total of 50 g of the puree was added into 500 mL ethyl acetate and stirred for maceration for 72 hrs at room temperature. The mixture was filtered using Whatman filter paper to separate the oversize from the filtrate. The oversize was then soaked in 500 mL ethanol and stirred for maceration for 72 hrs at room temperature. The two filtrates were dried with a rotary evaporator at 40°C until the extract was ready.

## 2.4 Preparation of *Plasmodium falciparum* culture

Fresh erythrocytes O+ was obtained from ethically approved source. The erythrocytes were washed for three times with sterile phosphate buffer solution to remove plasma and buffy coat. Approximately 1 – 2% of *P. falciparum* was inoculated into the erythrocytes and added with hematocrit until it reached 2 – 5%. The culture was inoculated at 37°C in a closed container with a suitable gaseous atmosphere. The culture is checked and ready for sensitivity test when the concentration reaches 3 – 5%.

## 2.5 Inhibition of parasitaemia assay

A stock solution of 10 mg/mL was prepared by dissolving 10 mg of the extract in 1 mL of DMSO 100%. Then, aqueous serial dilutions were made by diluting the stock solution with distilled water to produce concentrations of 1000 µg/mL, 100 µg/mL, 10 µg/mL, 1 µg/mL, and 0.1 µg/mL. A total of 2 µL of the sample solutions was pipetted into wells, followed by the addition of 198 µL of *P. falciparum* 3D7 strain culture. The plate was then kept in a chamber and exposed to a mixture of gases (5% O<sub>2</sub>, 5% CO<sub>2</sub>, and 90% N<sub>2</sub>). The plates were incubated for 48 hours at 37°C. The mixture from each well was smeared onto a thin blood film stained with 20% Giemsa. The films were analyzed by counting the number of infected erythrocytes per 1000 normal erythrocytes under a microscope magnification 1000×. The data was used to determine the percentage of

growth and inhibition with the following equations:

$$\% \text{ Growth} = \% \text{ Parasitemia} - D0$$

Where D0 = % growth at 0 hour

Followed by:

$$\text{Percentage of Inhibition (\%)} = 100\% - (Xu/Xk) \times 100\%$$

Where Xu is the percentage of growth in the test solution and Xk is the percentage of growth of the negative control.

A probit analysis was conducted based on the percentage of inhibition to determine the concentration of the test material that could inhibit the parasite's growth by as much as 50% (IC<sub>50</sub> value). The IC<sub>50</sub> values were calculated by applying the four logistic parameter regression curves to dose-response data using Graph Pad Prism 7.0 software (Graph Pad Co. Ltd., USA) (Hidayati et al., 2020).

## 3. Results

In this study, the ethanol and ethyl acetate extracts of *P. jiringa* skin were tested against *P. falciparum* in vitro for anti-malarial properties. This test was conducted by incubating the culture for 48 hrs, which corresponds to the asexual cycle of the parasite in the blood before the percentage of inhibition of the extracted compounds against parasites within erythrocytes was calculated (Okokon et al., 2007). Tables 1 and 2 tabulate the parasitemia inhibition of different concentrations of the ethyl acetate and ethanol extract of *P. jiringa* skin, respectively.

The results of the anti-malarial activity test showed a concentration-dependent effect of the extracts in inhibiting *P. falciparum* growth as shown in Figure 1 for the ethyl acetate extract of *P. jiringa* skin and Figure 2 for the ethanol extract of *P. jiringa* skin. The calculated IC<sub>50</sub> values using the probit transformed response for the ethanol and ethyl acetate extract were 4.897 µg/mL and 16.59 µg/mL, respectively (Figures 1 and 2). This indicates that the ethanol extract shows potent activity, while the ethyl acetate extract demonstrates moderate inhibition. According to World Health Organization (WHO) guidelines (2021), the crude extracts with IC<sub>50</sub> values ≤ 5 µg/mL exhibit significant antimalarial activity.

## 4. Discussion

The findings of this study demonstrated the potentiality of *P. jiringa* skin extracts as a source of antimalarial agents. The high IC<sub>50</sub> value of ethyl acetate extract of *P. jiringa* skin shows a promising anti-malarial

Table 1. Parasitemia inhibition of ethyl acetate extract of *Pithecellobium jiringa* skin.

Concentration (µg/mL)	R	% Parasitemia		% Growth	% Inhibition	% Inhibition Average
		0 hr	48 hrs			
Control (-)	1	1.10	8.30	7.20	-	-
	2	1.10	8.40	7.30	-	-
100	1	1.10	3.46	2.36	67.22	67.65
	2	1.10	3.43	2.33	68.08	
10	1	1.10	5.04	3.94	45.28	45.38
	2	1.10	5.08	3.98	45.48	
1	1	1.10	6.54	5.44	24.44	24.76
	2	1.10	6.57	5.47	25.07	
0.1	1	1.10	7.80	6.70	6.94	7.92
	2	1.10	7.75	6.65	8.90	
0.01	1	1.10	8.25	7.15	0.69	1.03
	2	1.10	8.30	7.20	1.37	

Table 2. Parasitemia inhibition of ethanol extract of *Pithecellobium jiringa* skin.

Concentration (µg/mL)	R	% Parasitemia		% Growth	% Inhibition	% Inhibition Average
		0 hr	48 hrs			
Control (-)	1	1.10	8.30	7.20	-	-
	2	1.10	8.40	7.30	-	-
100	1	1.10	0.00	0.00	100.00	100.00
	2	1.10	0.00	0.00	100.00	
10	1	1.10	4.25	3.15	56.25	56.89
	2	1.10	4.20	3.10	57.53	
1	1	1.10	5.56	4.46	38.06	38.21
	2	1.10	5.60	4.50	38.36	
0.1	1	1.10	7.70	6.60	8.33	8.62
	2	1.10	7.75	6.65	8.90	
0.01	1	1.10	8.10	7.00	2.78	3.17
	2	1.10	8.14	7.04	3.56	

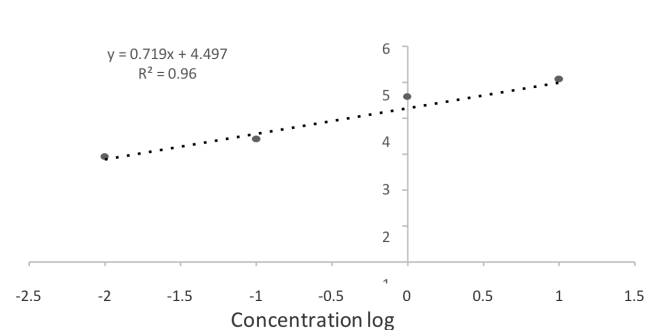
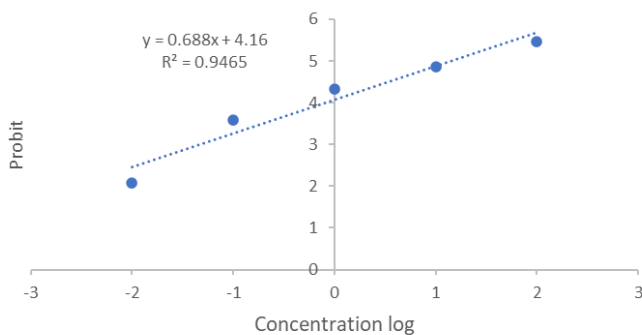


Figure 1. Probit transformed Response of ethyl acetate extract of *Pithecellobium jiringa* skin for  $IC_{50}$  value calculation. Using the equation  $y = 0.688x + 4.165$ , the x-value calculated was  $\log_{10} 1.22$  and converted to  $16.59 \mu\text{g/mL}$ .

Figure 2. Probit transformed Response of ethanol extract of *Pithecellobium jiringa* skin for  $IC_{50}$  value calculation. Using the equation  $y = 0.719x + 4.4975$ , the x-value calculated was  $\log_{10} 0.69$  and converted to  $4.897 \mu\text{g/mL}$ .

agent (Hidayati *et al.*, 2020). The superior activity of the ethanol extract can be attributed to the presence of secondary metabolites, particularly phenolic compounds. Previous studies have identified 1-(2,6-dihydroxy-4-methoxyphenyl) decan-1-one as a major component in the ethanol extract, suspected to play a key role in its antimalarial properties. Phenolic compounds are known to exert multiple bioactivities, including the disruption of cellular functions in parasites, which supports their antimalarial potential (Hidayati *et al.*, 2020).

Additionally, the bioactivity of *P. jiringa* extract is not limited to antimalarial effects. Extracts from other

parts of the plant have been reported to possess antimicrobial, antifungal, antioxidant, anticancer, and antidiabetic properties, further highlighting the versatility of the plant in therapeutic applications. These properties suggest the plant's extracts may serve as a rich source of bioactive compounds for various drug development pipelines. The results also highlight the importance of concentration-dependent effects of the extracts, where higher concentrations resulted in greater inhibition of parasite growth. This underlines the need for precise dosing strategies in potential therapeutic applications (Yanti *et al.*, 2023).

Future research should focus on the isolation, purification, and structural elucidation of the active compounds. Advanced techniques such as high-performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR), and mass spectrometry could help identify the specific compounds responsible for the antimalarial activity. Furthermore, in vivo studies and toxicity assessments are necessary to evaluate the extract's safety and efficacy in a biological system.

## 5. Conclusion

The ethanol and ethyl acetate extracts of *P. jiringa* skin were tested for their antimalarial activity against the growth of *P. falciparum* in vitro. The results revealed that both extracts exhibited a concentration-dependent inhibition of parasite growth. The ethanol extract of *P. jiringa* skin showed a lower IC<sub>50</sub> value of 4.897 µg/mL, meeting the WHO criteria for highly active crude extracts. This suggests the ethanol extract of *P. jiringa* skin can be used to develop novel anti-malarial agents and further combat malaria disease.

## Conflict of interest

The authors stated that there was no conflict of interest. This research is purely conducted to improve performance and scientific work as contributions to science.

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