

The Antimalarial Activity of the Water Extract of Simpur (*Dillenia Indica L*) Leaves against *Plasmodium Berghei* in Mice

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ABSTRACT

Malaria, caused by the climate of the subtropical area and the forest with many rivers and immovable water, is a contagious disease that still becomes a health problem in West Kalimantan. Simpur is a plant that is used by the locals to cure malaria. Therefore, this research aims to study the antimalarial activity *in vivo* and the 50% inhibitory concentration (IC₅₀) of the water extract of Simpur leaves (*Dillenia indica L*) against *Plasmodium berghei*. This research used Peter Test method that used 7 test groups based on the test solution namely positive control group that was given dihydroartemisinin+piperazine (DHP), negative control that was given aquabidestilata and the test group that was given the water extract of Simpur leaves with various doses of 20, 40, 60, 80 and 100 mg/Kg BB of mice, which each group was given the test solution for 3 days. The result shows that the water extract of Simpur leaves could lower the parasitemia count with IC₅₀ 19,22 mg/kg BB.

Keywords: *Dillenia indica L.*, antimalarial, *in vivo*, *Plasmodium berghei*

INTRODUCTION

Malaria is one of the contagious diseases that still become a global and national health problem (Malik, 2015). The disease is caused by a parasite called *Plasmodium sp* by infecting and spreading in human blood cell and could cause death (Rumagit, *et al*, 2013). Based on World Health Organization (WHO) data, malaria cases in 2016 are about 216 million cases in the world, 14,6 million cases in the Southeast Asia and 1,6 million cases in Indonesia (World Health Organization, 2017). Based on Indonesia Health Ministry data, in West Kalimantan, malaria cases in 2015 are about 4,4 million cases (Kementerian Kesehatan Republik Indonesia, 2016). The Features of the subtropical area in the forest that have many rivers and area with immovable water could give a good chance for mosquito bringing parasite to spread out (Laikad, 2011., Serafina, *et al.*, 2012).

Indonesian use empirically medicinal plant to cure diseases. Indonesian Health Ministry gives support for this using through research so that the efficacy of medicinal plant could be obtained to people with the right dose and safety (Menteri Kesehatan Republik Indonesia, 2010). Simpur, *Dillenia indica* is a plant in the forest of the regency of Sintang, West Kalimantan. People use it empirically to cure malaria by boiling it in water (Hidayat and Gusti, 2012,). Previous research proved that the methanol extract from the leaves and the fruit has the activity as antileukemia, antibacterial and antiinflammation

(Kumar, *et al.*, 2010, Apu, *et al.*, 2010, Yeshwante, *et al.*, 2009). The 80% ethanol extract of the leaves showed the activity of antimalarial *in vitro* (Puoplin, 2007). *Betulinic acid* is a compound that had been isolated from the methanol extract of the leaves fractioned in a nonpolar solvent that had been proved as antimalarial (Kumar, *et al.*, 2010, Steele, *et al.*, 1999, Muhid, *et al.*, 2010).

According to the data, we need to conduct the study of antimalarial activity of the water extract of Simpur leaves *in vivo* in an effort to obtain the information related to the antimalarial activity that could be used for people to cure malaria.

METHODOLOGY

Material

This research used *Plasmodium berghei* inoculated to the male mice of swiss strain as donor obtained from the Parasitology laboratory of the Medicinal Faculty of Brawijaya University. The samples were old fresh Simpur leaves that were not broken and obtained from Sungai Ambawang, the regency of Kuburaya, West Kalimantan, that had been determined as Simpur leaves in Biology Laboratory of Mathematics and Science Faculty of Tanjungpura University. The preparation of extract, the screening of secondary metabolite content and the antimalarial activity assay used material obtained from PT. Merck.

Method

Preparation of extracts

Old fresh Simpur leaves were collected from the sub-district of Sungai Ambawang, the district

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of Kubu Raya, the province of Kalimantan Barat, in March 2018. Sample was separated from the other parts. The leaves were washed with flowing water and cut into small pieces. The pieces were dried in an oven at 60°C and smashed to obtain a dry powder. The powder extracted three times using aquabides for 15 minutes at 90°C. The extract was concentrated using freeze-drying to obtain the dry extract.

The screening of secondary metabolite content of the extract solution

The extract was dissolved in aquabides. The extract was identified for alkaloid, steroid, terpenoid, flavonoid, saponin, tannin dan phenol contents. The screening used the method according to the book of Kristanti, *et al.*, 2008, that consist of the tube and the thin layer chromatography method (Kristanti, *et al.*, 2008).

The preparation of extract solution, positive control, and negative control.

The extract was weighed and dissolved in aquabides to obtain the concentration of solution of 20, 40, 60, 80, and 100 mg/Kg BB of mice. Positive control with human dose, 360 mg/70kg BB and 4 tablets dihydroartemisinin + piperazine (DHP), was converted into mice dose to obtain 18,72 mg/Kg BB of mice before solvated in aquabides. Negative control was prepared using only aquabides.

The preparation of donor mice and test mice

Animal for the test is mice strain Swiss with 20-30 gram. These were divided into donor mice and test mice. The test mice divided into 7 test groups based on the concentration of the extract. Group I, II, III, IV, and V are constitutively for the concentration of 20, 40, 60, 80 and 100 mg/kg BB. The group of positive control was given dihydroartemisinin+piperakuin (DHP) and the group of negative control was given only aquabides.

The inoculation of the parasite to donor mice and the infection of test mice

Plasmodium berghei from frozen stock was warmed by rotating using hand until it melts into the temperature of the mice body. This stock was injected 200 µL each mouse intraperitoneal to donor mice and parasite growth was observed. The blood was collected by decapitation when the amount of parasitemia reached 15%.

The blood obtained by decapitation was collected into tubes containing 0,5% trisodium citrate and solvated in saline solution (0,9%)

until 1 ml blood contains 5×10^7 of infected blood cell. 0,2 ml of the infected blood that contain 1×10^7 *Plasmodium berghei* was injected intraperitoneal into the test mice and counted as day-0.

Antimalarial assay

The assay was conducted using Peter test method (Peters, *et al.*, 1975). The solution of extract with doses constitutively of 20, 40, 60, 80, and 100 mg/kg BB of mice were given orally to the test mice. Positive control and negative control were also given orally to the test mice. The injection was started from the first day, namely 3 hours after the inoculation of parasitemia to the test mice. The solutions were continued to be injected orally each day until the third day.

The calculation of the inhibition percentage of parasitemia growth and IC50

The calculation used formulas as follow :
The calculation of parasitemia percentage

$$\% \text{ Parasitemia} = \frac{\text{The amount of infected erythrocyte}}{1000 \text{ erythrocyte}} \times 100 \%$$

The calculation of parasitemia growth percentage

$$\% \text{ Growth} = \frac{P[d2-d1] + P[d3-d2] + P[d4-d3]}{\text{The amount of days}-1}$$

The calculation of the inhibition percentage of parasitemia growth

$$\% \text{ Inhibition} = 100\% - \left(\frac{X_e}{X_k} \times 100\% \right)$$

Information= Xe: the percentage of parasite mean growth that is given dose extract; Xk: the percentage of parasite average growth of negative control; P(dx-dx-1): parasitemia percentage of day x minus parasitemia percentage of the previous day

The result of the inhibition percentage was analyzed against various doses of extract using probit analysis to obtain the dose of extract that could inhibit 50% of parasite growth (Purwaningsih, S., 2003). The result was also analyzed using the statistic package for the social science (SPSS) program, with one way ANOVA test to know the significance of inhibition percentage of the extract against parasitemia growth.

RESULTS AND DISCUSSION

The extract was brown, dry powder and had a typical smell. Rendement obtained was 0,507% with shrinkage drying was $6,25\% \pm 0,0256$. Screening result shows metabolites that may contribute to the antimalarial activity of the extract. The screening result is shown in the (Figure 1) below. The result shows tannin, phenol,

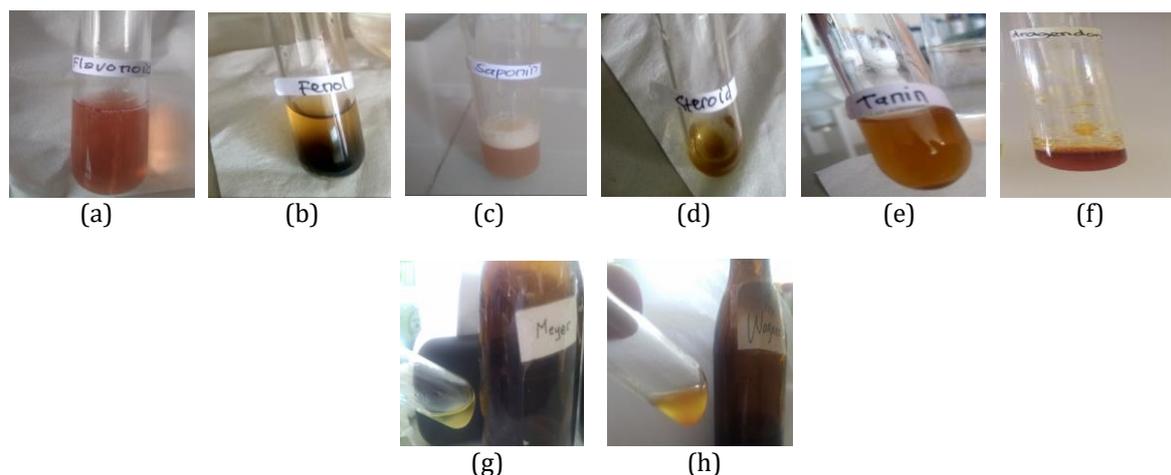


Figure 1. The screening result of secondary metabolites in water extract of Simpup leaves. (a) Flavonoid test using HCl and Mg metal; (b) phenol test using FeCl_3 in water; (c) Saponin test using shaking method in water; (d) steroid test using Ac_2O and H_2SO_4 ; (e) tannin test using Liebermann-Burchard reagent; (f) alkaloid test using dragendorff reagent; (g) alkaloid test using Mayer reagent; (h) alkaloid test using Wagner reagent.

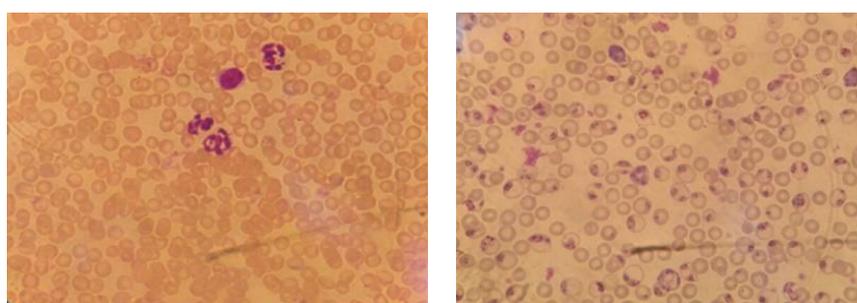


Figure 2. The blood cell of test mice before and after the infection using giemsa coloring

flavonoid, steroid, alkaloid, and saponin in the extract that conforms with previous research conducted by Bose *et al.*, 2010 that found flavonoid, steroid, alkaloid, and saponin in the methanol extract of Simpup leaves. The result also conforms with the previous research that found an isolate, betulinic acid, in the methanol extract of Simpup leaves that is a steroid that had been proven has antimalarial activity (Kumar *et al.*, 2010, Apu *et al.*, 2010, Yeshwante, *et al.*, 2009).

The antimalarial activity assay was conducted using a Peter test method that had been approved by the Ethics Committee in Medicine Faculty of Tanjungpura University. The assay was started by inoculation and infection of mice until parasite growth well in mice's blood. The result of the infection of test mice that were injected with infected blood of the donor mice (Figure 2). The violet color shows the cell has been infected.

The result of the antimalarial activity assay of the water extract of Simpup leaves shows

the potency of extract against parasitemia (Table I). Based on the statistic result of one way ANOVA, the result shows a significant difference between the test group and negative control that means water extract of Simpup leaves could decrease the amount of parasitemia. The result also shows no significant difference between the test group with extract dose of 60 mg/Kg BB of mice means that the extract and positive control could decrease the amount of parasitemia. Negative control shows no inhibition but positive control shows inhibition of parasitemia. This means that this assay conducted well and valid. In addition, the r-value of the regression formula used to calculate IC_{50} is 0,9848 or close to 1. This shows that the increasing of extract doses is proportional to the activity that means the method was conducted, including how researcher work, the procedure, tools, and the result presents the valid result. Moreover, this antimalarial activity conforms to the previous research that used 80% ethanol extract of Simpup

Tabel I. The Result of Antimalarial Activity Assay of The Water Extract of Simpurn Leaves (Peter test Method) n=4 Mice/Group.

No.	Doses (mg/Kg BB)	The inhibition percentage of parasitemia (%)	IC ₅₀ (mg/Kg BB)
1.	20	50,16	
2.	40	64,31	
3.	60	69,29	
4.	80	79,42	19,22
5.	100	96,30	
6.	Positive control	70,90	
7.	Negative control	0,00	

leaves, and Betulinic acid that had been isolated from the methanol extract of Simpurn leaves that has antimalarial activity.

This study needs to be continued to a clinical study to increase the result accuracy of antimalarial assay. Various factors such as genetic and species had been proved that could interfere with drug metabolism. Differences in distribution volume, organ volume and the profile of absorption, distribution, metabolism, and elimination of between test animal and human could cause differences in doses that are used to decrease parasitemia in the blood (Coleman, and Michael, 2005).

CONCLUSION

Based on the data that IC₅₀ of the extract is 19,22 mg/Kg BB, antimalarial activity of extract with dose 60 mg/Kg BB that are close to antimalarial activity of the positive control with dose 18,72 mg/Kg BB, the validity of the method and the steroid content in the extract that could be betulinic acid, those could be concluded that the water extract of Simpurn leaves has antimalarial activity in vitro.

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