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Research Article

IN VIVO TEST OF ANTIMALARIAL ACTIVITY FROM DICHLOROMETHANE-ETHYL ACETATE-METHANOL FRACTIONS OF MUNDU'S BARK (*GARCINIA DULCIS* (ROXB.) IN SWISS WEBSTER MICE

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ABSTRACT

Objective: Genus *Garcinia* is well-known having rich xanthone compound contents and several of those are having biological activity as antimalarial. The aims of this research were to determine *in vivo* antiplasmodial activity of dichloromethane-ethyl acetate-methanol fractions of Mundu's bark (*Garcinia dulcis* (Roxb.) Kurz), especially Fraction V against *Plasmodium berghei* and to determine effective dose 50 (ED_{50}).

Methods: Making Fraction V from ethyl acetate extracts of Mundu's bark with vacuum column chromatography method was applied using dichloromethane-ethyl acetate-methanol. The product was monitored by thin layer chromatography using silica gel GF-254 and a mobile phase of chloroform: Ethyl acetate (6:4). The same profiles from eluent composed Fraction 18 and 19 were categorized as Fraction V. Those were tested in each of groups animals with a dose of 12.5; 25; 50 and 100 mg/kg body weight. Antimalarial activity assessments have been performed with the *in vivo P. berghei* test. Plasmodial activity was obtained by calculating the percentage of parasitemia, parasitemia inhibition, and ED_{50} determination. ED_{50} value determined based on the relationship between dose and the percentage of parasite growth inhibition by probit analysis.

Results: In this research, Antiplasmodial activity of Fraction V from Mundu's bark displayed in a dose of 50 mg/kg bw and 100 mg/kg bw with inhibition parasite growth by values of 47.255% and 12.761%, respectively.

Conclusion: According to the research, Fraction V of Mundu's bark (*G. dulcis* (Roxb.) Kurz) had the most potential antiplasmodial activity by *in vivo* test for male Swiss-Webster mice. The value of ED_{50} was reached by 47.424 mg/kg bw.

Keywords: Mundu's bark, Antiplasmodial activity, Effective dose 50.

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INTRODUCTION

Malaria is still being a significant health problem around the world. World Health Organization predicted more than 40% residents in the world or about 2.3 billion people stayed in endemic areas of malaria. Malaria disease is also still found in all provinces of Indonesia. Based on annual parasite incidence's stratification, Eastern Indonesia was in the high stratification of malaria, mid-stratification was in Kalimantan, Sulawesi, and Sumatera then Jawa-Bali were in the lowest stratification [1].

Malaria predicted has infected 300-500 M people in the world and caused death in 1.5-2.7 M sufferer in every year. This is caused by four of protozoa's parasites; *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale,* and *Plasmodium malariae* that infect the human red blood cells. *P. falciparum* presented the resistant potency against mefloquine and haloforin, and perhaps, it had potential multiresistency to malaria's drug in Africa [2,3]. Analog artemisinins such as *artesunate* and artemether were introduced indicating the effectively potency toward *P. falciparum*. However, the results of study of drug induction and relation between the dose and neurotoxicity in animal were feared about emerged safety from this compound in the human body [4]. Increasing of resistance to antimalarial drug, particularly synthesized drug such as chloroquine insisted on the effort to find new drug as an alternative of quinine and chloroquine that safe and has an affordable price.

Use of natural resources to resolve various diseases was carried out. Plants as potential resources of antimalarial have begun with finding quinine from bark of Chincona sp. continued with artemisinins from *Artemisia annua*. One of the genus Garcinia has known consist of many xanthone compounds and some of them have biological activity as antimalarial [1,5]. One of medicinal plants showing antiplasmodial activity has reported, there was Kandis' bark [6]. Ethanolic extract of *Garcinia dulcis* Kurz also had antiplasmodial activity [7]. According to the study two kinds of xanthone compound from ethyl acetate fraction of Mundu's bark, there were 1,3,4,5,8-pentahydroxyxanthone and 1,4,5,8-tetrahydroxyxanthone [8].

Xanthone is plant contain that has phenolic nature, has also hydroxy group and is dissolved in semipolar solvent such as ethyl acetate [8]. Xanthone has biological activity and pharmacology as cytotoxic, antifungal, antimicrobial, antioxidant, antimalarial, anti-inflammatory, and also anti-HIV activities [5]. Fraction V contained dichloromethane-ethyl acetate-methanol. By *in vitro*, Fraction V had the biggest potency compared with Fraction IV, VI and VII.

This study was aimed to test the antiplasmodial activity of Mundu's bark-Fraction V by *in vivo* in male Swiss-Webster mice that was induced with *Plasmodium berghei* with parameters of percentage of parasitemia test, retardation of parasitemia, and determination of effective dose 50 (ED_{50}).

METHODS

Animals

Animals test used were 36 male and female Swiss-Webster mice with average weight of 20-30 g, 1.5-2.5 months old, induced with *P. berghei.* Permission and approval for animal studies were obtained from Faculty of Medicine, Gadjah Mada University, Yogyakarta.

Preparation of *P. berghei*

P. berghei was found from infected broodstock mice (with *P. berghei*), taken from Medical Faculty of Universitas Gadjah Mada, Yogyakarta. Afterward, infected mice was taken their blood, looked for *P. berghei* concentration based on erithrocyte total by hemocytometer. Smear was made from mice's blood with giemsa coloring to calculate infected eritrocyte to determine the concentration of Plasmodium. Concentration obtained was made up to 1×10^7 to be induced in other mice.

Plant material and fractionation

Mundu's plant was obtained from Sukoharjo, Central Java. A part that was used was bark of the plant. Determination was performed at Biological Pharmacy Laboratory of Gadjah Mada University. The powder of Mundu's bark was macerated with ethyl acetate for 5 days, extract was separated with vacuum column chromatography. Stationary phase used silica gel 60. From the separation was obtained 30 fractions. Fractions that had the same amount of chromatogram and Rf value was compiled so that was acquired 5 compiled fractions. Fraction V was a combination of 18 and 19.

***In vivo* test**

Research layout used in this study was fully random same direction pattern using 36 Swiss-Webster mice that inoculated with *P. berghei* in the 1st day (D_{+0}). *In vivo* antimalarial activity was performed based on Peter's test (The 4 days suppressive test) [9]. Animals test were divided into 6 groups treatment that contained 6 mice in every group. There were Group I (-): Carboxymethylcellulose (CMC) suspension; Group II (+): Chloroquine suspension with 5 mg/kg bw/day; Group III, IV, V: Fraction V suspension of Mundu's bark with dose of 12.5 mg/kg bw; 25 mg/kg bw; 50 mg/kg bw; 100 mg/kg bw. CMC suspension, chloroquine, Fraction V of Mundu's bark was given for 4 days (D_{+0} until D_{3+}) orally and the percentage of parasite growth was observed every day. Blood samples were taken to determine parasitemia level for 4 days. This result examined by making thin blood smear from mice's tail with the Giemsa stain on the object glass.

Statistical analysis

Data taken from antiplasmodial activity test were calculated the percentage of parasitemia, percentage of parasitemia obstacle, and determination of ED_{50}. Percentage of parasitemia used equation (1):

$$\%\ \text{Parasitaemia} = \frac{\text{Infected erythrocyte}}{\text{Erythrocyte total } (\pm 1000)} \times 100\% \quad (1)$$

Percentage of parasitemia obstacle used equation (2):

$$\%\ \text{Inhibition of parasitaemia} = \frac{\sum \text{parasitemia in negative control} - \sum \text{parasitemia in test}}{\sum \text{parasitemia in negative control}} \times 100\ \% \quad (2)$$

While determination of ED_{50} based on the relationship between dose and the percentage of parasite growth inhibition by probit analysis.

RESULTS

Calculation of infected erythrocyte was shown in the Tables 1 and 2. To calculate the percentage of parasitemia used equation (1).

According to Tables 1 and 2, control (+) shown negative results. In general, negative control and suspension of Fraction V administration presented increasing percentage of parasitemia both in male and female mice. In male mice, the average percentage of parasitemia was between 0.192% and 9.148%, while in female mice was just 8.403%. The bigger dose exposured in animals test, the less parasitemia value decreased in average, except for dose 100 mg/kg bw. It meant the higher of the average percentage of parasitemia, the more infectious *P. berghei* in Swiss-Webster mice conversely.

Table 1: Average percentage of parasitaemia in male Swiss-Webster mice

Test group (mg/kg bw)	Average percentage±SD (%)			
	D_{+1}	D_{+2}	D_{+3}	D_{+4}
Control (-)	0.192	1.758±0.933	6.865±3.229	9.148±5.922
Control (+)	-	-	-	-
12.5	-	0.645±0.161	4.357±1.903	10.058±4.015
25	-	0.907±0.455	4.283±2.987	9.151±5.597
50	-	0.416±0.388	1.626±1.565	4.825±4.195
100	-	0.678±0.522	2.782±1.646	7.981±3.870

SD: Standard deviation

Table 2: Average percentage of parasitemia in female Swiss-Webster mice

Test group (mg/kg bw)	Average percentage±SD (%)			
	D_{+1}	D_{+2}	D_{+3}	D_{+4}
Control (-)	0	1.867±1.016	4.750±3.916	8.403±6.606
Control (+)	0	0,000	0,000	0.000
12.5	0	1.587±0.798	4.760±3.642	7.766±6.900
25	0	0.806±0.599	3.822±3.116	8.589±5.252
50	0	0.420±0.328	1.742±1.714	4.830±4.067
100	0	1.679±0.468	2.490±1.337	7.547±3.868

SD: Standard deviation

Inhibition percentage of parasitemia

Determination of ED_{50} value based on percentage of inhibition parasite growing was observed in the end of administration (D_{+4}) using equation (2).

According to Tables 3 and 4, those can be seen the average of parasitemia obstacle by either male or female mice. Tukey's honestly significant difference analysis indicated that a dose of 50 mg/kg bw gave the differentiation with the negative control for inhibition parasite growth. In general, the less average percentage of parasitemia, the bigger average percentage of parasitemia obstacle. ED_{50} defined the ED_{50} required to inhibit a half of *P. berghei* that was infected in mice. Based on antiplasmodial activity test, the relationship between dose and the percentage of parasite growth inhibition by probit analysis the value of ED_{50} in male mice by Fraction V of Mundu's bark was obtained 47.424 mg/kg bw and in the female was obtained 8053,784 mg/kg bw.

DISCUSSION

In vivo-antiplasmodial activity test in this research was performed based on Peter's test (The 4 days supresive test). Inoculation of *P. berghei* in male Swiss-Webster mice had several advantages where male Swiss mice were quite sensitive toward the parasite infection and had more resistance against the infection that other mice. In the latest research conducted by Widodo and Rahayu [10], the highest antimalarial activity in the same condition as this research was achieved by a dose of 50 mg/kg bw of ethyl acetate fraction from Mundu's bark. The extract of Mundu's bark with n-hexane presented antiplasmodial activity through decreasing of parasitemia, particularly with a dose of 100 mg/kg bw could decrease parasitemia effectively [11]. In this research, antiplasmodial activity of Fraction V from Mundu's bark displayed in a dose of 50 mg/kg and 100 mg/kg bw with values of 47.255% and 12.761%, respectively.

Fraction V showing antiplasmodial activity from 4 various doses were 50 mg/kg bw and 100 mg/kg bw. This was caused by active compound showing antiplasmodial activity. There was xanthone that the most concentration was found in both doses. Besides, from the various dose

Table 3: Inhibition percentage of parasitemia at the fourth day in male mice

Dose (mg/kg bw)	% Inhibition parasitemia						
	Mice 1	**Mice 2**	**Mice 3**	**Mice 4**	**Mice 5**	**Mice 6**	**Average±SD**
12.5	−34.696	−21.349	-	-	−38.697	54.941	−9.950±43.892
25	-	97.967	15.184	−50.055	−49.497	−13.763	−0.033±61.182
50	8.013	73.798	81.941	56.777	−25.426	88.424	47.255±45.860
100	−7.313	−1.465	70.026	−37.527	-	40.085	12.761±42.309
Control (+)	100	100	100	100	100	100	100±0

SD: Standard deviation

Table 4: Inhibition percentage of parasitemia at the fourth day in female mice

Dose (mg/kg bw)	% Inhibition parasitemia						
	Mice 1	**Mice 2**	**Mice 3**	**Mice 4**	**Mice 5**	**Mice 6**	**Average±SD**
12.5	−60.788	−106.819	69.285	93.133	−20.433	71.141	7.587±82.113
25	91.765	58.681	−24.372	−65.893	−42.247	−31.227	−2.216±62.499
50	0.345	73.652	78.305	51.232	−33.583	85.148	42.517±48.401
100	−21.433	−5.784	67.107	−38.855	-	49.875	10.182±46.030
Control (+)	100	100	100	100	100	100	100±82.113

SD: Standard deviation

exposured, these doses were the biggest. However, the antiplasmodial activity shown different values between dose of 50 mg/kg bw and 100 mg/kg bw.

ED_{50} that can inhibit a half of parasite growing (ED_{50}) was counted based on the relationship between dose and percentage of inhibition parasite growing by compound test with prohibit analysis. Antiplasmodial activity of plant extract was divided into 3 types; the most potent extract (ED_{50}<100 mg/kg bw), potential extract (100 mg/kg bw <ED_{50} <100 mg/kg bw) and less potential (ED_{50}>500 mg/kg bw) [12]. From this research, ED_{50} was achieved by 47.424 mg/kg bw so that it has the most potential of antiplasmodial activity. In this research, it was yet conducted working mechanism of Fraction V. Fraction V contained xanthone, tannin, and flavonoid. Xanthone was the compound that respect to this antiplasmodial activity [1,6,7]. Even though antiplasmodial activity of xanthone has not clearly defined yet, but it is estimated forming dissolve complex compound with heme-protein to block hemozoin parasite forming [13]. Forming hemozoin is the process where parasite protects itself from toxic effect of releasing heme after the digestion of hemoglobin. This action model from xanthone probably interacts with monomer of heme, between Fe^{3+}-heme and oxygen from carbonyl group and interaction between both of aromatic system, and side group function of carboxylic between heme and xanthone in the fourth and fifth position [14].

CONCLUSION

According to the research, Fraction V of dichloromethane-ethyl acetate-methanol of Mundu's bark (*G. dulcis* (Roxb.) Kurz) had the most potential antiplasmodial activity by *in vivo* test for male Swiss-Webster mice, with an ED_{50} value was 47.424 mg/kg bw.

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