In Vivo Antimalarial Activity of Ethanol Extract and Ethyl Acetate Fraction of Alectryon serratus Leaves on Plasmodium berghei Infected Mice

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In Vivo Antimalarial Activity of Ethanol Extract and Ethyl Acetate Fraction of *Alectryon serratus* Leaves on *Plasmodium berghei* Infected Mice

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INTRODUCTION

Malaria was a major global public health concern due to the development of resistance by the most lethal causative species, *Plasmodium falciparum*. Natural products were potential sources of new antimalarial drugs (Bero, 2011; Nogueira, 2011). In vitro antimalarial activity screening of several Indonesian plants using HRP2 method showed that ethanol extract and ethyl acetate (EA) fraction of *Alectryon serratus* were active as an antimalarial (Widyawaruyanti, 2014). The aim of this study is to identify Thin Layer Chromatography (TLC) profile and investigate in vivo antimalarial activity of extract and fraction of *Alectryon serratus* leaves.

MATERIALS AND METHOD

Plant material and extraction

Leaves of *Alectryon serratus* was collected from Alas Purwo National Park, Banyuwangi, East Java, Indonesia, Authentication and identification of plant was carried out at the Purwodadi Botanical Garden, East Java. 1 kg of powdered material was extracted using 80% ethanol by ultrasonic assisted extraction (UaE) for two minutes, three times replication. The ethanol extract were filtered, pooled, and dried at 40°C using rotary evaporator and weighed afterwards. 100 grams of crude extract was suspended in distilled water and partitioned with dichloromethane and ethyl acetate successively, which were in turn concentrated to dryness in rotary evaporator. The crude extract and fractions were kept in air tight containers and were stored at 4°C for use in phytochemical screening and antimalarial bioassay.

Phytochemical screening

Dried crude extract and ethyl acetate fraction (10 mg) was dilute in methanol. The phytochemical screening was performed by Thin Layer Chromatography (TLC) method to determine the content of chemical compound of extract and fraction using certain optimized mobile phase and sprayed by 10% sulphuric acid reagent.

Animals

Male mice BALB/C strain were obtained from LPPT-Universitas Gajah Mada, Yogyakarta. They were weighting between 20-30 g and maintained on standard animal pellets and water ad libitum at Animal Laboratory of Institute of Tropical Disease, Universitas Airlangga. Permission and approval for animal studies were obtained from Faculty of Veterinary Medicine, Universitas Airlangga.

Rodent malaria parasite

Rodent parasites used were *Plasmodium berghei* ANKA strain. The parasite has been maintained at Institute of Tropical Disease, Universitas Airlangga by passage in male BALB/C mice.

In vivo antimalarial activity test

In vivo antimalarial activity was performed based on Peter's test (The 4-days suppressive test) (Phillipson, 1991).

Ethanol extract and ethyl acetat (EA) fraction were tested using 28 mice which divided to 7 groups. 3 groups were treated using extract at a dose of 100 mg/kgBW, 10 mg/kgBW, and 1 mg/kgBW, respectively. Meanwhile, 3 other groups were treated using EA fraction at a dose of 100 mg/kgBW, 10 mg/kgBW, and 1 mg/kgBW, respectively. One group was treated using CMC-Na 0.5% (as negative control). Each mice received 0,2 ml of diluted blood containing 5% *P. berghei* infected erythrocytes by intraperitonial route. Treatment of extract, EA fraction and negative control was given at one day after inoculation of parasite by orally at day-0 until day-3 (four consecutive days). Thin blood smears were made every day for 7 days (day-0 until day-6) and stained using 10% giemsa dye. Percentage of parasitemia and percentage of inhibition growth of *P. berghei* were calculated using the formula in below.

Percentage of parasitemia:

$$Xe/Xk \times 100\%$$

Percentage of inhibition:

$$100\%-(Xe/Xk \times 100\%)$$

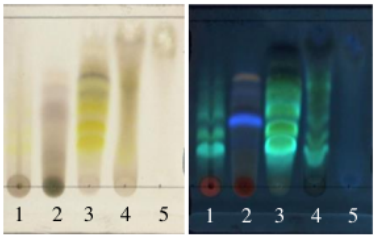
Xe: % parasitaemia growth of experimental group
Xk: % parasitaemia growth of negative control

Data analysis

The ED_{50} (Effective dose) were analyzed using probit analysis (SPSS software).

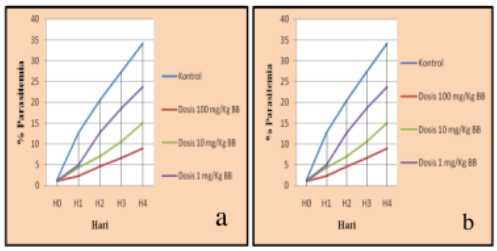
RESULTS DAN DISCUSSION

TLC profile showed that EA fraction of *A. serratus* (Picture 1, spot number 3) contained flavonoid compounds.



Picture 1. TLC Profile of extract *Alectryon serratus*

In vivo antimalarial assay of ethanol extract and EA fraction of *A. serratus* was done on *P. berghei* infected mice at a concentration of 100 mg/kgBW, 10 mg/kgBW and 1 mg/kgBW, respectively. The result showed that ethanol extract and EA fraction of *A. serratus* active as antimalarial agent with ED_{50} value of 13.82 mg/kg BW and 5.92 mg/kg BW. Flavonoid compound is considered to take effect in antimalarial activity of EA fraction from *Alectryon serratus* leaves. Further study to determine the active antimalarial compounds from *A.Serratus* leaves was needed.



Picture 2. Percentage parasitemia of ethanol extracts (a) and EA fraction (b) of *A. serratus* leaves against *P. berghei*.

Table 1. Activity of ethanol extract of *A. serratus* leaves on *P. berghei* infected mice

Dose (mg/Kg BW)	R	% Parasitemia					% Inhibition
		H0	H1	H2	H3	H4	
Negative control	1	1.50	12.2	22.67	29.87	38.50	
	2	1.44	13.0	19.89	25.60	32.43	
	3	1.55	13.1	20.05	29.40	35.78	
	4	1.38	12.9	19.50	24.71	30.18	
100	1	1.02	3.34	7.93	10.32	16.44	52.93
	2	0.90	2.70	5.20	7.48	10.32	71.25
	3	1.17	3.04	6.96	9.98	13.22	63.22
	4	1.05	2.65	5.10	7.50	10.20	72.07
10	1	1.58	5.06	9.98	14.05	17.85	50.34
	2	1.49	4.85	9.87	13.45	17.71	50.49
	3	1.59	4.98	10.60	14.21	18.10	49.60
	4	1.60	5.89	10.50	14.87	18.46	48.53
1	1	1.80	7.17	13.95	18.70	24.99	29.21
	2	1.50	6.40	13.56	17.63	25.24	27.53
	3	2.08	7.20	14.72	17.10	25.87	27.38
	4	1.98	7.28	14.21	18.85	25.67	27.69

Tabel 2. Activity of EA fraction of *A. serratus* leaves on *P. berghei* infected mice

Dose (mg/Kg BW)	R	% Parasitemia					% Inhibition
		H0	H1	H2	H3	H4	
Negative control	1	1.50	12.2	22.67	29.87	38.50	
	2	1.44	13.0	19.89	25.60	32.43	
	3	1.55	13.1	20.05	29.40	35.78	
	4	1.38	12.9	19.50	24.71	30.18	
100	1	1.09	2.31	4.42	6.20	8.24	78.17
	2	0.90	2.15	5.08	7.38	9.98	72.28
	3	1.00	2.10	4.64	6.97	8.95	75.73
	4	1.09	2.50	4.50	6.10	8.47	77.47
10	1	1.00	3.87	6.45	10.95	14.60	58.49
	2	1.10	4.05	6.37	9.89	14.34	59.58
	3	1.15	4.42	7.76	11.30	15.98	54.73
	4	1.20	4.85	7.50	10.25	15.50	56.35
1	1	1.22	5.23	12.78	18.22	23.30	32.60
	2	1.18	4.95	12.34	18.90	23.65	31.41
	3	1.20	4.80	12.10	17.86	23.10	33.15
	4	1.25	5.15	13.77	19.36	24.80	28.11

CONCLUSION

Ethanol extract and EA fraction of *A.serratus* were very active as antimalarial agent (very active if ED_{50}<100 mg/kgBW based on Munoz, 2000). EA fraction had higher antimalarial activity with ED_{50} value of 5.92 mg/kgBW and potential to be develop as a new antimalarial drug.

ACKNOWLEDGEMENT

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