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 40oC 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 4oC 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

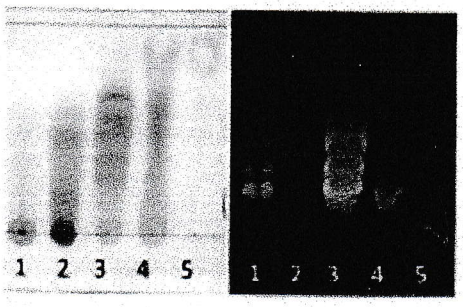
mg/kgBW, respectively. Meanwhile, 3 other groups were treated using EA fraction at a dose of 100 mg/kgBW, 10 mg/kgBW, and 1 mg/kgBW, respectively. One group was treated using CMC-Na 0.5% (as negative control). Each mice received 0,2 ml of diluted blood containing 5% P. berghei infected erythrocytes by 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 x 100%
Percentage of inhibition:
100%-(Xe/Xk x 100%)

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

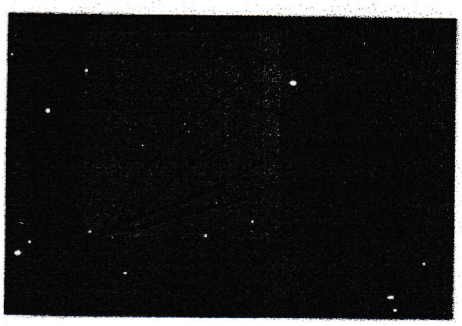
Data analysis
The ED50 (Effective dose) were analyzed using probit analysis (SPSS software).

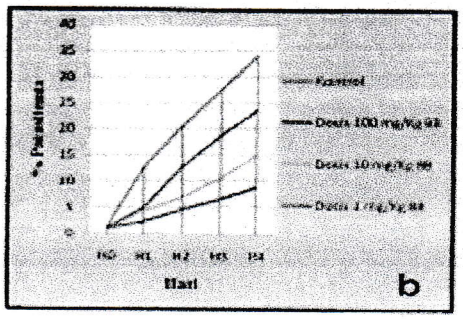
RESULTS DAN DISCUSSION
TLC profile showed that EA fraction of A. serratus (Picture 1, spot number 3) contained flavonoid compounds.



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 ED50 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	28.40	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	3.64	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	17.31	50.69
	3	1.59	4.98	10.69	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	12.56	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	
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	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	6.65	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 ED50<100 mg/kgBW based on Munoz, 2000). EA fraction had higher antimalarial activity with ED50 value of 5.92 mg/kgBW and potential to be develop as a new antimalarial drug.

ACKNOWLEDGEMENT

This study was supported by Directorate General of Higher Education DIPA BOPTN 2014, contract no 965/UN3/2014.

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CERTIFICATE

This is to acknowledge that

ATY WIDYAWARUYANTI

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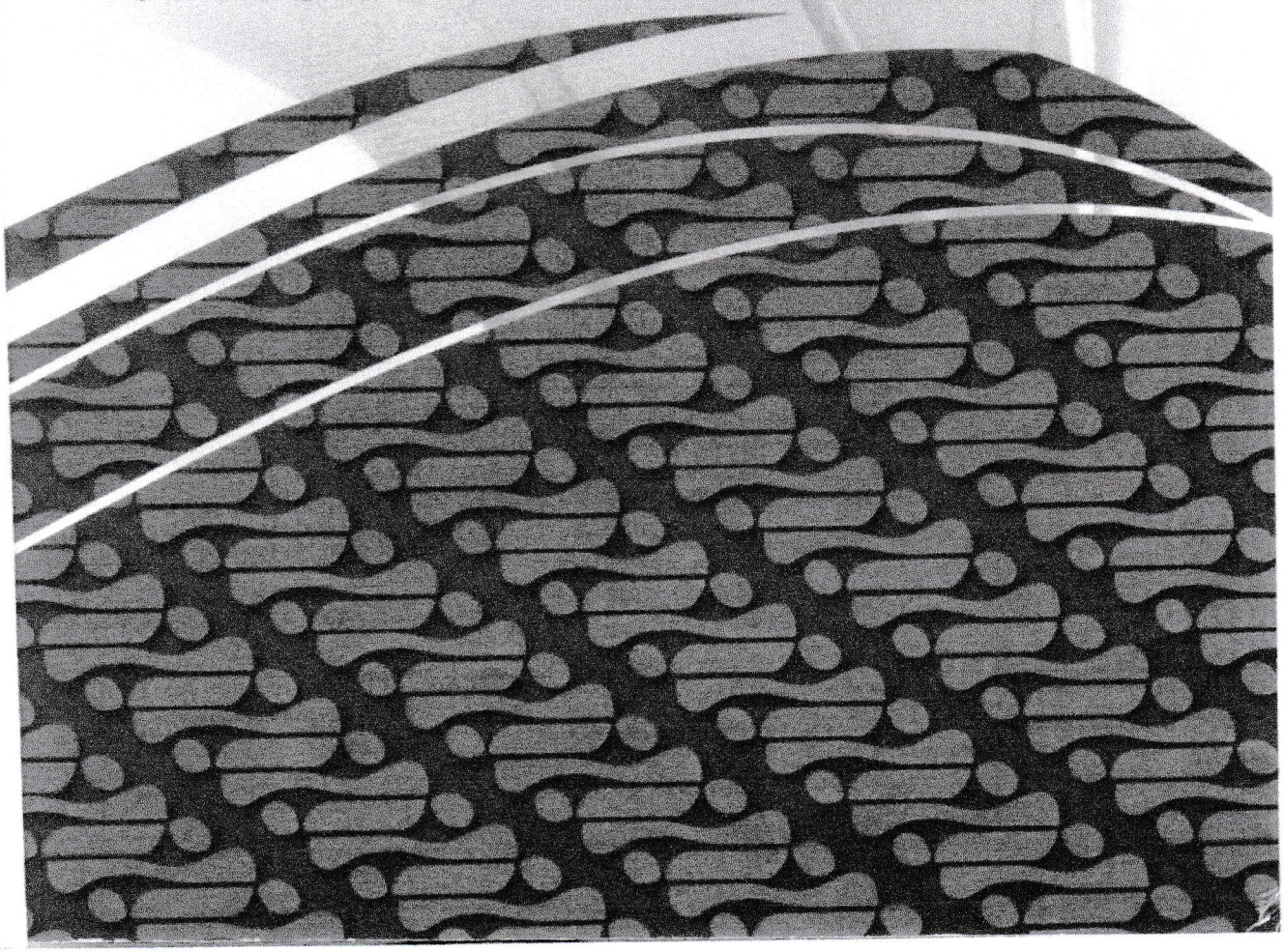

Proceeding

The 1st International Conference on Pharmaceutics & Pharmaceutical Sciences

Published and Organized by
Faculty of Pharmacy
Universitas Airlangga
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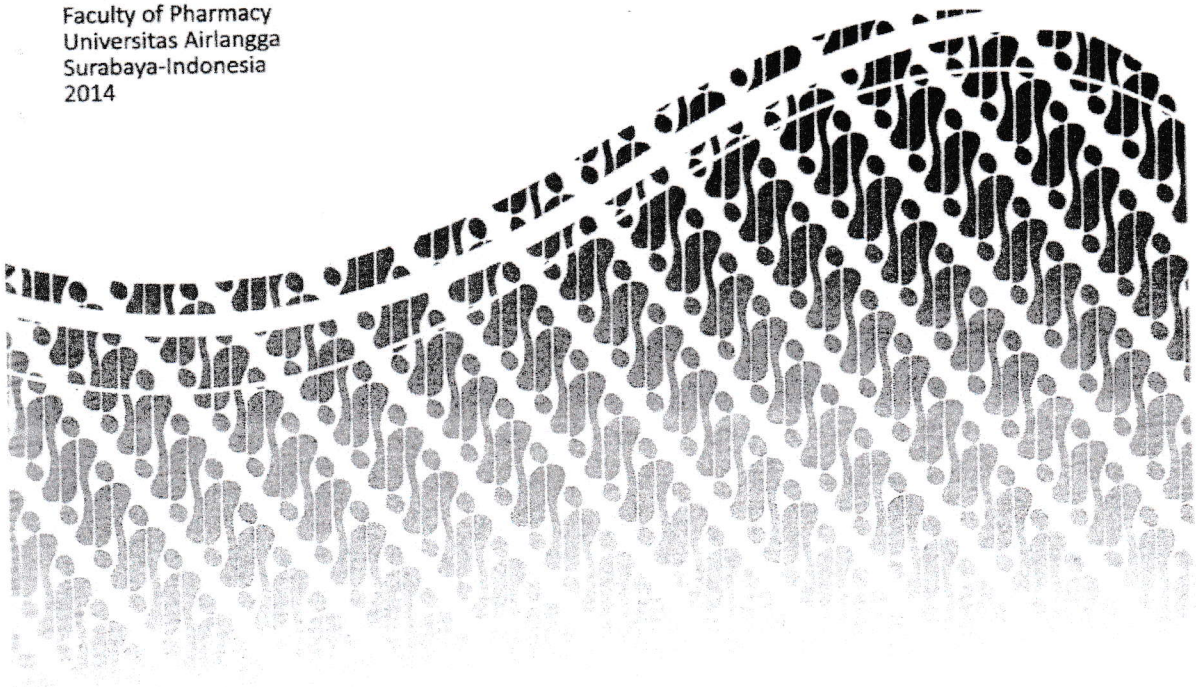

The 1st International Conference on Pharmaceutics & Pharmaceutical Sciences Proceedings

ISBN : 978-602-72333-0-0
(Letter of National ISBN Agency No. 4127/E.8/p/03.2015 Date 18 March 2015)

1st edition Proceeding
Published by:
Faculty of Pharmacy Universitas Airlangga
Surabaya, Indonesia

Address:
Kampus B Jl. Dharmawangsa Dalam
Surabaya 60286
Phone +62 31 5033710
Fax +62 31 5020514
Website: www.icpps2014.com or www.ff.unair.ac.id
Email: icppinfo@gmail.com

ISBN 978-602-72333-0-0

PREFACE From Chairman

It is our pleasure to present you the proceedings of The 1st International Conference on Pharmaceutics and Pharmaceutical Sciences (ICPPS) organized by The Faculty of Pharmacy Universitas Airlangga Surabaya Indonesia.

The proceeding was produced based on papers and posters presented at The 1st International Conference on Pharmaceutics and Pharmaceutical Sciences (ICPPS), held in Surabaya, Indonesia, 14-15 November 2014.

The proceeding clearly reflects broad interest, from the participants that coming from all around the world.

The papers presented were pharmaceutics and biopharmaceutics; requirements on how to evaluate molecules in discovery and their appropriateness for selection as potential candidate; their development in context of challenges and benefits, together with associated time and cost implications and also requirements to progress through pre-clinical and clinical.

In this an opportunity, I would like to express my appreciation to the editorial team of the proceeding who have been working hard to review manuscripts, and making the first edition of this proceeding be possible.

I would like also to thanks to all invited speakers and presenters who participated in The 1st International Conference on Pharmaceutics and Pharmaceutical Sciences (ICPPS) and your contribution to this proceeding.

Finally, I hope this proceeding will give contribution to the Pharmaceutics and Pharmaceutical Sciences research.

Chairman,

Dra. Esti Hendradi, MSI., Ph.D., Apt

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TABLE of CONTENT

AUTHOR INDEX