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Submission date: 16-Dec-2020 03:04PM (UTC+0800)
Submission ID: 1476576925
File name: C-43-_Fullpaper_malaria_Acmena-07112014.doc (434.5K)
Word count: 969
Character count: 5585

In Vitro Antimalarial Activity of Chloroform Subfraction of Salam Badak Leaves (*Acmena acuminatissima*)

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INTRODUCTION

Malaria is still an endemic disease in more than 90 countries, mainly in developing countries (Sanchez et al., 2004). Patients infected with malaria have doubled in the last two decades. This occurs mainly due to the emergence of resistant strains of *Plasmodium falciparum* malaria to the chloroquine and its derivatives (Trape et al., 2002). Perez et al. (1997) stated that the global spread of malaria parasites that are resistant to current antimalarial drug is a major health problem. Therefore, it is need to find new antimalarial substances to replace current drugs that are not sensitive anymore. One attempt to discover new antimalarial substances is through active exploration of the natural sources.

It is known that some species of the family Myrtaceae has an antimalarial activity. Previous studies shown that ethanol extract and chloroform fraction of *A. acuminatissima* leaves (Myrtaceae) were exhibited antimalarial activity with IC_{50} value of 0.040 μg/ml and 0.006 μg/ml, respectively. Therefore, the separation of the chloroform fraction was done to determine the antimalarial activity substances of *A.acuminatissima*.

MATERIALS AND METHODS

Plant materials

A.acuminatissia leaves was obtained from Purwodadi Botanical Garden on December 2012. Sample was authenticated by the authority of Purwodadi Botanical Garden, Pasuruan, East Java.

Fractionation

Chloroform fractions which obtained from the fractionation of ethanol extract of *A.acuminatissima* leaves was separated by vacum liquid chromatography (VLC) using hexane, chloroform, and ethanol at gradient condition. This separation was produced six subfractions, then evaporated using a vacum evaporator and dried. These six subfractions was then analyzed by TLC and tested for antimalarial activity.

In vitro antimalarial activity test

Antimalarial in vitro test was performed based on Budimulya et al. (1997). Sample prepared in serial dilution at concentration of 0.01; 0.1; 1; 10 and 100 µg/ml in microwells. Each microwell was added with 500 µl parasite culture (1% parasitemia, 5% haematocrit) and incubated for 48 hours in 37°C. After incubation, thin blood smears were made and stained using 20% giemsa dye. Percentage of parasitemia was determined by counting infected erythrocytes per 1000 total erythrocytes under microscope.

RESULTS AND DISCUSSION

The separation results of *A.acuminatissima* leaves chloroform fraction was produced six subfractions (A-F). Each subfraction was tested for in vitro antimalarial activity against *P. falciparum* (3D7). According to Rasoanaivo et al. (2004), an extract is very active if $IC_{50} < 5$ μg/mL, active 5 μg/mL $< IC_{50} < 50$ μg/mL, weakly active 50 μg/mL $< IC_{50} < 100$ μg/mL and inactive $IC_{50} > 100$ μg/mL. The test results showed that all chloroform subfractions classified as very active and subfraction E was the most active with IC_{50} value of 0.007 μg/mL. The result of antimalarial activity test of chloroform subfractions (A-F) can be seen in Table 1.

Identification of chloroform subfraction was performed by TLC method using silica gel GF_{254} as stationary phase and chloroform: methanol (98:2) as a mobile phase. Then observed under UV light in wavelength of 254 nm, 366 nm and sprayed with H_2SO_4 10%. TLC chromatogram profile showed that all subfractions contain purple spot (Fig.1). According to Sharifa et al. (2012), terpenoid compounds will form a pink to purple or violet after being sprayed with 10% H_2SO_4. It can be considered that all subfractions were containing terpenoids.

Sub fraction	% Inhibition at a concentration of (μg/ml)					IC_{50} (μg/ml)
	100	10	1	0.1	0.01	
A	100	100	75.32	65.18	56.19	0.012
B	100	83.39	63.95	47.69	24.32	0.158
C	86.41	83.94	41.38	35.17	20.40	1.469
D	96.90	92.48	64.83	36.5	26.40	0.172
E	100	85.70	70.42	69.84	56.75	0.007
F	97.19	91.41	68.41	59.81	54.61	0.014

Table 1. IC_{50} values of Chloroform subfractions against *P. falciparum* 3D7

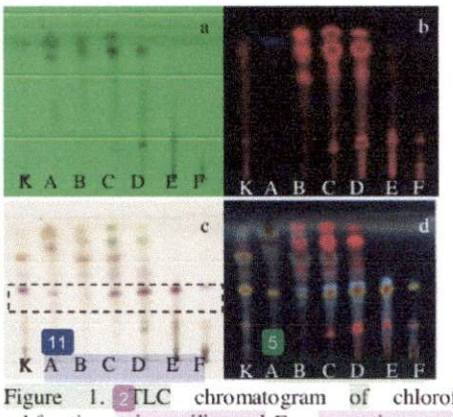

Figure 1. TLC chromatogram of chloroform subfraction using silica gel F_{254} as stationary phase and chloroform-methanol (98:2) as mobile phase, viewed under UV light : (a) 254 nm; (b) 366 nm; (c) after sprayed with 10% H_2SO_4 and heated at 105°C for 5 minutes. (d) 366 nm after sprayed with 10% H_2SO_4 and heated at 105°C for 5 minutes; K = chloroform fraction, A-F = subfraction.
[ ] = considered to contain terpenoids.

Subfraction A-F were exhibited antimalarial activity (IC_{50} value of 0.007-1.469 μg/ml) and containing terpenoids substances. Based on that result, it is possible to conclude that antimalarial activity of subfractions was derived from terpenoids substances.

CONCLUSION

Subfraction E of *A.acuminatissima* leaves was the most active as antimalarial with IC_{50} value of 0.007 μg/ml. The antimalarial activity was possible derived from terpenoids substances. Further study needed to isolate and identify the active compounds from *A.acuminatissima*

ACKNOWLEDGEMENT

The authors acknowledge Satreps Lab-Natural Product Medicine Research Development Division, Institute of Tropical Diseases Universitas Airlangga for suporting this research.

REFERENCES

1. Budimulya, AS., Syafruddin, Tapchaisri, P., et al. 1997. The sensitivity of Plasmodium protein synthesis to prokaryotic ribosomal inhibitors. *Mol Biochem Parasitol* 84:137-141
2. Perez, H., Diaz, F., Medina, JD., 1997. Chemical investigation and in vitro antimalarial activity of *Taebuina ochracea ssp. neochrysantha*. International Journal of Pharmacognosy 35(4) : 227-231.
3. Rasoanaivo, P., Dehar, OE., Ratsimamanga, U., et al. 2004. Guidelines for The Nonclinical Evaluation of The Efficacy of Traditional Antimalarials. In : Traditional Medicinal Plants and Antimalaria. CRC Press. USA 256-268.
4. Sanchez, BAM., Mota, MM., Sultan, AA., et al. 2004. *Plasmodium berghei* parasite transformed with green fluorescent protein for screening blood schizontocidal agents. *Int. J. of Parasitology* 34 : 485-490.
5. Sharifa, AA., Jamaludin, J., Kiong, LS., et al. 2012. Anti-Urolithiatic Terpenoid Compound from *Plantago major* Linn. (Ekor Anjing). *Sains Malaysiana*, Vol. 41(1): 33–39.
6. Trape. JF., Pison, G., Speigel, A., et al. 2002. Combating malaria in Africa. *Trends in Parasitology* 18 : 224-230.

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