ICPPS 2014

Proceeding

The 1st International Conference on Pharmaceutics & Pharmaceutical Sciences

Drug Delivery Systems:
From Drug-Discovery, Pre-formulation, Formulation and Technological Approaches for Poorly Soluble Drugs and Protein

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14-15 November 2014
PULLMAN Surabaya City Centre

Drug Delivery Systems:
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Organized by :
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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 occurred 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 IC50 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 vacuum 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 37oC. 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 IC50 < 5 µg/mL, active 5 µg/mL < IC50 < 50 µg/mL, weakly active 50 µg/mL < IC50 < 100 µg/mL and inactive IC50 > 100 µg/mL. The test re-

...showed that all chloroform subfractions classified as very active and subfraction E was the most active with IC50 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 GF254 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 H2SO4 10%. TLC chromatogram profile showed that all subfractions contain purple spot (Fig.1). According to Sharma et al. (2012), terpenoid compounds will form a pink to purple or violet after being sprayed with 10% H2SO4. It can be considered that all subfractions were containing terpenoids.

	% 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	97.39	91.41	68.41	59.81	54.61	0.014

Table 1. IC50 values of Chloroform subfractions against P. falciparum 3D7

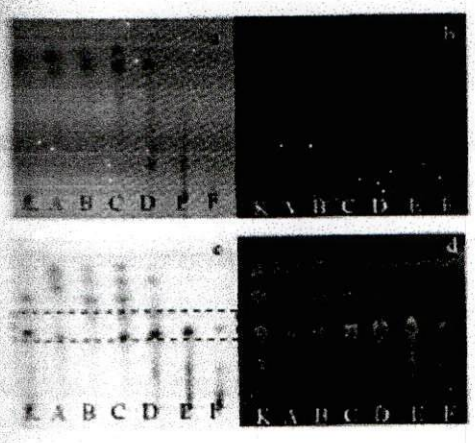

Figure 1. TLC chromatogram of chloroform subfraction using silica gel F254 as stationary phase and chloroform-methanol (98:2) as mobile phase, viewed under UV light : (a) 254 nm; (b) 366 nm; (c) after sprayed with 10% H2SO4 and heated at 105oC for 5 minutes. (d) 366 nm after sprayed with 10% H2SO4 and heated at 105oC for 5 minutes; K = chloroform fraction, A-F = subfraction.

[ ] = considered to contain terpenoids.

Subfraction A-F were exhibited antimalarial activity (IC50 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 IC50 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.

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