

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

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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 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 $0.040 \mu\text{g/ml}$ and $0.006 \mu\text{g/ml}$, 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. acuminatissima 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 vacuum 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 $\mu\text{g/ml}$ 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 $IC_{50} < 5 \mu\text{g/mL}$, active $5 \mu\text{g/mL} < IC_{50} < 50 \mu\text{g/mL}$, weakly active $50 \mu\text{g/mL} < IC_{50} < 100 \mu\text{g/mL}$ and inactive $IC_{50} > 100 \mu\text{g/mL}$. The test results showed that all chloroform subfractions classified as very active and subfraction E was the most active with IC_{50} value of $0.007 \mu\text{g/mL}$. 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 Sharifa 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.

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. IC₅₀ values of Chloroform subfractions against *P. falciparum* 3D7

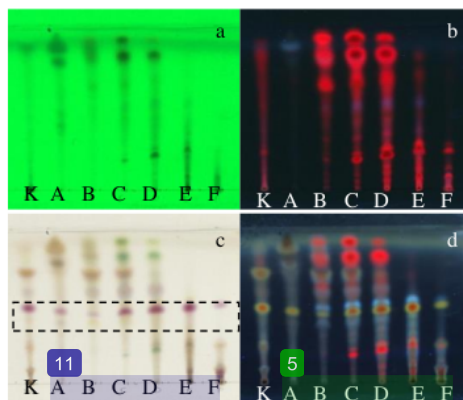


Figure 1. TLC chromatogram of chloroform subfraction using silica gel F₂₅₄ as stationary phase and chloroform-methanol (98:2) as mobile phase, viewed under UV light : (a) 254 nm; (b) 366 nm; (c) after sprayed with 10% H₂SO₄ and heated at 105°C for 5 minutes. (d) 366 nm after sprayed with 10% H₂SO₄ and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

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