

In Vitro Antimalarial Activity of Dichloromethane Sub-fraction of Eucalyptus globulus L. Stem against Plasmodium falciparum

by Aty Widyawaruyanti

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In Vitro Antimalarial Activity of Dichloromethane Sub-fraction of *Eucalyptus globulus* L. Stem against *Plasmodium falciparum*

Elis Suwarni, Akademi Farmasi Saraswati, Denpasar, Bali; Achmad Fuad Hafid, Aty Widyawaruyanti, Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286; Institute of Tropical Disease Universitas Airlangga, Campus C Unair Mulyorejo Surabaya 60115, aty-w@ff.unair.ac.id

INTRODUCTION

Malaria is a serious infectious disease caused by protozoan parasites in tropical and subtropical regions. In 2010, malaria was endemic in about 104 countries worldwide and approximately 219 million cases of malaria caused 660,000 deaths. Approximately 90% of malaria deaths occur in Africa (WHO, 2012). Global spread of multiple drug-resistant malaria has become a major health problem and efforts to search for new antimalarial are needed.

Eucalyptus globulus is a plant of the Myrtaceae family that in Indonesia commonly known as kayu putih and empirically used as an antipyretic (Backer, 1968). In Brazil, *E. globulus* is used as an antimalarial plants (Nagpal *et al.*, 2010). In Cameroon, *E. globulus*, *Carica papaya* and *Psidium guajava* leaves are mixed and boiled as a decoction that is drunk for the treatment of malaria (Titanji *et al.*, 2008). In Venezuela, *E. globulus* leaves is boiled as decoction for the treatment of malaria (Carballo *et al.*, 2004).

Our preliminary study showed that the 80% ethanol extract and dichloromethane fraction were very active as an antimalarial with IC_{50} of 0.090 $\mu\text{g/mL}$ and 0.022 $\mu\text{g/mL}$, respectively.

This study aims to separate the dichloromethane fraction and to test antimalarial activity of its subfractions.

MATERIAL AND METHODS

Plant Material

Eucalyptus globulus stem was obtained from Cangar Forest at Malang, East Java on April 2010. Sample was authenticated by the authority of Purwodadi Botanical Garden, Pasuruan, East Java.

Separation Method

Vacuum liquid chromatography (VLC) of dichloromethane fraction of *E. globulus* stem was performed using hexane- CHCl_3 (25% gradient) to CHCl_3 -MeOH (98:2, 96:4, 94:6, 90:10, 85:15 and 80:20).

Thin Layer Chromatography (TLC) Method

Sub-fractions obtained from Vacuum liquid chromatography (VLC) of dichloromethane fraction were monitored by TLC using silica gel F₂₅₄ as stationary phase and chloroform-methanol (98:2) as mobile phase. The separated spots were visualized under ultra-violet light of

two different wavelengths (UV₂₅₄ nm and UV₃₆₅ nm) and visible light before and after sprayed with 10% H_2SO_4 and heated at 105°C for 5 minutes.

In Vitro Antimalarial Activity Test

Antimalarial activity of sub-fractions was assessed against *Plasmodium falciparum* strain 3D7 which is sensitive to chloroquine. This strain was maintained in continuous culture in flask according to the methodology described by Tragger and Jensen (1976).

Percentage inhibition was calculated using formula:

$$\left[\frac{(\% \text{ parasitaemia in control wells} - \% \text{ parasitaemia of test wells})}{(\% \text{ parasitaemia of the control})} \right] \times 100$$
 (Ngemenya *et al.*, 2006).

IC_{50} values refers to the concentration required to inhibit 50% of parasite's growth (Mustofa *et al.*, 2007).

RESULTS AND DISCUSSION

Vacuum liquid chromatography of dichloromethane fraction produced 8 sub-fractions (D.1 - D.8 sub-fractions). TLC chromatogram of dichloromethane sub-fractions was shown in figure 1.

Antimalarial activity test showed that IC_{50} value of each dichloromethane sub-fractions was 10.284 $\mu\text{g/mL}$, 16.387 $\mu\text{g/mL}$, 0.053 $\mu\text{g/mL}$, 1.059 $\mu\text{g/mL}$, 0.318 $\mu\text{g/mL}$, 0.387 $\mu\text{g/mL}$, 0.150 $\mu\text{g/mL}$ and 0.040 $\mu\text{g/mL}$. D.8 sub-fraction has the lowest IC_{50} value of 0.040 $\mu\text{g/L}$. This activity was analysed in accordance with the norm of plants antimalarial activity of Rasoanaivo *et al.* (1992). According to this norm, 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 $IC_{50} > 100 \mu\text{g/mL}$. Based on this classification, result from this study of D.8 sub-fraction of *E. globulus* stem with IC_{50} of 0.040 $\mu\text{g/mL}$ is said to have very active antimalarial activity. The result of antimalarial activity test of dichloromethane sub-fractions (D.1 - D.8 sub-fractions) can be seen in Table 1.

TLC test of sub-fractions indicated the presence of the most dominant spot (spot D) on D.8 sub-fraction with R_f values of 0.40 which gave a red purple colour after sprayed with 10% H_2SO_4 and heated at 105 °C for 5 minutes. Spot D began to appear on D.6 sub-fraction which has the IC_{50} value of 0.387 $\mu\text{g/L}$. Colour intensity of spot D increased on D.7 and D.8 sub-fractions which have the IC_{50} values lower than that of D.6 sub-fraction (0.387 $\mu\text{g/mL}$). From

these data can be seen that the higher concentration of spot D, the lower IC₅₀ value of sub-fractions. Therefore, it can be presumed that spot D on D.8 sub-fraction is a substance that is responsible for activity of D.8 sub-fraction.

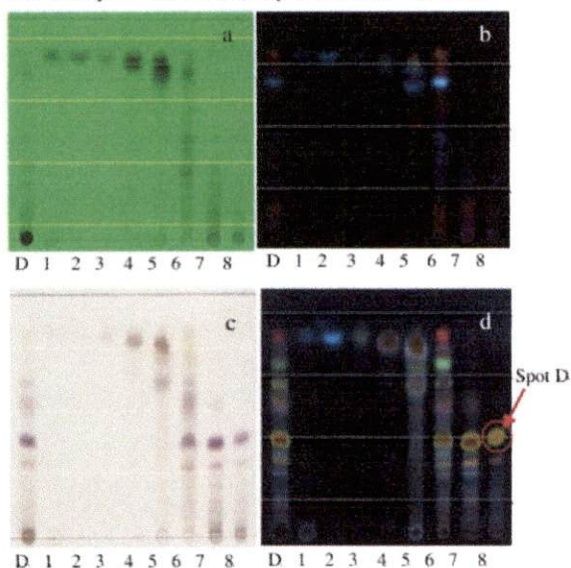


Figure 1. TLC chromatogram of dichloromethane sub-fraction using silica gel F₂₅₄ as stationary phase and chloroform-methanol (98:2) as mobile phase, viewed under UV light : (a) 254 nm; (b) 366 nm; (c) after sprayed with 10% H₂SO₄ and heated at 105°C for 5 minutes; (d) 366 nm after sprayed with 10% H₂SO₄ and heated at 105°C for 5 minutes; D = dichloromethane fraction, D.1-D.8 = sub-fraction.

Table 1. IC₅₀ values of dichloromethane sub-fractions of *E. globulus* L. stem against *P. falciparum*

Sam ple	Percent of average inhibitions at various doses ($\mu\text{g/mL}$)					IC ₅₀ ($\mu\text{g/mL}$)
	100	10	1	0.1	0.01	
D.1	73.46	40.42	31.63	21.42	16.32	10.284
D.2	63.27	46.95	29.77	10.38	1.63	16.387
D.3	89.83	79.10	69.52	51.22	41.12	0.053
D.4	87.40	54.41	44.55	39.60	21.58	1.059
D.5	92.41	75.86	52.80	43.27	24.13	0.318
D.6	90.20	70.94	55.43	36.34	27.30	0.387
D.7	89.50	73.96	58.95	45.41	36.15	0.150
D.8	92.24	80.80	72.99	53.92	41.85	0.040

CONCLUSION

D.8 sub-fraction of *E. globulus* possesses a very active antimalarial activity and might be a good candidate for antimalarial. Further work is suggested to isolate, identify and characterize the active principles from this substance.

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