



ICPPS 2014

# Proceeding

## The 1<sup>st</sup> International Conference on Pharmaceutics & Pharmaceutical Sciences

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

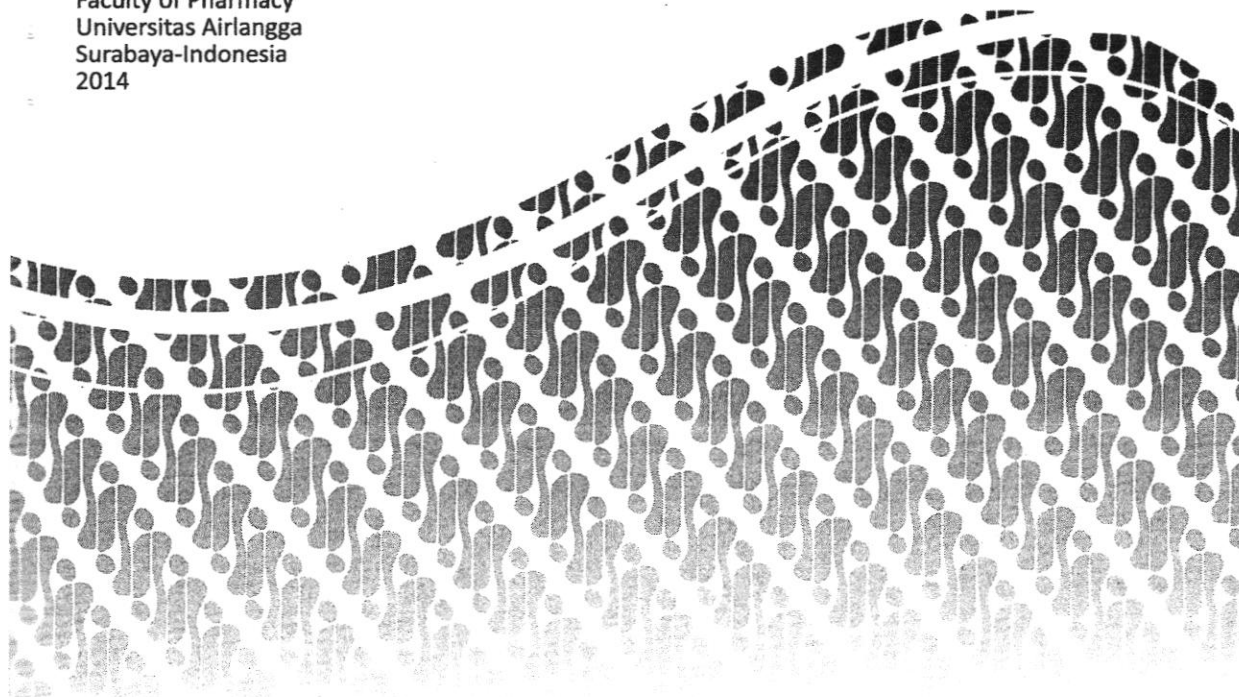
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2014

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## **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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## IN VITRO ANTIMALARIAL ACTIVITY OF DICHLOROMETHANE SUB-FRACTION OF *Eucalyptus globulus* L. STEM AGAINST *Plasmodium falciparum*

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### 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<sub>50</sub> of 0.090 µg/mL and 0.022 µg/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<sub>3</sub> (25% gradient) to CHCl<sub>3</sub>-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<sub>2</sub>SO<sub>4</sub> 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:

$$\left[ \frac{(\% \text{ parasitaemia in control wells} - \% \text{ parasitaemia of test wells})}{(\% \text{ parasitaemia of the control wells})} \right] \times 100$$

control)) x 100 (Ngemenya et al., 2006).  
IC<sub>50</sub> values refers to the concentration re-  
quired to inhibit 50% of parasite's growth  
(Mustofa et al., 2007).

### RESULTS AND DISCUSSION

Vacuum liquid chromatography of dichloro-  
methane 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<sub>50</sub> val-  
ue of each dichloromethane sub-fractions was  
10.284 µg/mL, 16.387 µg/mL, 0.053 µg/mL,  
1.059 µg/mL, 0.318 µg/ml, 0.387 µg/mL, 0.150  
µg/mL and 0.040 µg/mL. D.8 sub-fraction has  
the lowest IC<sub>50</sub> value of 0.040 µg/L. This activi-  
ty 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<sub>50</sub> < 5 µg/mL, active 5 µg/mL  
< IC<sub>50</sub> < 50 µg/mL, weakly active 50 µg/mL <  
IC<sub>50</sub> < 100 µg/mL and inactive IC<sub>50</sub> > 100 µg/  
mL. Based on this classification, result from  
this study of D.8 sub-fraction of *E. globulus*  
stem with IC<sub>50</sub> of 0.040 µg/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 pres-  
ence of the most dominant spot (spot D) on  
D.8 sub-fraction with R<sub>f</sub> values of 0.40 which  
gave a red purple colour after sprayed with  
10% H<sub>2</sub>SO<sub>4</sub> and heated at 105 oC for 5 min-  
utes. Spot D began to appear on D.6 sub-fraction  
which has the IC<sub>50</sub> value of 0.387 µg/L.  
Colour intensity of spot D increased on D.7  
and D.8 sub-fractions which have the IC<sub>50</sub> val-  
ues lower than that of D.6 sub-fraction (0.387  
µg/mL). From these data can be seen that the  
higher concentration of spot D, the lower IC<sub>50</sub>  
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.

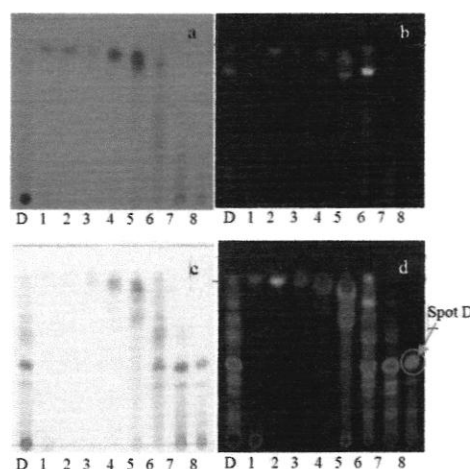


Figure 1. TLC chromatogram of dichloromethane sub-fraction using silica gel F254 as stationary phase and chloroform-methanol (98:2) as mobile phase, viewed under UV light : (a) 254 nm; (b) 366 nm; (c) after sprayed with 10% H<sub>2</sub>SO<sub>4</sub> and heated at 105oC for 5 minutes. (d) 366 nm after sprayed with 10% H<sub>2</sub>SO<sub>4</sub> and heated at 105oC for 5 minutes; D = dichloromethane fraction, D.1-D.8 = sub-fraction.

Table 1. IC<sub>50</sub> values of dichloromethane sub-fractions of *E. globulus* L. stem against *P. fal-  
ciparum*

Sam- ple	Percent of average inhibitions at various doses (µg/mL)					IC <sub>50</sub> (µg/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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