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The 1st International Conference on Pharmaceutics & Pharmaceutical Sciences

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

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on Pharmaceutics & Pharmaceutical Sciences

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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 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 tropical and subtropical areas of the world, such as in Indonesia (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 showed that ethanolic 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. 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 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 Rasoaivo 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-
sults 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 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.

<table>
<thead>
<tr>
<th>Subfraction</th>
<th>% Inhibition at a concentration of 100 μg/ml</th>
<th>IC50 (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>100</td>
<td>0.012</td>
</tr>
<tr>
<td>B</td>
<td>100</td>
<td>0.158</td>
</tr>
<tr>
<td>C</td>
<td>100</td>
<td>1.469</td>
</tr>
<tr>
<td>D</td>
<td>100</td>
<td>0.172</td>
</tr>
<tr>
<td>E</td>
<td>100</td>
<td>0.007</td>
</tr>
<tr>
<td>F</td>
<td>100</td>
<td>0.014</td>
</tr>
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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.

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

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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