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

COMMITTEE

Advisor : Dean of Faculty of Pharmacy Universitas Airlangga
Chairman : Dra. Esti Hendradi, M.Si., Ph.D, Apt.

Editor : Dr. Wouter LJ. Hinrichs (Netherlands)
Prof. Kozo Takayama (Japan)
Dr. Srinivas Mutalik (India)
Dr. Nuttakorn Baisaeng (Thailand)
Prof. Dr. Widji Soeratri, DEA (Indonesia)

Committee : Prof. Dr. Widji Soeratri, DEA, Apt.
Prof. Dr. Siswandono, MS., Apt.
Prof .Dr. Tutuk Budiati, MS., Apt.
Prof. Dr. rer.nat. Muhammad Yuwono, MS., Apt.
Drs. Hadi Poerwono, MSc.,Ph.D, Apt.
Drs. Marcellino Rudyanto, MSi., Ph.D, Apt.
Drs. Bambang Widjaja, M.Si, Apt.
Drs. Sugiyartono, MS, Apt.
Dra.Tutiek Purwanti, MSi, Apt.
Dra.Tristiana Erawati, MSi, Apt.
Dra.Retno Sari, MSc, Apt.
Dra.Dewi Isadiartuti, MSi, Apt.
Dra.Noorma Rosita, MSi, Apt.
Dr. Dwi Setyawan, SSi, MSi., Apt.
Dr. Riesta Primaharinastiti, SSi, MSi, Apt.
Dewi Melani Hariyadi, SSi, M.Phil., Ph.D, Apt.
Helmy Yusuf, SSi, MSc., Ph.D, Apt.
Dr. rer. nat. Maria Lucia Ardhani Dwi Lestari, SSi,MPharmSci., Apt.
Melanny Ika Sulistyowati, SFarm, M.Sc, Apt.
Febri Annuryanti, SFarm,M.Sc, Apt.
M. Faris Adrianto, MFarm, Apt.
Dini Retnowati, SFarm,Apt.
Abhimata P, SFarm, Apt.

CERTIFICATE

This is to acknowledge that

ELIS SUWARNI

SK No. : 024/SK-SKP/PP.IAI/IV/2014

has successfully attended the

as Poster Presenter & Participant

The 1st International Conference on Pharmaceutics & Pharmaceutical Sciences

14-15 November 2014
PULLMAN Surabaya City Centre

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

Organized by :
FACULTY OF PHARMACY AIRLANGGA UNIVERSITY

Dr. Umi Athiyah, MS, Apt
Dean
Faculty of Pharmacy Airlangga University

Dra. Esti Hendradi,MSi,PhD,Apt.
Chairman
ICPPS

Participant	: 12 SKP		
Committee	: 3 SKP	Oral/Poster Presenter	: 3 SKP
Moderator	: 3 SKP	Keynote Speaker	: 7 SKP

ICPPS 2014

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, Department of Pharmacognosy-Phytochemistry, Faculty of Pharmacy, Universitas Airlangga, Surabaya 60286; Institute of Tropical Disease Universitas Airlangga, Campus C Unair Mulyorejo Surabaya 60115; 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 IC50 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-$CHCl_3$ (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 105oC 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:

[(% parasitaemia in control wells – % parasitaemia of test wells)/(% parasitaemia of the

200 (Ngemenya et al., 2006).
values refers to the concentration required to inhibit 50% of parasite's growth (Basco et al., 2007).

AND DISCUSSION

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

antimalarial activity test showed that IC50 values of such dichloromethane sub-fractions was µg/mL, 16.387 µg/mL, 0.053 µg/mL, 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 IC50 value of 0.040 µg/L. This activity was analysed in accordance with the norm of Rasoanaivo et al. (2011). According to this norm, 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. Based on this classification, result from the study of D.8 sub-fraction of E. globulus stem with IC50 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 presence of the most dominant spot (spot D) on D.8 sub-fraction with Rf values of 0.40 which gave a red purple colour after sprayed with 10% H_2SO_4 and heated at 105 oC for 5 minutes. Spot D began to appear on D.6 sub-fraction which has the IC50 value of 0.387 µg/L. Colour intensity of spot D increased on D.7 and D.8 sub-fractions which have the IC50 values 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 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.

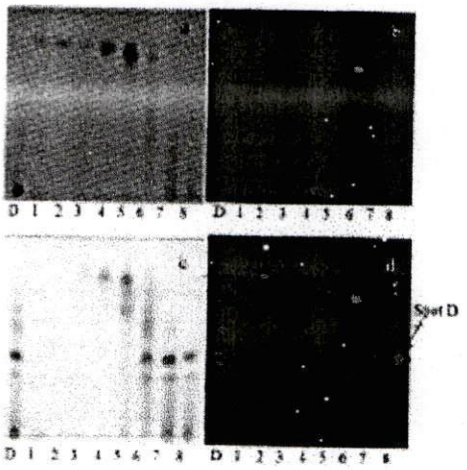

Figure 1. TLC chromatogram of dichloromethane sub-fraction using silica gel F254 as stationary phase and chloroform-methanol (98:2) as mobile phase, viewed under UV light : (a) 254 nm; (b) 366 nm; (c) after sprayed with 10% H_2SO_4 and heated at 105oC for 5 minutes. (d) 366 nm after sprayed with 10% H_2SO_4 and heated at 105oC 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

Sample	Percent of average inhibitions at various doses (µg/mL)					IC_{50} (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.

ACKNOWLEDGEMENTS

The authors acknowledge Satreps Lab-Natural Product Medicine Research Development Division, Institute of Tropical Disease Universitas Airlangga for supporting this research.

REFERENCES

1. Backer CA, van den Brink B. (1968). Flora of Java. vol 2, Noodhoff NV, Groningen, Nederlands, hal. 195, 203, 478.
2. Bloland PB, (2001). Drug Resistence in Malaria. WHO, Switzerland.
3. Caraballo A, Caraballo B, Acosta AR. (2004). Preliminary assessment of medic inal plants used as antimalarials in the southeastern Venezuelan Amazon. Revis ta da Sociedade Brasileira de Medicina Tropical 37(2) : 186-188.
4. Mustofa J, Sholikhah EN, Wahyuono S. (2007). In vitro and in vivo antiplasmodial activity and cytotoxicity of extracts of Phyllanthus niruri L. herbs traditionally used to treat malaria in Indonesia, South east Asian Journal of Tropical Medicine and Public Health, 38(4): 609-615.
5. Nagpal N, Shah G, Arora NM, et al. (2010). Phytochemical and Pharmacol ical Aspects of Eucalyptus Genus. Sci 1(12): 28-36.
6. Ngemenya MN, Akam TM, Yong JN, et (2006) Antiplasmodial activities of cru products from Turrenthus africanus liaceae), African Journal of Health Scie es, 13: 33 - 39.
7. Rasoanaivo P, Ratsimamanga-Urverg Ramanitrhasimbola D, et al. (1992). Criblage d'extraits de plantes de Mad gascar pour recherche d'activité anti dique et d'effet potentialisateur de la chloroquine. J Ethnopharmacol 64 126.
8. Titanji VPK., Zofou D, Ngemenya MN. (2008). The Antimalarial Potential of dicinal Plants Used for The Treatment Malaria in Cameroonian Folk Medicine Afr. J. Trad. Cam : 5(3) : 302 – 321.
9. Trager W, Jensen WB. (1976). Human Malaria Parasites in Continuous Culture In : Science 193:673-675.
10. WHO. (2012),. World Malaria Report 2012, http://www.who.int/malaria/publications/world_malaria_report_2012/en/index.htm.