



University of Jenderal
Soedirman



National Working Group for
Indonesian Medicinal Plant

INTERNATIONAL CONFERENCE ON MEDICINAL PLANTS 2012

Horison Hotel Purwokerto, 11-13 October 2012

PROCEEDING BOOK



**Scientification of Jamu
(Evidence-based Jamu Development):
A Breakthrough Program from Plant
to Medicine for Health Care**

**The 43rd Meeting of National Working Group
on Indonesia Medicinal Plant**

**"Exploration, Conservation, Development,
and Utilization of Indonesian Medicinal Plant"**

Penerbit :
**UNIVERSITAS JENDERAL SOEDIRMAN
PURWOKERTO**

PROCEEDINGS OF INTERNATIONAL CONFERENCE ON MEDICINAL PLANTS

Scientification of Jamu (Evidence-based Jamu Development): A Breakthrough Program from
Plant to Medicine for Health Care

In occasion of

**The 43th National Meeting of National Working Group on Indonesia
Medicinal Plant
11-13 October 2012
Purwokerto, Indonesia**

Editors:

dr. Agung Saprasetya Dwi Laksana, M.Sc., PH.
Heny Ekowati, M.Sc., Apt.
Dr. Agus Nuryanto, M.Si.
Dr. Puji Widodo, M.Sc.
Rehana, M.Si., Apt.

Lay-out:

Sarmoko
Prima Andika Citra
Revo Almando Harris

Published by.
Universitas Jenderal Soedirman

Perpustakaan Nasional RI: Katalog Dalam Terbitan

PROCEEDINGS OF INTERNATIONAL CONFERENCE ON MEDICINAL PLANTS

Scientification of Jamu (Evidence-based Jamu Development): A Breakthrough Program from Plant to Medicine for Health Care

© Universitas Jenderal Soedirman

Cetakan Pertama, 2012

Hak Cipta dilindungi Undang-undang

All Right Reserved

- Editor : dr. Agung Saprasetya Dwi Laksana, M.Sc., PH.
Heny Ekowati, M.Sc., Apt.
Dr. Agus Nuryanto, M.Si.
Dr. Puji Widodo, M.Sc.
Rehana, M.Si., Apt.
- Perancang Sampul : Panitia
- Penata Letak : Sarmoko
Prima Andika Citra
Revo Almando Harris
- Pracetak dan Produksi : Tim UPT. Percetakan dan Penerbitan Unsoed

Penerbit



UNIVERSITAS JENDERAL SOEDIRMAN
Jalan Prof. Dr. H.R. Boenyamin 708 Purwokerto
Kode Pos 53122 Kotak Pos 115
Telepon 635292 (Hunting) 638337, 638795
Faksimile 631802
www.unsoed.ac.id

ISBN: 978-979-9204-80-6

vii + 271 hal, 29.7 cm x 21 cm

Dilarang keras memfotokopi atau memperbanyak sebagian atau seluruh buku ini tanpa seizin tertulis dari penerbit



Committee

Sterring committee:

Dr. Hj. Retno Widiastuti, MS

Indah Yuning Prapti, SKM., M.Kes.

Prof. Ir. Totok Agung DH, M.P., Ph.D.

Prof. Suwijoyo Pramono, DEA., Apt.

Prof. Dr. Ir. Suwanto, MS.

Ir. Agus Margiwiyatno, MS., Ph.D

Dr. Bambang Heru Budianto, MS.

Dr. Ir. Akhmad Sodiq, M.Sc., Agr.

Sugiyono, Ph.D

Tuti Sri Suehesti, M.Sc.

Dr. Lesley Braun

Assoc. Prof. Wandee Gritsanapan

Chariperson : Dra. Warsinah, M.Si., Apt.

Contents

The Effect of Aqueous Extract of <i>Scrophularia Striata</i> on The Growth of <i>Leishmania Major</i> In Vitro and In Vivo Conditions <i>Adolhossein Dalimi, Saeid Soflaei, Fatemeh Ghaffarifar</i>	1
Antifungal Activity and Stability of Long Term Storage of Turmeric Cream Preparations <i>Mukda Jankasem, Wuthi-udomlert, Wandee Gritsanapan</i>	7
Antibacterial Activity of Ethanolic Extract of Mangosteen Skin Fruit (<i>Garcinia mangostana</i>) Against <i>Gardberella vaginalis</i> <i>Fuji Faujiah, Iskandar Sobri, Sunarto</i>	11
Potency of Methanol Extract of <i>Artocarpus champeden</i> Stem Bark to Inhibit the Growth of <i>Plasmodium falciparum</i> <i>Maximus Taek, Aty Widyawaruyanti Maria Nindatu</i>	17
The Most Four Utilise Medicinal Plants For Anti-Malarial Phytomedicine in Papua and Their Main Chemical Constituents <i>Wahyudi</i>	21
Antifungal Effect of Virgin Coconut Oil (VCO) to <i>Candida albicans</i> : in Vitro Study <i>Thianthy Silviningrum, Fitranto Arjadi</i>	29
Antibacterial Activity Test of Sambiloto Herbs Extracts Against <i>Staphylococcus aureus</i> ATCC 29213 and <i>Escherichia coli</i> ATCC 25922 <i>Yonas B.A., Martha Ervina, Dien Limyati, Lisa Soegianto, Hendrawati</i>	33
The Effect of <i>Hibiscus sabdariffa</i> Linn. Calyx Water Extract on Increasing of Lymphocyte Proliferation In Vitro <i>Nurkhasanah, Puti Ratna Puri</i>	37
Free Radical Scavenging and Antifungal Activities of Juice and Ethanolic Extracts Of <i>Garcinia mangostana</i> L Leaves <i>Elza Sundhani, Diniatik, Muhammad Taufiq Nugroho</i>	41
Antioxidant Activity by DPPH Method and Total Phenolic Content of Water Fraction of Methanolic Extract of <i>Piper betle</i> L. Leaf <i>C.J. Soegihardjo, Rollando</i>	47
In vitro antioxidant activity of <i>Amomum cardamomum</i> leaves and stem <i>Hery Winarsi, Nurtjahjo D. Sasongko, Agus Purwanto, I Gede Arinton, Indah Nuraeni</i>	53
Antioxidant Activity of Extracts and Fractions of Sambiloto Herbs and Salam Leaves <i>Hendra Kurniawan, Lannie Hadisoewignyo, Martha Ervina, Lisa Soegianto</i>	60
Xanthone Derrivate Compound Of Mangosteen (<i>Garcinia mangostana</i>) in Suppressing VEGF Expression of SP-C1 Tongue Cancer Cell Line <i>Arlette Setiawan, Roosje R. Owen, Supriatno, Willyanti Soewondo, Unang Supratman, HR Sidik</i>	65
Potency Extract of <i>Morus alba</i> L. As Chemoprevention Agent in Vitro and in Silico <i>Sarmoko, Nia K. Sholihat, Revo A. Harris, Guruh Pratomo, Desiainy S. Sahara, Firster Nugroho</i>	71
Cytotoxic Effects of Methanol Extracts of Soursop (<i>Annona muricata</i>) Leaves on MCF-7 Cell Line and Its Effect on Expression of Bcl-2 Protein <i>Annisa Nur Jannah, Eka Prasasti Nur Rachmani, Iskandar Sobri</i>	76
Identification and Cytotoxic Activity from n-Hexane Fraction of Red Mangrove (<i>Bruguiera gymnorhiza</i>) Against to Hela Cells <i>Warsinah, Sismindari, Ratna Asmah Susidarti</i>	81
Petroleum Ether, Ethyl Acetate, and Methanol Extracts of <i>Pseudocalymma allicium</i> (Lam.) Sandwith Leaves and Their Antiviral Activities Against Newcastle Disease Virus <i>Nuning Rahmawati, M. Kuswandi, Ratna Asmah Susidarti</i>	86
Hepatoprotective Effect of Curcumin and Piperine against Iron Overload in Rats <i>Heny Ekowati, Sarmoko, Joko Setyono</i>	89
Effect of Green Tea on Hematological Profile in Lead-Exposed Rat <i>Hernayanti, Sukarti Moeljopawiro, Ahmad Hamim Sadewa, Bambang Hariono, Subagus Wahyuono</i>	93

Potency of Methanol Extract of *Artocarpus champeden* Stem Bark to Inhibits The Growth of *Plasmodium falciparum*

Maximus M. Taek^{1*}, Aty Widyawaruyanti², Maria Nindatu³

¹Faculty of Mathematics and Natural Sciences, Widya Mandira Catholic University,
 General Achmad Yani Street No. 50-52 Kupang 85225, Indonesia
 *E-mail: maximusmt2012@gmail.com

²Faculty of Pharmacy, Airlangga University, Surabaya, Indonesia

³Faculty of Medicine, Pattimura University, Ambon, Indonesia

Abstract

Methanol extract obtained from a three-steps successively extraction of the Cempedak (*Artocarpus champeden*) stem bark was examined for its antimalarial activity. The extraction was done firstly with n-hexane, secondly with dichloromethane, and then with methanol. The methanol extract then subjected to examination with the chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum* according to an in-vitro testing procedure developed by Trager and Jensen. The examination was conducted with non-synchronized cultures of *P. falciparum* of the 3D7 strain (the chloroquine-sensitive) and the G-2300 strain (the chloroquine-resistant) with the concentrations of extract in the microwells were 100, 10, 1.0, 0.1 and 0.01 µg/mL for 48 hours incubation. Chloroquinediphosphates was used for positive control with the concentrations of 10, 1.0, 0.1, 0.01 and 0.001 µg/mL, and DMSO for negative control. The IC₅₀ values found for the methanol extract were 4.230 g/mL on the 3D7, and 16.580 µg/mL on the G-2300 strain. At the same test condition, the chloroquinediphosphates has the IC₅₀ of 0.003 µg/mL on the 3D7 strain. From the results of the examination, it can conclude that methanol extract *A. champeden* is categorized as strong or very active antimalarial against the chloroquine-sensitive *P. falciparum* strain, and moderate or active against the chloroquine-resistant strain.

Keywords: *Artocarpus champeden*, antimalarial activity, in-vitro, *Plasmodium falciparum*, chloroquine-sensitive and chloroquine-resistant.

Background

Malaria is a disease spread out by an insect whose prevalence is the highest in the world. Every year the malarial disease infect 300 to 500 million people, rendering the death of 1.5 to 2.7 million people among those infected. It is estimated that 40 to 50 percent of world population are exposed to the disease with the highest risk to prevail among children below 5 years old (Saxena *et al.*, 2003; Murningsih *et al.*, 2005). Malaria is one example of the classic diseases that affect the productivity of the individual, the family and society. This disease is commonly found among poor and developing countries. African countries are the place of highest prevalence followed by South East Asian countries, China and India (Saxena *et al.*, 2003).

Malaria is an infectious disease caused by single-cell parasites belong to protozoan group of *Plasmodium* family that live in the red blood cells and liver cells. The malarial disease is infected into the human body through the bites of female *Anopheles* mosquitoes. There are four species of *Plasmodium* that cause the malaria to take place in human body, namely, *P. malariae*, *P. vivax*, *P. falciparum* and *P. ovale*. Of the four species, *P. falciparum* is the most dangerous as it can cause severe and acute or even fatal infection (Sherman, 1998) for it can attack young and old erythrocytes and causes a high risk of death for non-immune individuals (Schlesinger, 1988).

The first anti-malarial medicine is quinine, an alkaloid isolated from *Cinchona* stem bark in 1820. However, due to its wide-ranged side-effects such as tinnitus, vertigo and eye dysfunctions, the quinine was later replaced by synthetic medicines such as chloroquine, amodiaquine, and so on (Phillipson, 1991). Chloroquine then became a very important anti-malarial medicine until very recently. But this time, *P. falciparum* has become resistant against chloroquine. Although artemisinin from *Artemisia annua*, and its semi-synthetic derivatives have been found as a substitute for chloroquine, due to its high price and the fact that the problem of resistance has not been finalized, researches are still being made to look for other anti-malarial medicines from other plants. Most of the researches still utilize ethno-pharmacology approach and bioassay-guided isolation (Schwickard and van Heerden, 2002; Saxena, *et al.*, 2003).

Indonesian traditional society has been familiar with and using some plants as anti-malarial medicines for long time ago, one of which is the Cempedak (*Artocarpus champeden*). The cempedak is widely found in Indonesia and used by many people as food, construction material and ingredients for traditional medication. In traditional

medication, the bark of the cempedak is used to cure patients infected by malaria, dysentery and various skin diseases (Heyne, 1987; Iwasaki and Ogata, 1995).

Former researches on anti-malarial activities of the cempedak conducted by Utomo (2003) and Hidayati (2004) indicated that the total methanol extract (the sample powder directly extracted with methanol), and chloroform fractions of cempedak stem bark through an in-vitro method can inhibit the growth of malarial parasites on Balb/C mice that had been infected with *P. berghei*. The total methanol extract shows the inhibition activities against the growth of parasites with the ED₅₀ of 6.95419 mg/kg of the mouse body weight (Utomo, 2003), and the chloroform fraction with ED₅₀ of 0.36479 mg/kg of the body weight (Hidayati, 2004). Therefore, it is assumed that the cempedak plant contains one or more anti-malarial active compounds that can presumably be developed into anti-malarial medicines.

In this research, an in-vitro test has been conducted to evaluate the anti-malarial activity of the methanol extract of cempedak stem bark. The methanol extract was obtained through a successively extraction technique. The objective of this research was to evaluate the potency of methanol extract to inhibit the growth of malarial parasites of both the still sensitive ones and those already resistant against chloroquine as the main referral anti-malarial medicine.

Materials And Methods

Plant sample: stem bark of the cempedak was obtained from Makbalim, Salawati district, Sorong, West Irian Jaya, and identified in the Research Office of the Purwodadi Botanical Garden, Pasuruan, East Java.

Plasmodium: *P. falciparum* of the 3D7 strain (chloroquine sensitive) and G-2300 strain (chloroquine resistant) were obtained from the Eijkman Biomolecular Institute, Jakarta, and from Tropical Disease Research Center of Airlangga University, Surabaya. The *Plasmodium* was then cultivated in microwells plate according to the methods developed by Trager and Jensen (1976). The cultivation utilized type O human red blood cells of with 5 percent haematocrit suspended into the medium of RPMI 1640 added with the inactivated type O blood serum. The cultivated plasmodia are then placed in a CO₂ incubator at the temperature of 37°C. The medium is replaced everyday with a new one until the cultivated plasmodia indicate the parasitaemia has reached the level of 1-5 percent which was observed by the smearing of thin blood film.

Comparative substance: chloroquinediphosphates was used for positive control and dimethylsulphoxide (DMSO) for negative control.

Cempedak stem bark extraction: dry powder of the cempedak stem bark was macerated successively with n-hexane, dichloromethane and methanol, each in three days. The methanol extract was then dried with rotary evaporator through decreasing pressure until the extract dries. The methanol extract was then tested for its anti-malarial activity against both *P. falciparum* strains through the in-vitro technique.

Antimalarial activity test: methanol extract was dissolved with DMSO and filtered sterilely with 0.22 µm membrane filter. This dissolved methanol extract was put into into a microwell that contains suspension of *P. falciparum* where the parasitaemia was 1 percent. It was diluted with mediums in a series of techniques so that final concentrations of tested materials in the microwells were as follows: 100, 10, 1.0, 0.1 and 0.01 µg/mL. Each microwell contains 4 percent haematocrit. DMSO for negative control was diluted with the same method to find the final concentration of 0.5 percent at most. Chloroquine diphosphate for positive control is also prepared with similar method and differentiated in the concentrations of 10, 1.0, 0.1, 0.01 and 0.001 µg/mL. The mixture of testing material and suspension of parasites is put in a CO₂ incubator at the temperature 37°C for 48 hours.

After the incubation of 48 hours the researchers applied a thin blood film on an object glass. The thin blood film was then dried, fixated with methanol, and stained with the Giemsa. After that the researchers counted the number of erythrocytes infected by *P. falciparum* per total countable erythrocytes under microscopy with 1000 times enlargement. The counting was made for around 5,000 erythrocytes.

From the data of number of erythrocytes infected by *P. falciparum* the researchers then counted the percentage of parasitaemia and the inhibition percentage of tested materials against the growth of *P. falciparum* with the following formula:

$$\%Parasitaemia = \frac{\text{Number of infected eritrocytes}}{\text{Total of eritrocytes}} \times 100\%$$

$$\%Inhibition = 100\% - \left(\frac{X_t}{X_c} \times 100\% \right)$$

Where:

Xt = parasitaemia in treatment group = % paracytemia (48 hours – 0 hour)

Xc = parasitaemia in control group = % parasitemia (48 hours – 0 hour)

[(Xp/Xk) x 100 %] = *Plasmodium* growth percentage

Based on the inhibition data of every extract concentration, the researchers then counted the value of IC₅₀ that is the extract concentration that inhibited the growth of 50% of *Plasmodium* with Probit Analysis from the SPSS version 15.0.

Result And Discussion

Test over antimalarial activities of methanol extract and chloroquine diphosphate against non-synchronized *P. falciparum* culture of the 3D7 and G-2300 strains was conducted in-vitro. The test was conducted in a duplicate technique with 48 hours incubation period. After 48 hours the culture was taken and applied in a thin blood layer on an object glass, fixated with methanol and stained with Giemsa, and then counted its parasitaemia percentage and the tested materials' inhibition percentage.

The computation result in the inhibition percentage of various extract concentrations against the growth of *P. falciparum* of strains 3D7 and G-2300 can be seen in Table 1. Based on the inhibition percentage data in Table 1, researchers then computed the IC₅₀ value of methanol extract against each *Plasmodium* strain where the result can be seen in Table 2. According to Rasanaiwo *et al.* (1992) in Ouattara *et al.* (2006), the extract that has the antimalarial activity with the IC₅₀ value smaller than 5µg/mL is said to be very active; 5–50µg/mL is categorized as active; 50–100µg/mL is categorized as less active (weak activity); and the IC₅₀ value more than 100 µg/mL is said to be not active as anti-malaria. Referring to this description, it is quite clear that methanol extract has a strong activity and categorized as very active as antimalarial against chloroquine-sensitive *P. falciparum* (the 3D7 strain), and active against the G-2300 strain of *P. falciparum* that has become resistant against chloroquine.

Table 1. Inhibition Percentage of Various Methanol Extract and Chloroquine Concentrations against *P. falciparum* growth in 48 hours

Concentration (µg/mL)	Average Inhibition Percentage against <i>P. falciparum</i> strains		Chloroquine 3D7
	Methanol extract 3D7	Methanol extract G-2300	
100,000	100	69	-
10,000	41	36	100
1,000	39	29	83
0,100	3	21	70
0,010	0	0	55
0,001	-	-	49

Note: – (No tests have been conducted for the concentrations)

Table 2. The IC₅₀ values of methanol extract and chloroquine against *P. falciparum* for 48 hours

Materials	IC ₅₀ (µg/mL)	
	3D7	G-2300
Methanol extract	4.230	16.580
Chloroquine	0.003	-

Compared to the chloroquine, the activity of methanol extract is much weaker, around 1/1,410 times the activity of chloroquine against chloroquine-sensitive *P. falciparum*. However, as already known, the chloroquine is no longer active as antimalarial medicine, due to the resistance of *P. falciparum* against this remedy. Therefore it is quite probable that other compounds those are active as antimalarial agents then have prospect to be developed as antimalarial drugs to replace the chloroquine. Compounds whose molecular structure and functional groups are different from the chloroquine are quite possible to possess different action mechanisms from that of the chloroquine to kill or to inhibit the growth of *Plasmodium*, and as such it is expected that those compounds can kill or inhibit the growth of *Plasmodium* that has become resistant against the chloroquine.

Maximus (2009) indicates that the methanol extract of the cempedak stem bark contains some phenolic carbonyl compounds such as flavonoid. The phenolic compounds are known to possess various biological activities. Referring to research results reviewed by Rowe *et al.* (1989), Schwikkard and van Heerden (2002), and Saxena, *et al.* (2003), it is quite clear that many compounds that indicate the antimalarial activity have one or more phenol groups in the structure of their compounds. The phenolic compounds may consist of tannins, flavonoids, xantons, lignans, stilbenes, coumarins, quinones, and so on.

Conclusion

The methanol extract of the cempedak stem bark indicates an in-vitro inhibition potency that categorized as strong or very active against the chloroquine-sensitive *P. falciparum* strain, and moderate or active against *P. falciparum* strain that has become resistant against chloroquine.

References

- Heyne, K., 1987. *Tumbuhan Berguna Indonesia*, jilid II. Yayasan Sarana Warna Jaya. Jakarta, pp. 669-670.
- Hidayati, A.R., 2003. Uji Aktivitas Antimalaria Fraksi Kloroform Kulit Batang Cempedak (*Artocarpus champeden*) terhadap *Plasmodium berghei in-vivo*. *Skripsi*, Fakultas Farmasi Unair, Surabaya.
- Iwasaki, T., and Ogata Y., 1995. Medicinal Herbs Index in Indonesia, 2nd edition. PT. Eisai Indonesia.
- Maximus, M.T., 2009. Aktivitas Antimalaria Isolat Flavonoid dari Kulit Batang *Artocarpus champeden* terhadap *Plasmodium falciparum*. *J. Nat. Sains*, 1(1), Juli, pp. 1-4.
- Murningsih, T., *et al.*, 2005. Evaluation of the Inhibitory Activities of the Extracts of Indonesian Traditional Medicinal Plants against *Plasmodium falciparum* and *Babesia gibsoni*. *J. Vet. Med. Sci.* 67(8), pp. 829-831.
- Ouattara, Y., S. Sanon, Y. Traore, V. Mahiou, N. Azas, and L. Sawadogo, 2006. Antimalarial Activity of *Swartzia madagascariensis* Desv. (Leguminosae), *Combretum glutinosum* Guill. & Perr. (Combretaceae) and *Tinospora bakis* Miers. (Menispermaceae), Burkina Faso Medicinal Plants. *Afr. J. Trad. CAM*, 3 (1), pp. 75-81.
- Rowe, J.W. (ed.), 1989. *Natural Products of Woody Plants*, vol.II. Berlin: Springer-Verlag, p. 1077.
- Saxena, S., Pant, N., Jain, D. C., and Bakhuni, R. S., 2003. Antimalarial Agents from Plant Sources. *Current Science*, vol. 85, no. 9, November, pp.1314-1329.
- Schlesinger, P.H., *et al.*, 1988. Antimalarial Agents: Mechanims of Action. *J. Antimicrobial Agents and Chemotherapy*, June, pp.793-798.
- Schwikkard, S., and F.van Heerden, 2002. Antimalarial Activity of Plant Metabolites. *Natural Products Report*, 19, September: pp. 675-692.
- Utomo, N.D.W., 2003. Aktivitas Antimalaria Ekstrak Methanol Kulit Batang Cempedak (*Artocarpus champeden* Spreng.) terhadap *Plasmodium berghei in-vivo*. *Skripsi*, Fakultas Farmasi Unair, Surabaya.