

IN VITRO INTRAERYTHROCYTIC ANTIMALARIAL ACTIVITY OF AKAR KUNING (*Arcangelisia flava* (L.) Merr.) STEM AQUEOUS EXTRACT IN *Plasmodium falciparum*

Eric Robhets Lovin¹, Heny Arwati², Ramadhani RB³

¹Medical Doctor Study Program, ²Department of Medical Parasitology,

³Department of Medical Pharmacology, Faculty of Medicine, Airlangga University, Surabaya

ABSTRAK

Akar kuning (Arcangelisia flava (L.) Merr) telah digunakan sebagai obat tradisional turun-temurun di berbagai daerah di Indonesia untuk mengobati berbagai penyakit, salah satunya adalah malaria. Umumnya, masyarakat tradisional menggunakan batang tanaman ini untuk mengobati malaria. Pemberian ekstrak air batang tanaman ini telah digunakan pada penelitian ini untuk menguji aktivitas antimalaria terhadap *Plasmodium falciparum* strain 3D7 in vitro. Setelah 48 jam inkubasi, parasitemia dihitung berdasarkan apusan tipis pewarnaan Giemsa dari kultur ekstrak, kontrol positif, dan kontrol negatif. Persentase parasitemia ditentukan pada 1000 sel darah merah, dan digunakan untuk menghitung persentase pertumbuhan dan penghambatan. Berdasarkan persentase penghambatan, IC50 ditentukan dengan menggunakan program SPSS 17. Hasil menunjukkan bahwa ekstrak air memiliki tanaman ini memiliki aktivitas antimalaria terhadap pertumbuhan *P. falciparum* strain 3D7 dan IC50 ekstrak sebesar 1,811 µg/mL. (FMI 2012;48:90-95)

Kata kunci: ekstrak air, in vitro, *Arcangelisia flava* (L.) Merr, *Plasmodium falciparum*, aktivitas antimalaria

ABSTRACT

Akar kuning (Arcangelisia flava (L.) Merr) has been used as hereditary traditional medicine in many regions of Indonesia for treating various diseases, one of which is malaria. Generally, traditional communities use the stem of this plant for treating malaria. The aqueous extract of stem of this plant has been used on this study to test the antimalarial activity against *Plasmodium falciparum* 3D7 strain in vitro. After 48 hours incubation, the parasitemia has been calculated based on Giemsa-stained thin smears of the extract-treated culture, positive control, and negative control. Parasitemia percentage was determined within 1000 red blood cells, and used to calculate the growth percentage and inhibitory percentage. Based on the inhibitory percentage, IC50 was determined by means of SPSS 17 program. The results indicate that the aqueous extract of this plant possesses antimalarial activity against the growth of *P. falciparum* 3D7 strain and the IC50 of the extract was 1.811 µg/mL. (FMI 2012;48:90-95)

Keywords : Aqueous extract, in vitro, *Arcangelisia flava* (L.) Merr, *Plasmodium falciparum*, Antimalarial activity

Correspondence: Eric Robhets Lovin, Medical Doctor Program, Faculty of Medicine, Airlangga University, Jalan Prof dr Moestopo 47, Surabaya 60131, Indonesia. Phone: 62-31-5020251.

INTRODUCTION

Malaria is the most important tropical diseases, widespread throughout the tropics, but also occurred in temperate regions. Malaria has become a global problem. It is endemic in 105 countries and results in more than 300-500 million clinical cases and more than one million deaths every year. During the 1950s and 1960s a vigorous campaign to eradicate malaria was launched successfully worldwide. In some areas the disease is in the process of elimination. But over the past few decades, the emergence of this disease occurred again. The dream of global malaria eradication began to fade with the increasing number of cases, the rapid spread of drug resistance in patients, and insecticide resistance in mosquitoes is increasing (World Health Organization 2007).

Approximately 1.216 million people or 70% of the total population of Southeast Asia are at risk for malaria. Of these about 29% of the population is moderate to high risk, 71% low risk, while the remaining 30% of the population is free from malaria. Approximately 96% of the population at risk is moderate to high in Southeast Asia are those who live in Bangladesh, India, Indonesia, Myanmar and Thailand, and contributed to 95% of malaria cases and deaths confirmed (World Health Organization 2011).

In Indonesia, until the year 2009, approximately 80% of districts/cities are still including malaria endemic category and about 45% of the population live in areas at risk of contracting malaria. While the number of cases reported in 2009 a total of 1,143,024 people. The

distribution of malaria can be divided into non-endemic and endemic areas. Said to be non-endemic area in the area where there is no malaria or malaria incidence (Annual Parasite Incident = API) zero. Including non-endemic areas are the provinces of Jakarta, Bali and Riau Islands (Barelang Binkar). While malaria-endemic areas are divided into highly endemic, endemic moderate and low endemic. Said to be endemic when the API is larger than 50 per 1,000 population that is in the province of Maluku, North Maluku, Papua, West Papua, North Sumatra (Kab. Nias and Nias Selatan), and NTT. Were endemic when its APIs ranged from 1 to less than 50 per 1,000 inhabitants in the province namely (Kab. Siemeulu), Bangka Belitung, Riau Islands (Kab. Lingga), Jambi (Kab. Batang Hari, Merangin, and Sorolangun), Borneo Central (Kab. Sukamara, waringin City west), Mura), Sulawesi (Kab. Toli-Toli, Banggai, Banggai Islands, Poso), Southeast Sulawesi (Kab. Muna), NTB (west Sumbawa, Dompu, Kab. Bima, and Sumbawa), Central Java (Wonosobo, Banjarnegara, Banyumas, Pekalongan and Sragen), West Java (Sukabumi, Garut, and Kudat). Endemic low when its APIs 0-1 per 1,000, including parts of Java, Kalimantan and Sulawesi (Ministry of Health of the Republic of Indonesia, 2010).

Malaria is caused by Plasmodium parasites. There are four types that cause malaria in humans: *Plasmodium falciparum*, *P. vivax*, *P. malariae*, *P. ovale*. *P. falciparum* and *P. vivax* are the most common. Among them, *P. falciparum* is the most deadly. In recent years, several cases of human malaria caused by *P. knowlesi* also, that malaria in monkeys that occurs in certain forest areas of Southeast Asia (World Health Organization, 2010)

Malaria belongs to the group of old infectious diseases that pose new problems or re-emerging disease (Ministry of Health, Republic of Indonesia, 2010). Some of the factors associated with this outbreak of malaria among the increasing antimalarial drug-resistant parasites, insecticide resistance, and as a result of the mobility of vulnerable populations from nonendemic areas to areas with malaria (Korgstad 1996). Imported malaria is still a new problem around the world due to the increasing mobility of international travelers and become a threat to travelers visiting malaria endemic areas (Zein 2009).

Parasite resistance to antimalarial drugs has emerged as one of the biggest challenges in the face of current malaria controL. Resistance to antimalarial drugs have been found on two of the four types of malaria parasites that naturally infect humans, *P. falciparum* and *P. vivax*. *P. falciparum* has developed resistance to almost all currently used antimalarials, although the geographical

distribution of resistance to some antimalarial very varied (Bloland 2001).

Malaria parasites resistant to antimalarial drugs in Indonesia, particularly chloroquine distribution is uneven, but all provinces have reported cases were classified as resistant to the drug (Zein 2009). Standard antimalarial drug chloroquine as reported is no longer effective for the treatment of falciparum malaria, sulfadoxine-pyrimethamine, while still relatively effective in some studies, and quinine reported to have shown a decrease in efficacy (Tjitra 2005).

Chloroquine-resistant falciparum malaria in vitro or in vivo have been reported in 27 provinces of Indonesia with the degree of RI-RIII. Sulfadoxine-pyrimethamine in 9 provinces (Irian Jaya, Lampung, Central Java, North Sumatra, Aceh, Riau, South Sulawesi, Jakarta and East Kalimantan) with the degree of RI and RII. Quinine in 5 provinces (West Java, Central Java, NTT, Irian Jaya and Kalimantan), whereas mefloquine in 3 provinces (Central Java, Irian Jaya and Kalimantan) with degrees RII - RIII and halofantrin in one province (Kaltim) (Tjitra 1994).

Artemisinin Combination Therapy (ACT) is now the first-line treatment of falciparum malaria worldwide (Maude et al, 2009). Artemisinin is often given in combination with other antimalarial variety has a longer half-life (Porter-Kelley et al, 2010). However, the use of artemisinin derivatives incorrectly may decrease the susceptibility of *P. falciparum* (Shahinas et al 2010).

Artemisinins interact and selectively inhibit PfATPase6, which is the only pump SERCA-type Ca²⁺ + -ATPase in the genome of *Plasmodium falciparum*. Sebuah other in vitro studies in Guiana, France showed *Plasmodium falciparum* with an increase in the IC₅₀ of artemisinin have a specific point mutation at codon S769N of loci ATPase6 (Mugittu et al 2006). A similar study showed that *P. falciparum* can develop stable resistance to artemisinin with high inhibitory concentration (IC₅₀), which indicates the potential for the emergence of resistance in vivo after extensive use and long term these drugs (Tahar et al 2009).

Thus, the development of resistance to artemisinin has the potential for widespread. Due to the ever increasing threat of resistance of *P. falciparum* to antimalarial drugs in the world it is necessary to look for other types of drugs that may be found in Indonesia and has potential as an antimalarial that can be developed at a later date (Zein 2009).

In Permenkes RI in 2010 stated that traditional medicine is the ingredient or ingredients in the form of plant

material, animal ingredients, mineral materials, preparation sarian (galenic), or mixtures of these materials that have historically been used for the treatment, and can be applied in accordance with the prevailing norms in society. (Ministry of Health of the Republic of Indonesia, 2010) The preparation of traditional medicines used by the people that come from medicinal plant materials is very necessary at this time to be researched and developed so-called herbal medicine or Phytopharmaca which can then be used a means of primary health care and increase the types of drugs that will selected (Zein 2009). Indonesia has abundant natural resources, including the potential crops to be used as medicine. However, the enormous potential of this otherwise good use certainly would not have any meaning, so it should be considered that the use of medicinal plants to support the need for drugs that are more urgent and to obtain a replacement drug if the drug resistance of the parasite to the widespread and not available other new drug types (Zein 2009).

Plant yellow root (*Arcangelisia flava* (L) Merr) is one of the medicinal plants that have been passed down is used by local communities in Central Kalimantan (Galingging & Bhermana 2010). Decoction of the stem of this plant is used to treat jaundice, indigestion, intestinal worms, strong medicine/tonic, fever, menstrual laxative, and thrush (Subiandono & Heriyanto 2009). In Central Sulawesi and South Sulawesi, the stem of this plant is commonly used for the treatment of malaria, diabetes, and urinary stones (Meistiani 2001). *A. flava* (L) Merr is included in group members Menispermaceae tribe (Ariyanti 2001).

Chemical compounds found in plants are alkaloids, including berberine, columbamine, jatrorhizine, palmatine, shobakunine, limacine, homoaromaline, dehydrocorydalmine, 8-hidroksiberberine, picnarrhine, and thalifendine (Ariyanti 2001). Berberine has several functions, such as anti-protozoan, chloretic, kolagog, cardiotonic, anti-cholinergic, anti-arrhythmic effects, and anti-platelet aggregation (Wongbutdee 2008). Berberine has been shown to inhibit telomerase activity of *P. falciparum* in a dose range of 30-300 μM . (Sriwilaijareon et al 2002)

A study in Vietnam proved that the antiplasmodial activity of plant extracts of *A. flava* (L) Merr against *P. falciparum* strains Colombia/FcB1 IC50 of chloroquine-resistant has varied between 0.4 to 1.1 $\mu\text{g/mL}$. In the study, classified high antiplasmodial activity if IC50 below 1 $\mu\text{g/mL}$, well if IC50 between 1 and 10 $\mu\text{g/mL}$, and moderate if IC50 above 10 $\mu\text{g/mL}$ (Nguyen-Pouplin et al 2007) From the description of the antiplasmodial activity the study was high. However, different strains of the parasite is likely to have different susceptibility to

the same medicine. Therefore, in this study will be tested on *P. falciparum* strain 3D7. The purpose of this study was to determine the antimalarial activity of the water extract of the roots of the plants yellow (*A. flava* (L.) Merr) against *P. falciparum* in vitro.

MATERIALS AND METHODS

This research is a kind of experimental laboratory. The population in this study was a smear of blood on the 0 hour and 48 hours after incubation with varying concentrations of water extract of yellow root plant (*A. flava* (L.) Merr). Thin blood smears were prepared from each well in the microplate were tested with plant roots extract water yellow (*A. flava* (L.) Merr). From each well we made one blood smear done while each concentration of 2 replicates. The concentration used for the test is 100; 10; 1; 0.1 and 0.01 $\mu\text{g/mL}$. A positive control was 1 well as well as one negative control well and the blood smear for hours-0 so that the total number of blood smear sample is 14. The sampling was done at the end of the test at the 48th hour by taking the blood as much as 2 μL and made blood smear thin and painted with 5% Giemsa.

Yellow root plant stems that have been obtained are washed with clean water. The rod then swabbed with 70% alcohol to remove the existing microorganisms. Once the rods are cut into small pieces of ± 2 cm. Trunk then dried by being placed in a clean tray and covered with newspaper and then stored at room temperature for several days. Controlled conditions on a daily basis until the rod stem dries. Dried stems removed and weighed 1 kg. Then 1 kg of dried stems are boiled with 2 liters of distilled water in a clean pot. 1.5 liter infusion then converted into powder by using a freeze dryer (lyophilizer). The powder obtained is then weighed, so that the known weight of powder that can be obtained from infusion 2 kg of yellow root plant stems. Malaria parasites were used in this study are sensitive 3D7 strain of *P. falciparum* to chloroquine were obtained from the Eijkman Institute for Molecular Biology, Jakarta and bred in the Department of Parasitology, Faculty of Medicine, University of Airlangga.

Materials for culturing the parasite is NaCl (Merck), RPMI 1640 (Gibco), HEPES buffer (Sigma), NaHCO_3 (Sigma), hypoxanthine (Merck), gentamicin sulfate (Sigma), serum and red blood cells of human type O were obtained from the Cross Merah Indonesia (PMI), Surabaya, sterile distilled water for irrigation (Otsuka), Giemsa dye solution with phosphate buffer, and oil immersion.

The process of implementation of pulverizing infusion yellow root crops conducted in the laboratory Pharmacognosy and Phytochemistry-section of Nature Materials Science, Faculty of Pharmacy, Airlangga University, Surabaya. Breeding and testing of *P. falciparum* parasitaemia malaria parasite strains 3D7 in vitro carried out in the Department of Parasitology, Faculty of Medicine, Airlangga University, Surabaya. The study was conducted starting in July 2011 s.d. In November of 2011.

As the test material is a powder yellow root plant stems are diluted with distilled water to obtain a concentration of 100; 10; 1; 0.1; 0.01 µg /mL. Way of providing this solution prepared in aseptic conditions. Positive control used was Chloroquin diphosphate 200 µg/mL. How to make it is Chloroquin diphosphate 20,000 µg/mL pipette with a micropipette of 10 µL and diluted with 990 µL CM (2000 µg/mL) was then pipetted 50 µL of Chloroquin diphosphate in 2000 µg/mL and diluted in 450 µL (CM, IRBC, RBC) and then inserted into the test wells F1. This procedure is performed replication and put in test wells F2. Negative controls were made with micropipette pipette with 50 µL CM then added with 450 µL (CM, IRBC, RBC) and then inserted into the test wells G1. This procedure is performed replication and put in test wells G2.

The testing procedure antimalarial effect in vitro is by ring-stage parasites with $\pm 1\%$ parasitaemia synchronized cultured together with test materials and controls using 96-well microplate with each well filled volume of 200 µL. The culture is put in wells containing water extract of the test material were then pipetted to mix culture and test materials. Candles were lit and the desiccator was closed. Having extinguished the candle in a desiccator, a desiccator put in an incubator and incubated at 37° C for 48 hours.

Observations were made on thin blood smears were painted with 5% Giemsa at hour 0 and 48 hours to determine the% parasitaemia. Based on% parasitaemia, calculated% growth. After getting the% growth is then calculated% inhibition. Based on data% inhibition then performed probit analysis by creating a relationship between the probit curve (Probability units)% inhibition and the logarithm of concentration of test material using a linear regression line equation to determine IC50. IC50 is an effective inhibitory concentration capable of inhibiting 50% growth of *P. falciparum* in vitro.

RESULTS

One kilogram of dried stems of plants roots and yellow made from a drip infusion is made of powder with a

lyophilizer and obtained 33.3 mg of powdered yellow root plant stems. Preparation of the infusion and powder conducted at the Faculty of Pharmacy, University of Airlangga. Test Results antimalarial activity

The observation of thin blood smears that have been painted with the staining of *P. falciparum* cultures were treated, positive control, negative control, and 0 h (Table 1).

Table 1. Percent parasitaemia, percent growth, and the percent inhibition of the test antimalarial activity of water extract of the roots of the plant stem yellow against *P. falciparum* 3D7 strain after 48 h of incubation

	Concentration (µg/mL)	% Parasitemia	% Growth	% Inhibition
Akar kuning Aqueous Extract	100	0.45	0.09	91
	10	0.88	0.52	48
	1	0.95	0.59	41
	0.1	1.01	0.65	35
	0.01	1.51	1.15	(-) 15
Positive control		0.26	-	-
Negative control		1.36	1	-
Paracyte (D0)		0.36	-	-



Figure 1. Graph Probit analysis of the inhibition of water extract of the roots of the plant stem yellow on the growth of *Plasmodium falciparum* in 48-hour incubation

Percentage inhibition data obtained is then used to obtain IC50 values with probit analysis on SPSS 17. From the probit analysis obtained Inhibitory Concentration (IC50) of the water extract of the roots of the plant stem yellow on the growth of *Plasmodium falciparum* in 48-hour incubation. The IC50 value indicates the magnitude of the concentration of the extract that can inhibit 50% of parasite growth. IC50 of the extract of the roots of the plant stem yellow water is 1.811 µg/mL.

DISCUSSION

Test antimalarial activity in vitro is needed to determine the potential antimalarial extract water from the plant stem yellow roots on the growth of *Plasmodium falciparum* strain 3D7 and the IC₅₀ values of these materials. The results of this study showed that the water extract of the roots of the plant stem yellow have antimalarial activity.

After incubation for 48 h, parasite cultures treated with the extract concentration of 0.01; 0.1; 1; 10; 100 µg/mL, positive control, and negative control skizogoni erythrocytic life cycle repeats, of skizont issued merozoites then rupture. Subsequently released merozoites infect other erythrocytes and formed immature stage trophozoite (ring form), mature trophozoites, and skizont. Therefore, observations were made at 48 hours after incubation, both in cultured parasites with and without treatment, it can be seen that the growth of the parasite. Parasitaemia is an indicator of parasite growth expressed as a percent.

There was a decrease percent growth and an increase in percent inhibition of parasite with increasing concentrations of the test material. At a concentration of 0.1 µg/mL obtained percent growth of 0.65% and the percent inhibition of 35%, a concentration of 1 µg/mL obtained percent growth of 0.59% and the percent inhibition of 41%, a concentration of 10 µg/mL was obtained 0.52 percent growth µg/mL and the percent inhibition was 48%, and a concentration of 100 µg/mL obtained percent growth of 0.09% and the percent inhibition of 91%. Meanwhile, at a concentration of 0.01 µg/mL obtained percent growth of 1.15%. Percent growth is close to or nearly equal to the percent growth in the untreated negative control or 1%. This is due to the content of the extract at a concentration of 0.01 µg/mL is very low, so almost no effect on the growth of the parasite. Thus parasitaemia obtained at these concentrations is almost the same as in culture untreated parasites.

It is known that extracts of the plant stem yellow root contains berberine (Ariyanti 2001, Nguyen-Pouplin et al 2007) Berberine on the test material to inhibit the activity of telomerase enzyme (Sriwilaijareon et al 2002). The enzyme telomerase is required by microorganisms to maintain the integrity of the coding region of the DNA, so that DNA replication can continue and the cells will continue to replicate (Wikipedia, 2011). Inhibition of this enzyme by Berberine causes telomere shortening each time the parasite replicates, and at one time the replication results contain DNA that has lost a number of coding

regions that are essential for the parasite's life further. Thus, the parasite will experience death.

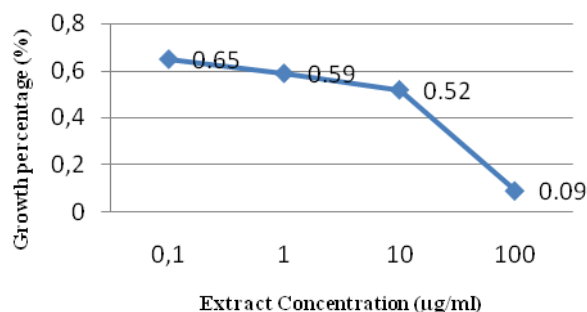


Figure 2. Percent growth of *P. falciparum* strain 3D7-treated water extract of the roots of the plant stem yellow after 48 hours incubation

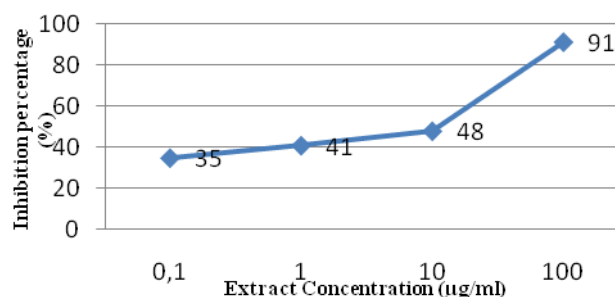


Figure 3. Percent inhibition of *P. falciparum* strain 3D7-treated water extract of the roots of the plant stem yellow after 48 hours incubation

Thin smear observation of the number of erythrocytes infected with parasites per 1000 erythrocytes were observed, with 1000 times magnification using a light microscope. From these observations calculated the percentage of parasitaemia both 0 and 48 h, positive control, negative control, and each concentration of the test material in the 48th hour. These calculations were obtained by percentage growth. Then the percentage of growth was used to obtain the percentage of inhibition for each treatment. Furthermore, the data the percentage of inhibition, the concentration of the test material, and the average number of erythrocytes were observed in appendix 3 are variables used to derive the IC₅₀ values of test materials on probit analysis using SPSS 17.

In probit analysis with SPSS 17, IC₅₀ values obtained were 1.811 µg/mL. The IC₅₀ value indicates the magnitude of the concentration of the extract that can inhibit 50% of parasite growth. According Pouplin et al (2007) the plant stem yellow root contains berberine as antimalarial active ingredients. However, the water extract of a plant still contained other materials is

unknown, so it is possible that the material is also supporting its activity as an antimalarial. Therefore it is necessary to conduct further research to assess the potential of root crops as antimalarial yellow, for example using pure berberine isolates of yellow root crops and in vivo studies as well as a comparison.

CONCLUSION

Extracts of the plant stem water yellow root (*A. flava* (L.) Merr.) has antimalarial activity. IC50 water extract of the roots *A. flava* (L.) Merr. against *P. falciparum* 3D7 strain was 1.811 µg/mL.

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