PHYTOCHEMICAL TESTED AND IN VITRO SCREENING ANTI-MALARIA ACTIVITY OF *BELILIK* (*Brucea javanica* (L)). MERR AGAINST *Plasmodium falciparum*

Henny Helmi, Idha Susanti

Biology Department, Bangka Belitung University

Corresponding Author e-mail:hennyhelmi24@gmail.com, Phone/fax: (0717)422145

ABSTRACT

*Screening of anti-malaria compound from nature is one of important thing to cure malaria disease especially in Bangka Belitung province. One of plant that as used as traditional medicine to cure malaria is Belilik (***Brucea javanica** *(L.)Merr. The aim of this study was to investigate the present of some kind of biochemical compound, and to evaluate in vitro anti-malaria activity of ethanol extracts of* ***Bruce javanica*** *fruits and roots against* ***Plasmodium falciparum****. A visual method was allowed to evaluate the in vitro anti-malaria activity of the extracts against* ***P. falciparum****. The number of parasites per 5,000 erythrocytes on thin Giemsa stained smears was calculated microscopically. IC50 values were determined by probit analysis of SPSS 13 program. The result showed, that the ethanol extract of Belilik contained some biochemical compound such as alkaloid, flavonoid and phenol in fruits and alkaloid, phenol and saponin in roots. The anti-malaria activity of ethanol extracts of* ***Brucea javanica*** *fruits and roots on* ***P. falciparum*** *were very effective as IC50<0,01 µg/mL. The highest inhibition of 100 µg/mL of* ***Brucea javanica*** *fruits and roots were 5.95% and 16.99% respectively.* ***Brucea javanica*** *could inhibit* ***Plasmodium falciparum*** *in vitro with very small dosage of extract and very potential to develop as malaria medicine.*

Key words: anti-malaria, ***Brucea javanica*** *(L), compound,* ***Plasmodium falciparum***

INTRODUCTION

Malaria is a major problem disease to the world population, especially in tropical country. Currently, Malaria is an endemic disease in more than 90 countries especially in developing country (Sanchez *et al*., 2004). For about 100 years, the world has not been able to provide a clear contribution to the treatment of this disease (Riley 2000).

In Indonesia, malaria was found scattered throughout the islands. Usually malaria attack people who live in the endemic areas or who are traveling to areas which have high transmission rate (Prabowo 2004). One of the endemic area is province of Bangka Belitung. Based on some Malaria disease, 48% of malaria caused by *Plasmodium falciparum* infections.

Patients who infected with malaria in the last of 2 decades has doubled mainly due to the emergence of strains of *Plasmodium falciparum* with the characteristic drug-resistant malaria that usually available such as is chloroquine and its derivatives (Trape *et al*, 2002). The deaths event that caused by malaria can also caused by the low immunity patients. The weak of immunity patient is one of the causes of malaria death since the age of the child (Kuby, 1997).

In some malaria endemic areas, malaria drugs are often not available or the price is not affordable by the community and also the rising cases of malaria parasite resistance to drugs available (Koch *et al*, 2005). Perez *et al* (1997) stated that the global spread of malaria parasites that are resistant to multiple drugs available is a major health problem that it takes effort to get the source material newer antimalarial drugs (Koch *et al*, 2005).

One of the plant is Simarubaceae family or usually called Ki Pahit (*Picrasma javanica*) is reported to have the ability antiplasmodial (Hidayat, 2003). One of the plants that one family with Ki Pahit is *Belilik* (*Brucea javanica*). Some exploration and ethnobotanical research conducted in Bangka and Belitung showed that *Belilik* fruit (*Brucea javanica* (L.) Merr) is often used to treatment malaria disease in Lom community, Bangka Belitung. Lom community is the name of ethnic communities in the District Belinyu, Bangka (Adelia, 2010). Exploration in several districts in the two site, namely Central Bangka and South Bangka shows that this plant is also widely used both fruit and roots (Haryati, 2001; Maysoroh, 2010; Sitompul, 2010). Given the kinship between plants within a family that is Simarubaceae, *Belilik* plants also have the possibility of chemical components that also have similar biological activities. In addition to the fruit of this plant is also used part of the roots to treatment the malaria disease.

This plant has a high erutility value based on the community assessment and the traditional healers to treatment the malaria disease. Information on the biological properties and phytochemicals, as well as the process of in vitro testing of this plant is not a done deal. Therefore, it is felt important to do this research. The aim of this research are to know the optimalization of extraction process of anti-malaria compound from *Belilik* (*Brucea javanica* (L.) Merr) root and fruit and also to perform screening of active compound of anti-malaria from *Belilik* (*Brucea javanica* (L.)Merr) by in vitro.

MATERIAL AND METHOD

Belilik (*Brucea javanica* (L.) Merr) Root and fruit extraction

Extraction were done by using soxlet method. A total of 100 g of *Belilik* root and fruit were crushed, then were soaked in 96% alcohol and heated to get the perfect extract and the solvent became clear. The solvent then were dried by using a vaccum rotary evaporator. Then the total percentage yield of extraction were weighed and counted.

Phytochemical test of *Belilik* (*Brucea javanica* (L.) Merr) root and fruit

Phytochemical analysis were analyzed by using qualitative method. This analysis was use to know the active compound of *Belilik (Brucea javanica* (L.) Merr) fruit. There are some active compound that analyzed such as alkaloid, saponin, triterpenoid, steroid, flavonoid, and phenol.

Belilik(Brucea javanica (L.) Merr)anti-malaria test

Preparation of non complete medium for growing *P. falciparum*

Incomplete medium were made by mixing 10.4 g of RPMI-1640, 5.96 g of HEPES, 2.1 g of sodium bicarbonate, 0.05 gr of

hipoxanthin, and 0.5 ml of gentamicin. Then were added with aqua DM until 1000 mL. The solution were filtered with a filter paper with pore size 0.22 µm, then were inserted into a Scott bottle, and were incubated at 37° C and pH 7.3 – 7.4 before use.

Preparation of complete medium

The complete medium were made by mixing 90 mL of incomplete medium with 10 mL of human serum.

Breeding of *P. falciparum* parasite culture

Culturing procedur based on Trager dan Jansen (1976) method. Tube that containing frozed parasite were thawed at 37° C and added sodium chloride 3.5% then transferred into sentrifuge tube. The sample were sentrifuged at 1500 rpm for 5 minutes and at 4° C and the supernatant was discarded. This step was repeated three times. The precipitate were suspended with 4,5 ml complete medium and 0.5 mL of erythrocytes 50%, thed were mixed slowly. The culture was transferred into a petri dish and put in candle jar and were stored in CO_2 incubator at 37° C.

Preparation of material test

A total of 1mg of crude extract of fruits and roots *Belilik* (*Brucea javanica* (L.) Merr) were dissolved in 100 mL of DMSO (as a stock). The stock solvent then were taken 10 ml and added 490 ml of complete medium, so will obtain a solution with concentration 200 µg/ml. the variations of stock solution concentration were made to 10, 1 and 0.1 µg/ml. Preparation of test solutions were performed aseptically and Duplo.

Negative control

Negative controls were made from parasites in the media with out the test material and the solvent DMSO at a concentrationof 0.5% in the500mL of aqua DM and made Duplo.

Testing procedure

The wells were filled with 1000 µL of complete medium in row 1 dan 3, 80 µL of complete medium were added (except negative control), 120 µl of material test were added into a the well number 1 and mixed until homogeny, 120 µl of well number 1 were poured into well number 2 (then repeated until well number 5), 120 µl of solution from well number 5 were discarded, a total of 80 µl from each well were discarded (except negative control), 500 µl of each well were poured into a the well on the side, and a total of 500 µl parasite were added into each well.

After incubated for 72 hours, the culture were harvested and were made a thin layer blood smears on the slide glass. Then the preparat were fixed in methanol solution. After drying, the preparat were coloured with Giemsa 20%. The sample were observed using microscope with 100x magnification. The percentage of parasitemia were counted by counting the number of infected erythrocyte per 5000 cell with formula:

a. % parasitemia = (number of infected erythrocyte/5000 cell) x 100%;

b. % growth = % Paracite (48 hour - 0 hour);

c. % inhibition = 100% - (Xp/Xk) x 100%,

Xp = Treatment parasitemia and Xk = control negative paracitemia (-).

The LC50 were determined by probit analysis using SPSS 13.

RESULT

Extraction of *Belilik* (*Brucea javanica* (L.) Merr) root and fruit

The result showed that the extraction by using alcohol solvent produces different extract mass between root and fruit of *Belilik*. The yield of fruit extraction resulting 2.9%, whereas from root resulting 8.39%. The color of the fruit extract darker than the root extract. This may be caused of the *Belilik* ripe fruit have black color. Visually, fruit extract look oily and slimly. The type of solvent may determine the component of active compound. The result of phytochemical analysis by using qualitative method indicated that the belilik fruit contained alkaloids, flavonoids, and phenols, whereas the *Belilik* root contained alkaloids, phenols, and saponin.

The result of anti-malaria tested of *Belilik* fruit's extract

Based on the anti-malaria test showed that the use of ethanol extract can inhibit the *Plasmodium falciparum* effectively. The treatment of *Belilik* root did not increase the population of parasitemia. The smallest concentration began with 0.01 µg/mL until 100 µg/mL resulted 100% percentage barrier. Belilik's fruits had IC50 values <0.01. Statistical analysis was not performed becausethe extract is able to inhibit 100% of parasite populations. The data results are presented in Table 1.

The result of antimalaria tested of Belilik root's extract

Based on the preliminary test showed that the use of ethanol extract can inhibit *Plasmodium falciparum* larvae effectively. The threatment of Belilik root extract did not increase the population of parasitemia. The smallest concentration in

Tabel 1. The results of the testing of anti-malarial compound from *belilik* fruit extracts against *Plasmodium falciparum* 3D7 counted in 5000 erythrocytes by in vitro

Concentration (µg/mL)	R	% Parasitemia		% growth	% inhibition	% inhibition mean	IC_{50} (µg/mL)
		0 hour	48 hour				
Control (-)	1	1,18	4,94	3.76	-	-	<0,01
	2	1,18	4,74	3.56	-		
0.01	1	1,18	0,72	-0,46	100	100	
	2	1,18	0,77	-0,41	100		
0,1	1	1,18	0,65	-0,53	100	100	
	2	1,18	0,69	-0,49	100		
1	1	1,18	0,60	-0,58	100	100	
	2	1,18	0,57	-0,61	100		
10	1	1,18	0,28	-0,90	100	100	
	2	1,18	0,35	-0,83	100		
100	1	1,18	0,17	-1,01	100	100	
	2	1,18	0,21	-0,97	100		

Note: R=repetition, IC (inhibition concentration)

this experiment is 0.01 µg/mL and this concentration can inhibit 100 % of parasit. The belilik root had IC50 value < 0.01. Statistical analysis was not performed because the extract was able to inhibit 100% of parasite populations. The data results were presented in Table 2.

DISCUSSION

Belilik fruit is one of the plants that used by the people of the Bangka Belitung Islands as a malaria drug. In South Kalimantan this plant is also used as a malaria drug and called tantaran gayung , especially in districts Kotabaru (Arnida & Jonah, 2009; Chen *et al*, 2013). *Belilik* fruit seeds called Ya - and - zi in Chinese is widely used to treat cancer disease (Yoshimora *et al*, 1985; Chen *et al*, 2013), but it also can serve as an anti-pyretic, detoxification, anti-inflammatory and anti-virus with the level of toxicity low (Chen *et al*, 2013).

Tabel 2. The results of the testing of anti-malarial compound from *belilik* root extracts against *Plasmodium falciparum* 3D7 counted in 5000 erythrocytes by in vitro

Concentration (µg/mL)	R	% Parasitemia		% growth	% inhibition	% inhibition mean	IC_{50} (µg/mL)
		0 hour	48 hour				
Control (-)	1	1,18	3,53	2,35	-	-	< 0,01
	2	1,18	3,90	2,72	-		
0.01	1	1,18	0,71	-0,47	100	100	
	2	1,18	0,60	-0,58	100		
0,1	1	1,18	0,44	-0,74	100	100	
	2	1,18	0,52	-0,66	100		
1	1	1,18	0,28	-0,90	100	100	
	2	1,18	0,37	-0,81	100		
10	1	1,18	0,22	-0.96	100	100	
	2	1,18	0,26	-0,92	100		
100	1	1,18	0,18	-1,00	100	100	
	2	1,18	0,20	-0,98	100		

Note: R=repetition, IC (inhibition concentration)

According Fidock *et al*, (2004), an anti-malarial compound is effective as if it had IC50 1-5 µg/mL. The IC50 values < 0,01 indicate that the anti-malarial compounds against *Plasmodium falciparum* from *Belilik* fruit (*Brucea javanica*) from the Bangka island in this study is very good compared to other studies from Indonesia and other countries. The high of IC50 value of plant *Belilik* from Bangka island caused by anti-malarial compounds that derived from secondary metabolites of *Belilik* plant that grown on acidic soil pH conditions or in conditions of stress is higher than in other regions.

Research O'Neil *et al*, (1987) using nine kinds of kuasinoid compound that treated orally to mice that infected with *Plasmodium falciparum* K-1 (resistant to chloroquine) by in vivo method resulted the IC50 value ranged from 0.0046 to 0.0008 mg/ml. Four of the nine compounds are also active against *Plasmodium berghei* by in vivo method after treated orally. O'Neil *et al*, (1987) also stated that the effectiveness of bruseolid that found in *Brucea javanica* also relatively high when compared to chloroquine against *Plasmodium berghei* by in vivo experiments with mice.

The ability of *Belilik* to inhibit *Plasmodium* because this plant produces a variety of active compounds such as alkaloids (brucamarine, yatanine), glucoside (brucealin, yatanoside), and phenol (bruceno and bruceolic acid). The seeds of *Belilik* containing brusatol and bruceine A, B, C, E, F, G, H. The flesh of *Belilik* fruit contains fatty oil, oleic acid, linoleic acid, stearic acid, and palmitic acid. Based on all that compound, the most were brucein (Wijayakusuma, 2008; Deharo & Ginsburg, 2011; Chen *et al,* 2013).

Pharmacological effects of *Belilik* are the bitter taste, cold, and poisonous. Generally, the active compounds of *Belilik* plant able to clear heat and toxins, anti-dysentery, anti-malarial, erythropoiesis, an immunostimulant, parasiticidal. Oleic acid and Brucei A and B are anticancer (anti-neoplastic) by inhibiting DNA synthesis of cancer cells, stimulates the formation of red blood cells in the bone marrow and increasing the phagocytes and macrophages (Wijayakusuma, 2008; Chen et al, 2013).

The effectiveness of *Belilik* on both of root and fruit research as anti-malarial compounds by in vitro cause the plant should be developed on a scale higher studies and conservation efforts. This plant is also quite widely spread in Indonesia. In some places, this plant has many regional names, such as *Dadih-dadih*,*Tambursipago*, or *Tamban Bui Melur* (Sumatra); *Kendang Pencang, Kipades*, or *Trawalot* (Java);*Tambara Marica* or *Amber Marica* (Sulawesi), and *Nagas* (Maluku). Development of research or drug product ready for consumption is a step that can be taken for the development of subsequent research.

The use of *Belilik* as anti-malaria drugs can solve the resistancy of *P. falcifarum* to commercial anti-malaria drug, such as chloroquinone (O'Neil *et al*,1987). Intensity the use of chloroquinone as antimalaria drug make the resistancy of *P. falcifarum* to grow faster so that the capability of *Belilik* as anti-malaria can prevent and solve of resistancy problems. Besides, the use of *Belilik* and other natural compound as drugs can give less effect to body than chemical compound.

The conclusion of the study is that the *Belilik* fruit and root extracts produces yield 2.9% and 8.39% respectively. The *Belilik* fruits contain alkaloids, flavonoids and phenols while *Belilik* roots contain alkaloids, saponins, and phenols. Based on the anti-malarial compound test indicate that the IC50 value of fruit and root *Belilik* amounted to 0,01 µg/mL so the *Belilik (Brucea javanica* (L.) Merr.) is highly active to inhibit the growth of *Plasmodium falciparum* in vitro in very small dosage and potential to develop to the next level test.

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