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 artemisinine 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 (Schwikkard 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 ED_{50} of 6.95419 mg/kg of the mouse body weight (Utomo, 2003), and the chloroform fraction with ED_{50} 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 inhibis 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 CO_2 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 0.01μg/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 0.001μg/mL. The mixture of testing material and suspension of parasites is put in a CO_2 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:

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

$$\%\text{Inhibition} = 100\% - \left(\frac{Xt}{Xc} \times 100\% \right)$$

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 IC_{50} 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 IC_{50} value of methanol extract against each *Plasmodium* strain where the result can be seen in Table 2. According to Rasanaivo *et al.* (1992) *in* Ouattara *et al.* (2006), the extract that has the antimalarial activity with the IC_{50} 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 IC_{50} 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 *P. falciparum* strains		
	Methanol extract		Chloroquine
	3D7	G-2300	3D7
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 IC_{50} 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.