New candidate for traditional anti-malarial medicine from Kebiul seed (Caesalpinia bonduc) as a substitute for chloroquine in preclinical testing of mice

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Abstract. Kebiul seeds (Caesalpinia bonduc) are seeds from the Kebiul plant that are widely used by the community of Bengkulu in southern, Indonesia, especially by the Sarawai tribe as a cure for malaria. The purpose of this study was to scientifically prove the efficacy of Kebiul seeds as an anti-malaria medicine through preclinical testing in mice by measuring percent (%) chemosuppression of crude extract of Kebiul seeds. Twenty-five mice were divided into 5 groups, K-, K+, P1, P2 and P3, each was infected with Palsmodium berghei. Three days after infection, the number of parasitemia of mice was calculated. When the number of parasites had reached 20-30%, mice were treated orally for 3 days according to their group. Group (K-) was treated with olive oil, group (K+) with chloroquine, and group P1, P2 and P3 with crude extract of Kebiul seeds at a dose of 0.028 g / kg bw, 0.056 g / kg bw and 0.084 g / kg bw. The average number of parasitaemia developments was calculated from the 1st day of treatment to the 7th day through thin blood smears drawn from the tail. Calculation of the percentage (%) of chemosuppression was done by comparing the mean number of parasitaemia in the negative control (K-) and one in the treated group. As the result, the average percentage (%) of chemosuppression in the administration of crude extract Kebiul seeds at a dose of 0.028 g / kg bw was 51.21% higher than the administration of chloroquine (50.77%). Moreover, it has been reported in several areas including Bengkulu that malaria is resistant to chloroquine. Therefore, based on this result, Kebiul seed extract at a dose of 0.028 g / kg bw can be developed as a new traditional medicine as a substitute for anti-malarial medicine chloroquine.

1. Introduction
Bengkulu, one of the provinces in Indonesia, has 26.000 Ha areas of TNKS (Kerinci Seblat National Park) protecting several species of medicinal plants including the medicinal plant Kebiul (Caesalpinia bonduc) which contains secondary metabolites namely flavonoid, saponin, tannin and alkaloids [1]. On the contrary, Bengkulu is declared as an endemic area because the number of malaria sufferers ranks sixth among other regions in Indonesia. Malaria is a dangerous tropical disease currently querying an immediate solution from WHO. This disease can cause death for high risk groups such as babies, children under five year old and pregnant women [2]. In some areas, malaria is reported to be resistant chloroquine [3]. Correspondingly,
alternative medicine is needed. Medicinal plants are potential medicine to be researched and developed as alternative anti-malaria drugs to replace it [4].

This research is intended to scientifically prove the efficacy of Kebiul seeds as an anti-malaria medicine through preclinical testing in mice and also carried out as an effort to find new traditional medicines from Kebiul seeds for anti-malaria medicine. At the same time, this research was carried out in order to maintain cultural heritage because the knowledge of traditional medicine was obtained by the community through hereditary knowledge from the ancestors.

2. Methods

*Kebiul Seeds* sample was taken from southern Bengkulu Indonesia. The selected *Kebiul seeds* were peeled and air-dried for 1 week. The *Kebiul* seeds were then mashed using blender followed by the extraction process.

2.1. Extraction

The extraction of active compounds in *Kebiul seeds* was carried out by maceration. A total of 1 kg of *Kebiul seeds* was macerated with 2 L of technical ethanol 96% for 7 days, stirred periodically. The filtrate obtained was evaporated using a rotary evaporator. The ethanol extract from the evaporation results was used for pre-clinical trials in mice. The maceration process is the process of equilibrium between the concentration of the active compound in the cell and the concentration of the solvent outside the cell [5].

2.2. Development of *plasmodium berghei*

P. Berghei transfer was done by drawing the blood of mice from the heart by using a 0.2 mL spluit which contains 0.5 mL of anti-coagulant EDTA, then injected intraperitonially into healthy mice of ± 0.1mL. Infected mice were examined for the number of parasites. When the number of parasites had reached 20-30%, the mice were then used for anti-malaria trials.

2.3. Treatment of mice

A total of 25 male mice were divided into 5 treatment groups namely, K (-), K (+), P1, P2, and P3 consisting 5 mice. Each group was treated with the sample orally according to Table 1.

| Group | Day to 1-3 after *P. berghei* infection | Day to 4-7 after *P. berghei* infection |
|-------|----------------------------------------|---------------------------------------|
| K(-)  | Treatment olive oil                     | Calculation of parasitaemia            |
| K(+)  | Treatment chloroquine 0,6 g/kgbw        | Calculation of parasitaemia            |
| P1    | Treatment Kebiul seed extract 0,028 g/kgbw | Calculation of parasitaemia            |
| P2    | Treatment Kebiul seed extract 0,056 g/kgbw | Calculation of parasitaemia            |
| P3    | Treatment Kebiul seed extract 0,084 g/kgbw | Calculation of parasitaemia            |

2.4. Observation of parasitaemia

Observation of *P. bergheli* was done through blood smear. After dried, it went through fixation process with 3% methanol for 3 minutes, washed under running water and dried at room temperature. It was then given 10% Giemsa solution for 45 minutes then rewashed under running water and dried at room temperature. The calculation of the number of *P. bergheli* was carried out using a light microscope with a strong magnification (10 x ocular lens and 100 x objective lens) [6].

The percentage of parasitaemia was calculated with the modification of the formula adopted from Gboeloh [7,8].

\[ PP = \frac{TPRBC}{TRBC} \times 100 \] (1)
PP is the percentage parasitaemia, TPRBC is the total number of parasitized red blood cells, and TRBC is the total number of red blood cells.

The percentage chemosuppression of parasitaemia was calculated with a modification of the formula adopted from Ebiloma [9].

\[
APC = \frac{(APCG - APTG)}{APCG} \times 100
\]  

(2)

APC is the average percentage chemosuppression, APCG is the average percentage parasitaemia in control group (infected untreated group), and APTG is the average percentage parasitaemia in the test group.

3. Results and discussion

Ethanol is a very effective solvent in extracting active organic compounds such as flavonoids, saponins, tannins and alkaloids which are also contained in Kebiul seeds. In this study, Kebiul seed ethanol extract was clinically tested in mice infected with P. berghei. Firstly, 25 healthy male mice were purposely infected with P. berghei by peritonially injecting blood from P. berghei infected donor mice. In several days, P. berghei in mice grew. After 3-5 days, the number of parasitaemia was examined. When it had reached 20-30%, the mice underwent some treatments including negative control (K-) administration by olive oil, positive control (K +) giving chloroquine, and administration of Kebiul seeds extract at a dose of 0.028 g / kgbw (P1), at a dose of 0.056 g / kgbw (P2), and 0.084 g / kgbw (P3). Parasitaemia in the mice blood was examined for 7 consecutive days [10] starting from the administration of Kebiul seed extract.

The average parasitaemia in each treatment group and the percent (%) chemosuppression in each treatment can be seen in Figure 1.

![Figure 1](image-url)

Where: K- is the olive oil, K+ is the chloroquin, P1 is the Kebiul seed extract at doses 0.028/kgbw, P2 is the Kebiul seed extract at doses 0.056/kgbw, and P3 is the Kebiul seed extract at doses 0.056/kgbw

**Figure 1.** The average (%) parasitaemia and (%) chemosuppression in each treatment.

Based on Figure 1, it can be seen that the treatment of K (-) whose infected mice were given olive oil have the average number of parasitaemia 26.78%, with 0 % of chemosuppression. It means that the treatment of olive oil has no emphasis on the development of parasitaemia. The infected mice in group K (+) were administered by chloroquine have the average the number of parasitaemia 13.18 %, with 50.77% of chemosuppression. The chloroquine in this study was able to suppress the development of parasitaemia, smaller than the ability of Kebiul seeds extract at a dose of 0.028 g / kgbw which is able to suppress the development of parasitaemia by 51.21%. Giving Kebiul seed extract at a dose of 0.028g / kgbw (P1) is an effective dose in suppressing the average development of parasitaemia for 7 days observation. Therefore, Kebiul seed extract at a dose of 0.028g / kgbw can be an alternative as an antimalarial medicine instead of chloroquine.
*P. berghei* in infected mice was able to develop because it digests haemoglobin in the vacuole of erythrocytes to produce heme. This content affects the cell metabolism by inhibiting enzymes, peroxidizing membranes and producing oxidative free radicals which is very toxic even for itself [11]. Therefore, *P. berghei* converts the heme through the process of polymerizing heme to hemozoin (β-hematin) or malaria pigment which is not toxic to itself [12-15]. Chloroquine and a number of other quinolin antimalarial drugs have the ability to inhibit the formation of β-hematin [16]. The mechanism of inhibition of hemozoin formation is considered a powerful way in designing antimalarial drug discovery [17,18].

Secondary metabolites in *Kebiul* seed extract given to mice (infected with *P. berghei*) orally enter the stomach followed by small intestine. In the small intestine, absorption occurs through epithelial cells which then enter the blood vessels. Secondary metabolites that enter the blood vessels will accumulate in the vacuole of *P berghei* food so that it can inhibit the detoxification of heme to hemozoin. Secondary metabolites in *Kebiul* seeds (flavonoids, saponoin, tannins and alkaloids) either work alone or work synergistically to inhibit the polymerization of hematin by binding to oxo dimers, thereby inhibiting the formation of hemozoin [19-21]. see the reaction of the formation hemozoin (Figure 2) [22].

![Diagram](image)

**Figure 2.** The formation hemazoin and kompleks HEME-Flavonoid.

4. Conclusion
*Kebiul* seed extract at a dose of 0.028 g / kg bw has a better treatment effect compared to chloroquine in suppressing the growth of parasitaemia (percent chemosuppression: 51.21%) in mice, thus *Kebiul* seed extract at a dose of 0.028 g / kg bw can be used as an anti-malaria drug instead of chloroquine.
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