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ANTIMALARIAL ACTIVITY OF CASSIARIN A FROM THE LEAVES OF *CASSIA SIAMEA*

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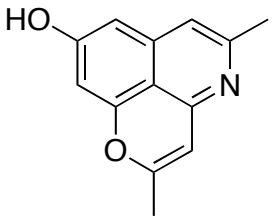
Abstract – The extracts and cassiarin A from the leaves of *Cassia siamea* showed promising antimalarial activities. The ethanol and chloroform extracts, alkaloid fraction, cassiarin A, and chloroquine as a positive control exhibited the inhibition against *Plasmodium falciparum* (IC_{50}: 7.06, 2.41, 0.24, 0.005, and 0.006 μg/mL, respectively). The *in vivo* antimalarial activity was assessed using the 4-day suppressive test procedure. The ethanol, chloroform extracts, and alkaloid fraction were found to give an ED_{50} 34.7, 19.59, and 0.47 mg/kg, respectively (p.o.). The ED_{50} values of cassiarin A and chloroquine were 0.17 and 0.21 mg/kg, respectively (i.p.). The result showed that cassiarin A deserved further investigation toward development of a promising antimalarial drug.

Malaria is one of the crucial infectious disease in the world and continues to cause morbidity and mortality on a large scale in tropical countries.[1] According to WHO, it is a threat to over 2 billion people living in areas of high incidence.[2] A major contributor to malarial morbidity and mortality is almost certainly the increasing resistance of malaria parasites to available drugs.[3] Such a situation has heralded the need for alternative antiplasmodial therapy. Antimalarial potential of drugs derived from plants has been proven by examples such as quinine from *Cinchona* species and artemisinin from *Artemisia annua*.[4] Selection of plants to be screened for antimalarial activity has been done on the basic traditional reputation of particular plants for efficacy in the treatment of malaria.[5]



cassiarin A

Indonesia is known with its green tropical vegetation and forest, which is an advantage for many plant research applied for therapy. One of Indonesia plants that has traditionally used as antimalarial medicines is Johar (*Cassia siamea* Lamk, Leguminosae).[6] Recently, two new alkaloids, cassiarins A and B, and a dimeric chromone, chrobisamone A have already been isolated from the leaves of *C. siamea*.[7] Due to the traditional use of the leaves of *C. siamea* by the Java people (Indonesia) to treat malaria, this paper describes studies on the extracts and cassiarin A isolated from the alkaloid fraction of the leaves of *C. siamea* for *in vitro* and *in vivo* antimalarial activities.

In Vitro Antimalarial Activity

The antimalarial activities of the extract and isolated compounds were determined by the procedure described by Budimulya *et al.*[8] Each compound was separately dissolved in DMSO (10^{-2} mol L^{-1}). The malarial parasite *P. falciparum* was propagated in a 24-well culture plate in the presence of a wide range of concentrations of each compound. The growth of the parasite was monitored by making a blood smear fixed with MeOH and stained with Geimsa stain.

High levels of chemosuppression against *Plasmodium falciparum* isolates from Myanmar (chloroquine-resistant clone) were produced at high doses of the extract in a concentration-dependent manner (ethanolic extract, IC_{50} 7.06 μg/mL; chloroform extract, IC_{50} 2.41 μg/mL). Alkaloid fraction of *C. siamea* leaves showed the most potent inhibitory activity (IC_{50} 0.24 μg/mL) as shown in Figure 1. Cassiarin A showed a potent antiplasmodial activity (IC_{50} 0.005 μg/mL) comparable to that (IC_{50} 0.006 μg/mL) of chloroquine diphosphate against *P. falciparum* strain 3D7 (chloroquine sensitive clone) (Figure 2).

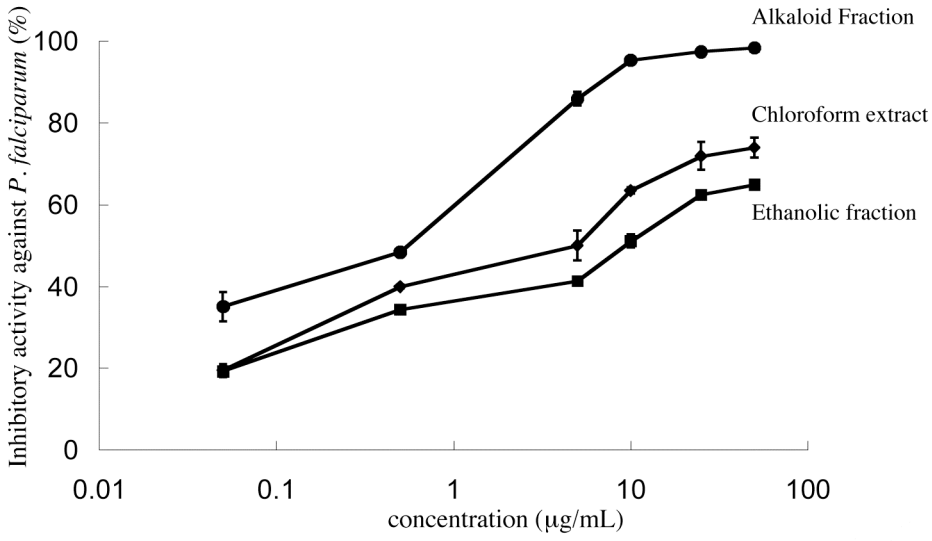


Figure 1. Antimalarial Activity of *Cassia siamea* Leaves against *P. falciparum*

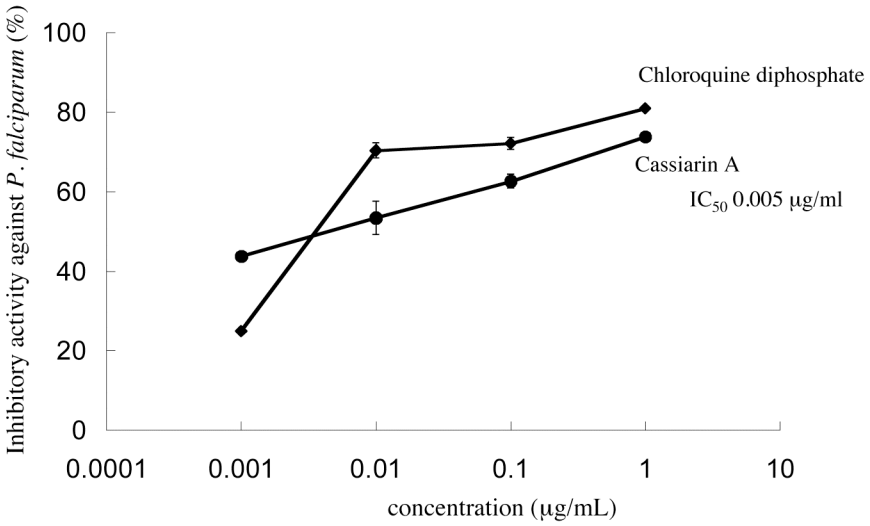


Figure 2. Antimalarial Activity of Cassiarin A against *P. falciparum* 3D7

In Vivo Antimalarial Activity

The antimalarial activities of the extracts of *C. siamea* in mice parasitized with *P. berghei* were summarised in Figures 3 and 4 at different doses. Five doses from 6.25 to 500 mg/kg body weight (p.o.) were screened for the decoction (ED_{50} 83.77 mg/kg), ethanol extract (ED_{50} 34.69 mg/kg), and chloroform fraction (ED_{50} 19.59 mg/kg). For alkaloid fraction, four doses from 1.5 to 12.5 mg/kg body weight were also screened. Activity was concentrated into the alkaloid fraction (ED_{50} 0.47 mg/kg) while that of chloroquine is 0.16 mg/kg (p.o.). By intraperitoneal administration, cassiarin A and chloroquine were tested from 0.002 to 2 mg/kg. The result indicated that cassiarin A showed more potent antimalarial activity (ED_{50} 0.17 mg/kg) than that of chloroquine (ED_{50} 0.21 mg/kg).

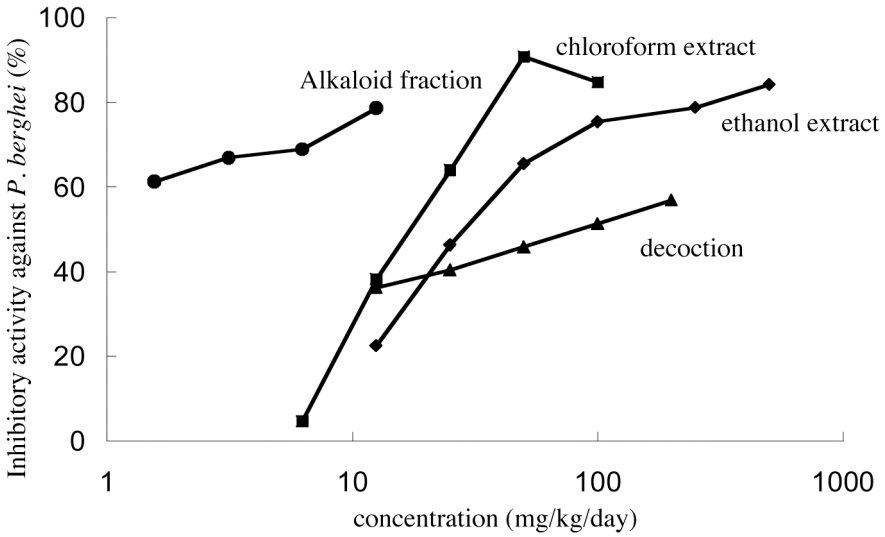


Figure 3. Antimalarial Activity from *C. siamea* Leaves against *P. berghei* in Mice (p.o.)

Although primate models provide a better prediction of efficacy in human than the rodents models, the latter have also been validated through the identification of several conventional antimalarials, such as chloroquine, halofantrine, mefloquine, and more recently artemisinin derivates.[11] *P. berghei*, which was an appropriate parasite for the study, was used in the prediction of treatment outcomes. As this parasite was sensitive to chloroquine, this drug was employed as a standard drug in this study.

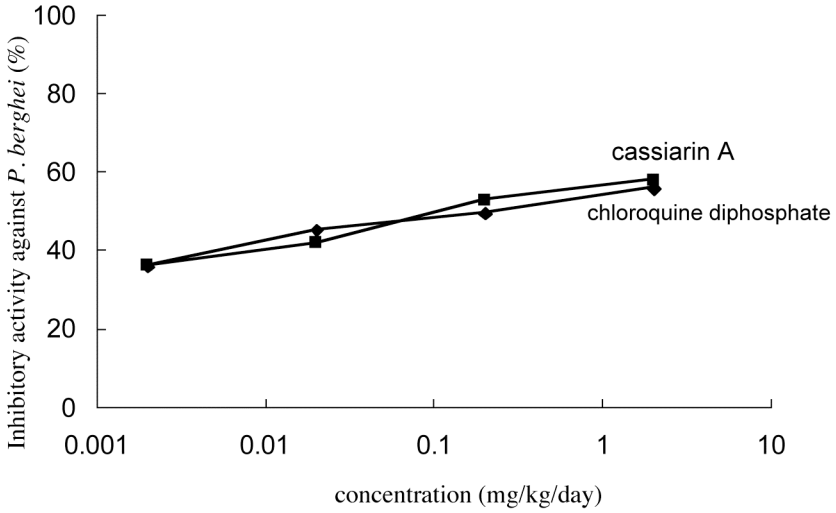


Figure 4. Antimalarial Activity of Cassiarin A against *P. berghei* in mice (i.p.)

It was proved in *P. berghei* infected mice, when treated with cassiarin A, the activity was more effective than that of chloroquine as a positive control (i.p.). In conclusion, the present work showed, in an animal model of malaria, efficacy of cassiarin A from the leaves of *C. siamea* showed that it deserved further investigation toward development of promising antimalarial drugs.

EXPERIMENTAL

Plant Material. The leaves of *C. siamea* were collected from Purwodadi Botanical Garden, Pasuruan, Indonesia and a voucher specimen was deposited at the herbarium.

Extraction and Isolation. The powdered and defated leaves (1.53 kg) were extracted with 90% EtOH containing 1 % tartaric acid at rt. The concentrated extract was alkalinized with NH_4OH (pH 8) and extracted with $CHCl_3$ to give a brown solid (80 g). The $CHCl_3$ extract (10 g) was fractionated by a silica gel column with hexane–$CHCl_3$–EtOH solvent system as a mobile phase. The alkaloid fraction eluted with hexane–$CHCl_3$–EtOH = 0 : 7 : 3 was subjected to a silica gel column with the same solvent system, followed by preparative TLC ($CHCl_3$-EtOH, 8.5 : 1.5) to yield cassiarin A (0.0008% yield). The decoction of the leaves of *C. siamea* was prepared from 20 g powdered leaves with 200 ml of distilled water during 15 min, then freeze-dried to yield the extract (4.3 g).

***In Vitro* Antiplasmodial Assay.** The antimalarial activities of the extract and isolated compounds were determined by the procedure described by Budimulja *et al*.[8] Stock solution of the samples were prepared in DMSO (final DMSO concentrations of < 0.5%) and were diluted to the required concentration with complete medium (RPMI 1640 supplemented with 10% human plasma, 25 mM HEPES and 25 mM $NaHCO_3$) until the final concentration of samples at well culture plate were : 10; 1; 0.1; 0.01; 0.001 μg/mL. The malarial parasite *P. falciparum* 3D7 clone was propagated in a 24-well culture plate in the presence of a wide range of concentrations of each compound. The growth of the parasite was monitored by making a blood smear fixed with MeOH and stained with Geimsa solution. The antimalarial activity of each compound was expressed as an IC_{50} value, defined as the concentration of the compound causing 50% inhibition of parasite growth relative to an untreated control.

The percentage of growth inhibition was expressed according to following equation: Growth inhibition % = 100 - [(test parasitaemia/control parasitemia) × 100. Chloroquine: IC_{50} = 0.0061 μg/mL.

***In Vivo* Antiplasmodial Assay.** The 4-day suppressive test was employed as described by Peters *et al*.,[9] which has been a standard test commonly used for antimalarial screening and the determination of percent inhibition of parasitemia has been the most reliable parameter.[10] Adult BALB/c male mice of ± 25 g body weight were infected intraperitonially with 1×10^7 parasitised red blood cells containing *Plasmodium berghei* strain ANKA. The infected mice were divided into groups of five mice per group. After infection, each group of mice was treated for 4 days with decoction, ethanol, $CHCl_3$ and alkaloid fraction of *C. siamea* leaves at various doses (p.o.). The negative control group was treated with CMC-Na. Cassiarin A and chloroquine was administered as a single dose by intraperitonial for 4 days. Chloroquine disphosphate was used as positive control.

Thin film of tail blood were made daily for 5 day starting from the first day of treatment, stained with Giemsa and parasite count of each blood film was under oil immersion. The total number of red blood cells for each blood film was first counted and then total number of parasitized red blood cells in order to assess the degree of parasitemia in percentage and the ED_{50} was determined. The ED_{50} representing 50% suppresion of parasite when compared with untreated control. The percentage inhibition of parasite growth for each plant fraction was calculated as :

$$\% \text{ inhibiton of parasite growth} = 100 - 100 \times \frac{(\text{mean \% parasite growth in treated mice})}{(\text{mean \% parasite growth in control mice})}$$

ACKNOWLEDGMENTS

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