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Effects of Morachalcon a Compound Isolated from *Artocarpus champeden* Spreng Stembark on the Morphology of the Malaria Parasites.

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ABSTRACT

Morachalcon A compound of prenylated chalcones was isolated from the stembark of *artocarpus champeden* and the structure was determined by the basis of spectroscopic analysis. The antimalaria activities of Morachalcon A were determined against the growth of *Plasmodium falciparum* in vitro. The malaria parasite *P. falciparum* 3D7 clone was propagated in a 24-well culture plate in the presence of 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. The antimalarial activity of compound was expressed as a IC_{50} value, defined as the concentration of the compound causing 50% inhibition of parasite growth relative to an untreated controll. The results indicate that all compounds exhibited antimalarial activity with IC_{50} of 0,28µg/ml.The findings indicate that Morachalchon A compounds of the *A. champeden* exhibited potent antimalarial activities. Therefore, the compounds are potentially developed into novel antimalarial drugs.

Keywords: *Artocarpus champeden* stembark, Morachalcon A , antimalarial

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INTRODUCTION

Malaria is one of the most prevalent parasitic diseases. It is estimated that nearly half the world population is at risk, with fatal rates being extremely high among young children below 5 years old. Worldwide prevalence of this disease is estimated to be more than 500 million acute illnesses and more than 1 million deaths each year [1]. Until now the battle against this deadly disease has not been successful due to the appearance of drug-resistant strain.Therefore, high efforts to develop alternative antimalarial drug are being sought thoughout the world. Increase efforts in antimalarial drug discovery is urgently needed. The goal must be to develop safe and affordable new drug to counter the spread of malaria parasites that are resistant to exiting agents.

Artocarpus champeden Spreng. (syn. A. integer Merr.), belongs to the family moraceae, is widely cultivated thoughout the tropical and subtropical regions of south-east Asia. In Indonesia, this plant is commonly known as"Cempedak"and its stembark is traditionally used for treatment of fever, diarrhoea, and malaria [2]. The antimalarial activities of many flavonoid compounds have been reported. Xanthones from *Garcinia dulcis* (Guttiferae) showed inhibitory effects on the growth of *P. falciparum* [1].

Several prenylfavonoids from *A. champeden* have also been reported to possess cytotoxic antimalarial activities [3,4]. In addition, mannose binding lectin isolated from its seed was also reported to have mitogenic activity and interaction with lgE and lgM [3-7]. Recently, our preliminary test also revealed that CH2Cl2 and methanolic extracts of the stembark of *A. champeden* exhibited potent antimalarial activities against *Plasmodium falciparum* in vitro and *P. berghei* ANKA in vivo.

The antimalarial activity of chalcone, a flavonoid compounds isolated from *Artocarpus heterophylus* was found to be mediated through inhibition of hemoglobin degradation [8]. Further, prenylated chalcones was proved to interfere with the haemin-degradation process in *P. falciparum* [9-11]. Hemoglobin is ingested by the malaria parasite during its development in the erythrocyte and digested inside the food vacuole of the parasite [12-13]. The present study aims to determine the antimalarial activities of Morachalcon A compound isolated from *A. champeden* stembark as well as their potential effects on the malaria parasite.

MATERIALS AND METHODS

Plant Material

Artocarpus champeden dried stembarks were collected from "mak Balim" village, Sorong Irian Jaya, Indonesia. A voucher specimen was identified by the staff from Herbarium Bogarienes, Bogor Botanical Garden, Bogor, Indonesia, and stored at the Herbarium.

Extraction and Isolation

The dried plan material (4.8 kg) was grounded and extracted with Methanol (MeOH) by maceriton. Fractionation of the MeOH extract (5.85g) of the stembark was done using vacuum-liquid chromatography over Si-gel as stationary phase using a hexane-EtOAc gradient as mobile phase to yield 5 different fractions. Isolation of the fraction using repeated column and preparative thin layer chromatographies.

Parasite cultivation in vitro

P. falciparum (strain 3D7) was propagated in vitro following the procedures published previously [14] in 24-well culture plates. The cultures were maintained in a candle jar placed in a 370 C, 5%, CO2 incubator and the parasitemia was monitored every day by making blood smears.

Determination of the antimalarial activity

The antimalarial activity of the isolated compounds was determined by the procedure described by Budimulja *et al* [15]. The malaria parasite *P. falciparum* 3D7 clone was propagated in a 24-well culture plate in the presence of a wide range of concentrations of the compound. Using concentrations ranged from 0,0001-10 µg/ml.The growth of the parasite was monitored by making a blood smear fixed with MeOH and stained with

Geimsa. The antimalarial activity of compound was expressed as a IC50 value, defined as the concentration of the compound causing 50% inhibition of parasite growth relative to an untreated control.

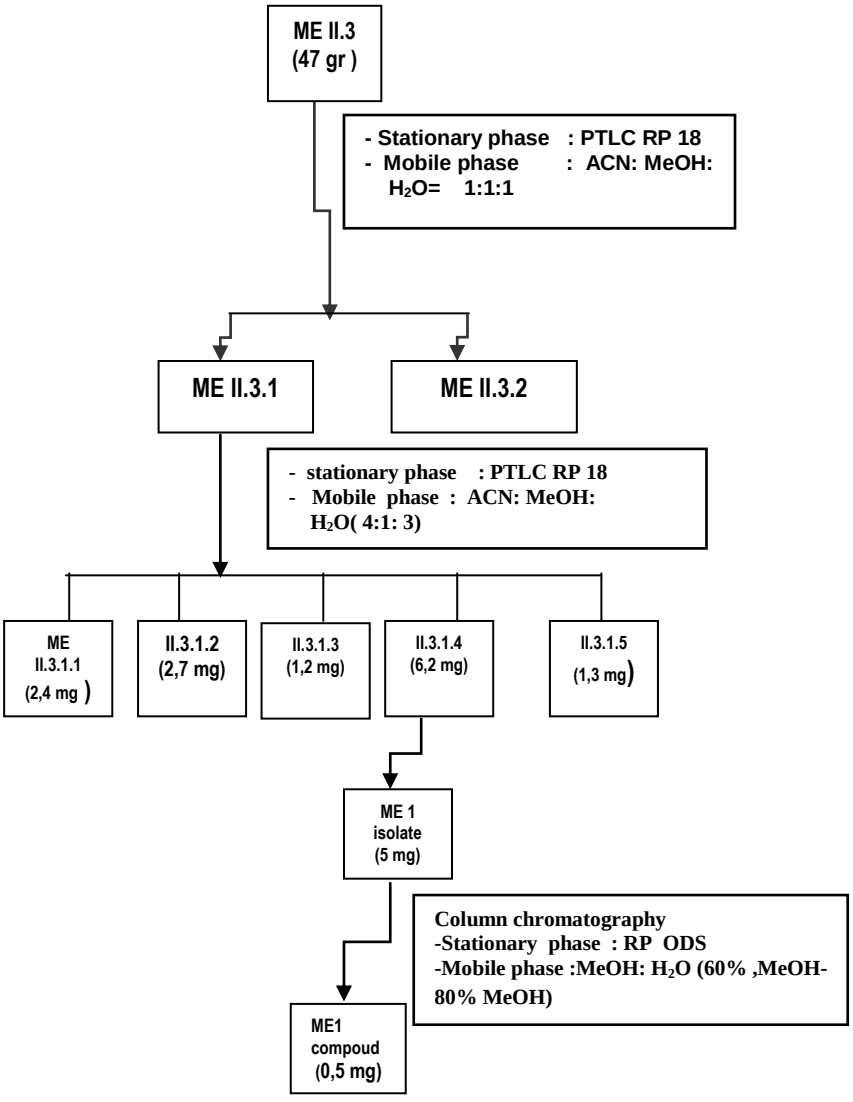
Effects of morachalcon A on the morphology of the malaria parasite

To determine the effect of the compounds at the subcellular structure, we evaluated the morphologies of 3D7 strain parasites after incubation with the compounds for certain period of time.

RESULTS AND DISCUSSION

Fractionation of the MeOH extract (5.85g) of the stembark using column chromatography on silica gel with hexane/EtOAc gradient system solvents gave rise to five different fractions (MEI-V). Fraction ME II was identified using thin layer chromatography (TLC), stained with cerrisulphate and eventually produced an orange-colored band. This band indicates that it contained flavonoid compound. Fraction ME II was further separated using open column chromathograpy and gave rise to seven sub frations (MEII. 1-7). Sub fractions MEII.3 and MEII.4 was identified using TLC, stained with cerrisulphate and eventually produced an orange-colored band indicated that the band contained flavonoid compound. The schematic presentation of the fractination procedures is shown in Figure 1. Sub fraction MEII.3 was subjected to reversed-phase preparative TLC with ACN:MeOH:H_2O to give rise to isolate 1 (5.0 mg) and isolate 1 was indentified and yielded one new flavonoid compound, morachalcon A (Fig. 1).

Figure 1: The schematic of the fractinated proceduresis sub fraction ME II.3 of methanol extract of cempedak (*A.champeden* Spreng) stembark using reversed-phase preparative thin layer chromatography (TLC) and column chromatography.



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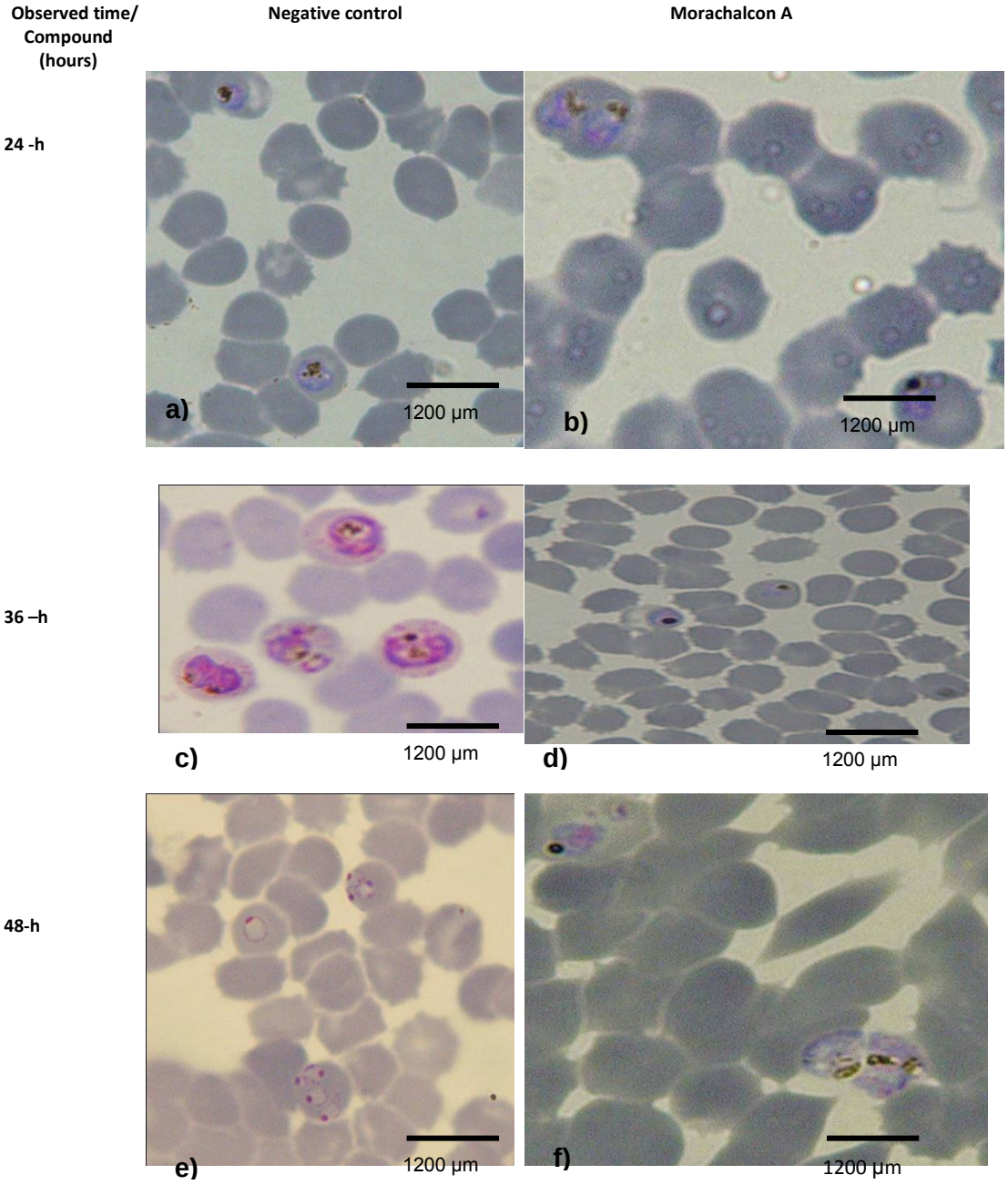
Morachalcon A was isolated as a amorphous yellow powder. UV (λmax) absoptions at 320 and 380 nm, indicated the presence of unsaturated binding (diena and polydiena), carbonyl groups, α,β- unsaturated, aromatic ring. IR absorptions at 3412,2919,1384 and 1616 cm^{-1} indicated the presence of hydroxyl, CH aromatic, C aromatic and carbonyl groups, respectively. Moreover, the 'H NMR spectrum of morachalcon A of the signals for two ortho-coupled aromatic protons (one of which is clearly peri to the carbonyl group owing to the lowfield chemical shift) required the placement of the prenyl group at C-3', thus revealing the substitution pattern of the B-ring . The characteristic proton signals for a isoprenylated group (δ 1,66 dan 1,79 singlet 3H, 2x Me). This was confirmed by analysis of the COSY, NOSY,HMQC and HMBC. Thus, morachacon A was assigned as 2,4,2',4'-tetrahydroxy-3'-prenylated-chalcone.

Figure 2: Development models of *P. falciparum* (strain 3D7) incubation of Morachalcon A compound at 24-48 h. (a,c,e) negative control, (b,d,f) morachalcon A, using light microscope (Olympus);10x100 magnification power. Scale 1200 µm.



The antimalarial activity of the Morachalcon A compound using concentrations ranged from 0,0001-10 µg/ml, displayed a significant dose-dependent inhibition, with IC_{50} of 0.28 µg/ml. In addition, the growth

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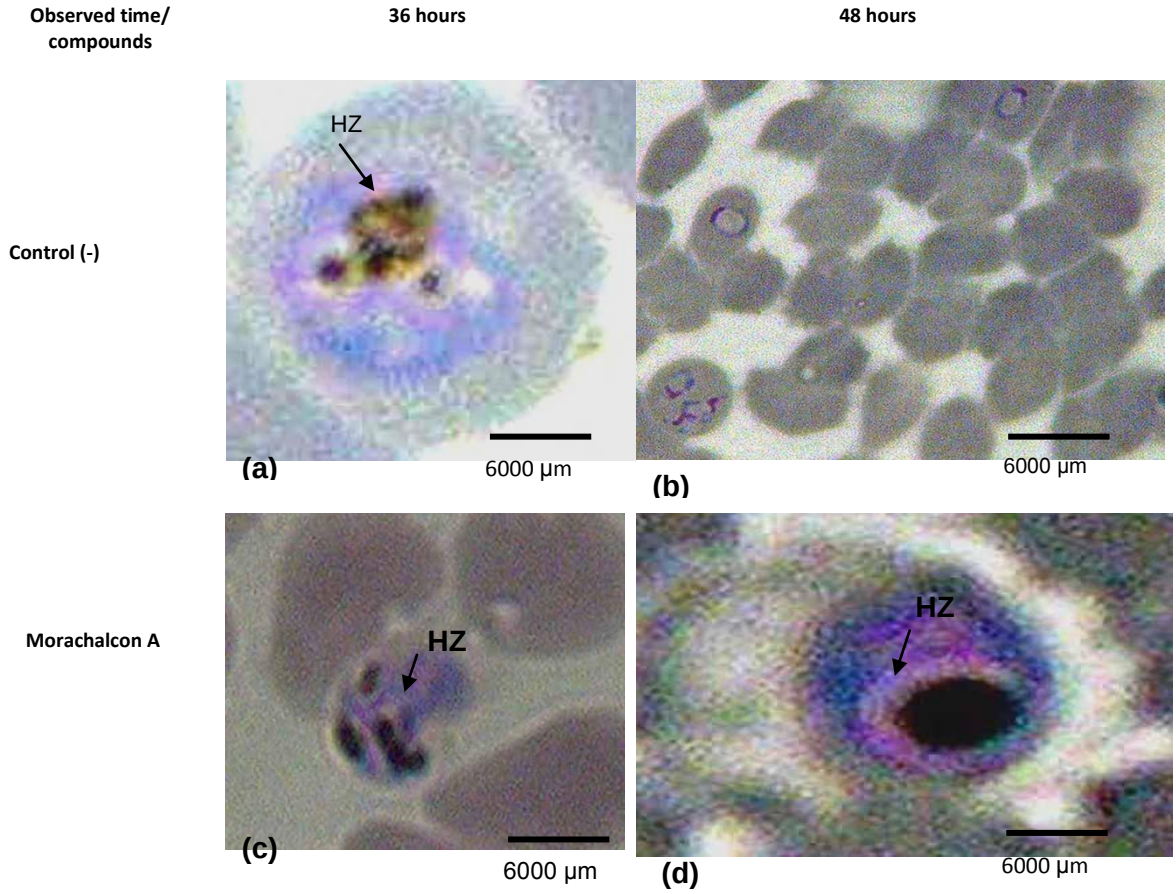
of the parasite was also found to be delayed in comparison to the untreated group. After 48-h, the untreated group mainly contained new ring stage parasites where as treated groups contained only few or on new ring from and trophozoites.

We evaluated the morphologies of 3D7 strain parasites after incubation with the compound for certain period of time. Examination under light microscope for 24-48h (Fig. 2-3) revealed the extraerythrocytic location of trophozoites with intracellular membrane (Fig. 3-a-b), distention of food vacuole, absence or darkly stained and aggregated hemozoins (Fig. 3 c-d). In addition, the growth of the parasite was also found to be delayed in comparison to the untreated group. After 48-h, the untreated group mainly contained new ring stage parasites where as treated groups contained only few or one new ring of trophozoites.

Figure 3: Development models of trofozoit stadium of *P. falciparum* (strain 3D7) incubated of Morachalcon A compound at 36-48 h. (a-b) negative control, (c-d) morachalcon A, using light microscope (Olympus);10x100 magnification power. FV = food vacuola of *P. falciparum*; HZ = Hemozoin, Nk= nucleus. Scale 6000 µm.



The mechanism underlying the antimalarial activity of the Morachalchon A compound has been analysed. The compound exhibed the potent antimalarial activity. Effect of flavonoid compound of Morachalcon A caused morphological changes of *P. falciparum* 3D7 strain by light microscope revealed the extraerythrocytic location of trophozoites with intraselular membrane, distention of food vacuole, absence, darkly stained and aggregated hemozoins. In addition, the growth of the parasite was also found to be delayed in comparison to the untreated group extraerythrocytic location of *P. falciparum* trophozoites with intraselular membrane (parasitophorous vacuolar membrane) PVM as reported previously[16-20].

On the other hand, effect of flavonoid antimalarial on hemoglobin degradation pathway by direct interaction between the hydroxyl groups. The precise mechanism by which this occurs in the parasite food vacuole is still uncertain but the other author suggested that dihydroartemisinin from of artemisinin exhibit hydroxyl group plays a relevant role to react to ferrous hemoglobin and also seems to play a role in the

antioxidant activity[21-25]. The inhibitory activity of these prenylated chalcon supports the traditional use of the dried stem bark of *A. champeden* as novel antimalarial drug.

CONCLUSION

The antimalarial activity of the isolated compounds displayed a significant dose-dependent inhibition, with IC_{50} of 0.28 µg/ml.

Effect of flavonoid compound caused morphological changes of *P. falciparum* 3D7 strain by light microscope revealed the extraerythrocytic location of trophozoites with intraselular membrane, distention of food vacuole, absence, darkly stained and aggregated hemozoins.

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