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Preliminary study on antimalarial activities of Thai herbal medicines

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Abstract

Malaria remains one of the most serious causes of mortality and morbidity in the tropics. The problems of multi-drug resistance *Plasmodium falciparum* have been aggravating and up to present, there is still no new clinical effective antimalarials to replace artemisinin and derivatives. It is therefore an urgent need to search for new promising antimalarial drug targets. In this study, we assess the *in vitro* antimalarial activity of the ethanolic extracts of the four Thai medicinal plants/regimen against the chloroquine(CQ)-resistant (K1) and CQ-sensitive (3D7) strains of *P.falciparum.* The ethanolic extracts of all medicinal plants/regimen showed promising antimalarial activity against both CQ-resistant and CQ-sensitive strains of *P.falciparum*, of which the extract of *Piper chaba* Hunt exhibiting the most potent antimalarial activity with IC_{50} of less than 10 μg/ml against both CQ-resistant and CQ-sensitive strains. Proteomics study of the extracts of these four plants/recipe are being investigated to identify their molecular targets of antimalarial action.

Keywords : *Plasmodium falciparum*, Thai herbal medicine, antimalarial, proteomics, drug resistance

Introduction

Malaria remains one of the most serious causes of mortality and morbidity in the tropics. The problems of multi-drug resistance *Plasmodium falciparum* have been aggravating and up to present, there is still no new clinical effective antimalarials to replace artemisinin and derivatives (1). It is therefore an urgent need to search for new promising antimalarial drug targets. Thai medicinal plants have been increasingly applied as an alternative treatment for various infectious diseases including malaria. The ultimate goal of the study was to screen a total of 30 medicinal plants/recipes for their *in vitro* antimalarial activity against a total of 30 *P. falciparum* isolates collected from the Thai-Myanmar border. Promising candidates were planned for investigation of their protein targets. Here, were present the preliminary results on the antimalarial activity of the ethanolic extracts of three Thai medicinal plants namely *Piper chaba* Hunt, *Atractylodes lancea*, *Zingiber officinale* Roscoe, and one recipe-- Prasapraoyai, against the laboratory strains—chloroquine (CQ)-resistant K1 and CQ-sensitive 3D7. Standard antimalarial drugs CQ, mefloquine (MQ) and artesunate (ARS) were used as control drugs for activity against K1 and 3D7 strains. The plants/recipe have been used for centuries in Thai folklore medicine to treat various diseases. The *Piper chaba* Hunt is a well-known medicinal plant for the treatment of a variety of symptoms such as stomachache and fetch-up. The *Atractylodes lancea* has been claimed to be an effective diuretics and antipyretics. The *Zingiber officinale* Roscoe is commonly used to treat several symptoms including common cold, anarcartharsis and diarrhea. The Prasapraoyai recipe has been used to treat fever in children. In addition, further study on proteomics was also planned, in order to identify protein targets of these plant extracts.

Methods

Plant Materials*:* The ethanolic extracts of the four medicinal plants/recipe were prepared at the Applied Thai Traditional Medicine Center, Faculty of Medicine, Thammasat

University. Plant materials were collected from various parts of Thailand and some were purchased from the city markets. Authentication of plant materials was carried out at the herbarium of the Department of Forestry, Bangkok, Thailand, where the herbarium vouchers have been kept. A duplicate set has been deposited in the herbarium of Southern Center of Thai Medicinal Plants at Faculty of Pharmaceutical Science, Prince of Songkhla University, Songkhla, Thailand.Stock solutions of the ethanolic extracts were prepared at concentration of 1 mg/ml.

Parasite Culture: CQ-sensitive (3D7) and CQ-resistant (K1) strains of *P. falciparum* were maintained in continuous culture in O^+ human erythrocytes suspended in RPMI 1640 culture medium (at 37°C under a gas mixture of 5% CO_2, 5% O_2, and 90% N_2) according to the standard method described by Trager and Jensen (2). The culture medium was supplemented with 25 mM sodium bicarbonate, 10 mg/ml gentamicin sulfate, 25mM HEPES (pH7.4), 80 ml/l human B or AB serum. To obtain specific life cycle stages of *P. falciparum*, 5% sorbitol treatment was used (3).

Assessment of Antimalarial Activity In Vitro: Antimalarial activity screening of the four medicinal plants/recipe were performed in a 96-well microtiter plate based n SYBR green-I-based assay (4-5). Experiments were repeated three times, and triplicate in each experiment. The concentration range of the extracts used was 0.78- 100 µg/ml. Assays were initiated at 2% parasitemia and 1% hematocrit. Fifty percent inhibitory concentration (IC_{50}) values were calculated for each plant extract based on dose effect analysis (CalcuSyn^{TM} software).

Results

Figure 1A, B, C and D showed dose response curves of the four medicinal plants/recipe in 3D7 and K1 strain *P.falciparum in vitro*. The median (range) IC_{50} values of the ethanolic extract of *Piper chaba* Hunt for 3D7 and K1 strains *P.falciparum* were 4.1 (4.7-3.8) and 5.3 (7.1-3.9) µg/ml, respectively (Figure 1A). The IC_{50} values for the extract of *Atractylodes lancea* in 3D7 and K1 strains were 17.3 (57.8- 17.0) and 19.5 (49.5-7.1) µg/ml, respectively (Figure 1B). The extract of Prasapraoyai recipe showed median (range) IC_{50} values of 12.6 (15.8- 9.1) and 16.3 (32.6- 15.7) µg/ml, respectively (Figure 1C). The corresponding IC_{50} values of the extract of *Zingiber officinale* Roscoe were 36.7 (125.6-27.0) and 16.9 (68.8-15.2) µg/ml, respectively (Figure 1D).

Table1. *In vitro* antimalarial activity of *Piper chaba* Hunt, *Atractylodes lancea*, Prasapraoyai regimen, and *Zingiber officinale* Roscoe against 3D7 and K1 *P. falciparum* strains. CQ, MQ and ARS were used as control drugs

Ethanolic extract or drugs	Median IC_{50} (range) (µg/ml) of parasite strain	
	3D7	K1
1.*Piper chaba* Hunt	4.1 (4.7-3.8)	5.3 (7.1-3.9)
2.*Atractylodes lancea*	17.3 (57.8- 17.0)	19.5 (49.5-7.1)
3.Prasapraoyai regimen	12.6 (15.8- 9.1)	16.3 (32.6- 15.7)
4.*Zingiber officinale* Roscoe	36.7 (125.6- 27.0)	16.9 (68.8-15.2)
5.Chloroquine*	9.4 (11.6-9.3)	128.7 (139.2-109.3)
6.Mefloquine*	20.8 (28.0-19.2)	10.4 (10.7-10.3)
7.Artesunate*	2.1 (2.5-2.0)	1.9 (2.1-1.9)

* Unit of median IC_{50} (range) is nM.

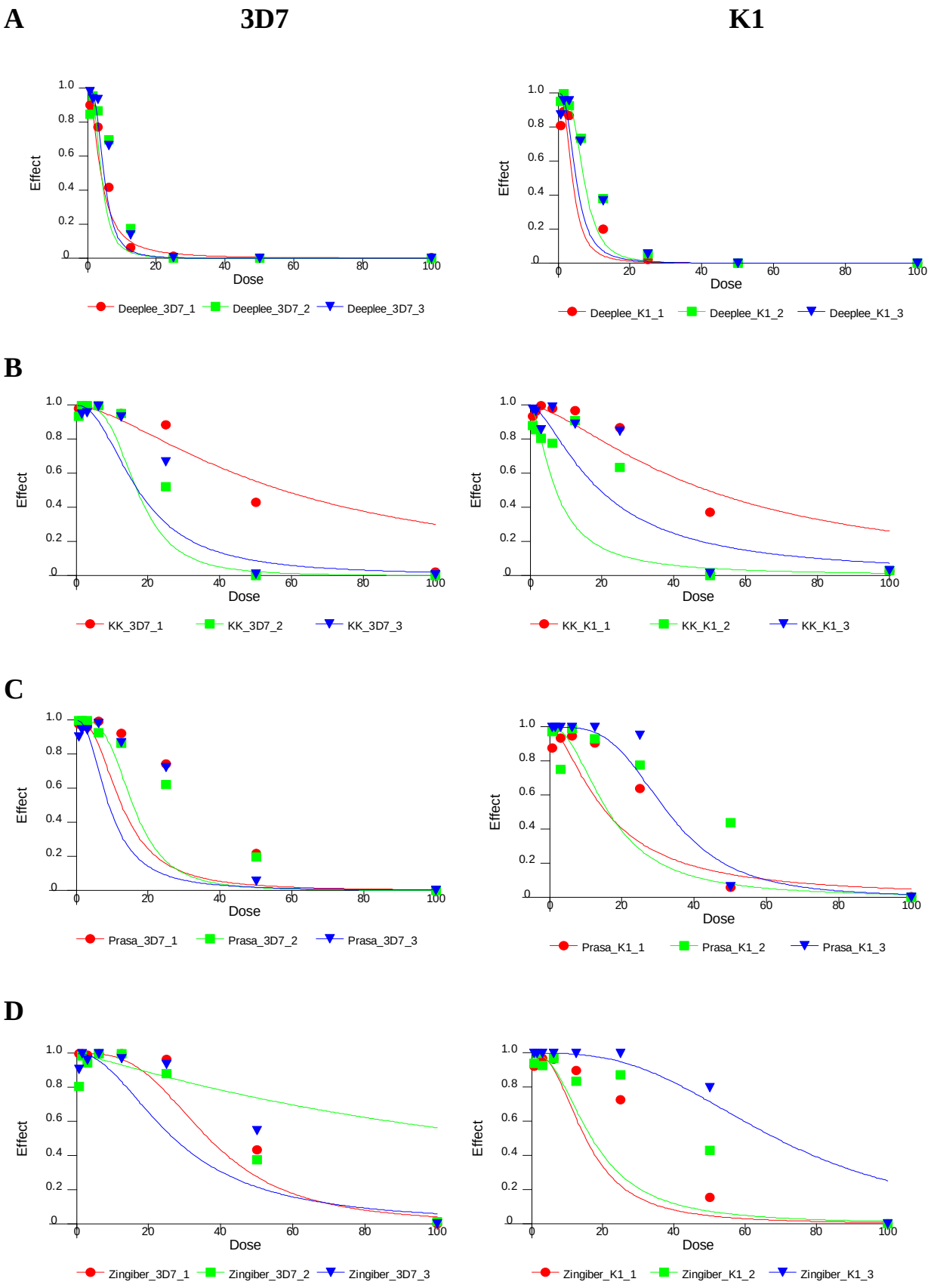

Figure 1 Dose response curves of the four medicinal plants/regimen (A) *Piper chaba* Hunt, (B) *Atractylodes lancea*, (C) Prasapraoyai regimen, and (D) *Zingiber officinale* Roscoe in 3D7 and K1 *P. falciparum* strains.

Discussion

The ethanolic extract of *Piper chaba* Hunt exhibited the most potent antimalarial activity with IC_{50} of less than 10 μg/ml against both CQ-resistant and CQ-sensitive strains. Based on the criteria for categorization of plant extracts with antimalarial activity (6), it could be classified as 'high activity' (6). The antimalarial activity of *Atractylodes lancea*, Prasapraoyai regimen and *Zingiber officinale* Roscoe are classified as 'moderate activity' with IC_{50} varying between 10-100 μg/ml. It is noted however that the antimalarial activity of all extracts except that of *Zingiber officinale* Roscoe, were similar against CQ-sensitive and CQ-resistant falciparum strains. Proteomics study of the extracts of these four plants/recipe are being investigated to identify their molecular targets of antimalarial action.

Conclusion

Ethanolic extracts of the four medicinal plants/recipe namely *Piper chaba* Hunt, *Atractylodes lancea, Zingiber officinale* Roscoe, and Prasapraoyai recipe showed promising antimalarial activity against both CQ-resistant (K1) and CQ-sensitive (3D7) *P. falciparum* strains.

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