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Komunikasi Pendek/*Short Communication*

In vitro Antiplasmodial Activity and Cytotoxicity of Ten Plants Used as Traditional Medicine in Malaysia

(Aktiviti Antiplasmodium dan Sitotoksisiti Secara *In vitro* Sepuluh Tumbuhan yang Biasa Digunakan dalam Rawatan Tradisional di Malaysia)

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ABSTRACT

Dichloromethane and methanolic extracts of each plant were tested for their antiplasmodial activity on chloroquine-resistant strain of Plasmodium falciparum (FCB strain), based on lactate dehydrogenase activity. Cytotoxicity was assessed with the MTT test on MRC-5 human diploid embryonic lung cells. Most extracts of ten selected plants used in Malay traditional medicine in Malaysia had activity in vitro. This supports continued investigations of traditional medicine in the search for new antimalarial agent. The compounds responsible for the observed antiplasmodial effects are under investigation.

Keywords: Plasmodium falciparum, Plant, Antiplasmodial activity, Cytotoxicity, Malaysia.

ABSTRAK

Ekstrak diklorometana dan metanol setiap pokok telah diuji aktiviti antiplasmodium terhadap Strain Plasmodium falciparum yang rintang klorokuin (Strain FCB) berdasarkan aktiviti dehydrogenas laktat. Sitotoksisiti diukur melalui ujuan MTT pada sel paru-paru embrionik diploid manusia MRC-5. Kebanyakan ekstrak sepuluh tumbuhan yang digunakan dalam perubatan tradisional Melayu mempunyai aktiviti secara in vitro. Ini menyokong penelitian berterusan perubatan tradisional dalam pencarian agen antimalaria baru. Sebatian yang memperlihatkan kesan antiplasmodium, sedang dalam penyelidikan.

Kata kunci: Plasmodium falciparum, Tumbuhan, Aktiviti antiplasmodium, Sitotoksisiti, Malaysia

Despite decades of intense research, malaria remains a deadly worldwide disease. Drug-resistance to limited available antimalarials, in part, has contributed to the persistence of this infectious disease. Likewise, the use of antimalarials such as artemisinin, though effective in global malaria control programs, is hampered by high cost and limited supply (World Health Organization 2008). Therefore, identification of an antimalarial drug that is easy to isolate and produce, is inexpensive, and demonstrates little toxicity across a diverse population represents the ideal agent needed for global malaria control programs and eradication of this deadly disease. These compounds have exhibited promising antimalarial activities *in vitro* and *in vivo* (Wan Omar et al. 2007). However, limitations such as toxicity, low bioavailability and/or poor solubility have probably restricted the scope of use for several plant products in humans. (Lee et al. 2009; Rajakumar & Shivana 2009). Plants provide novel leads, which can be developed into safe drugs by synthetic strategies as exemplified by artemether and quinoline class of antimalarials (Kaur et al. 2009). In this direction, semi synthetic approaches to newer and modified antimalarials have provided useful insights into their applicability in antimalarial drug discovery. As part of a project to identify new compounds active on malarial parasites, we tested the in vitro antiplasmodial activity of ten plants traditionally used to treat malaria and fever in Malaysia. The ethanobotanical information of the ten selected plants obtained from Malay traditional healers (bomoh) are indicated in Table 1. The protocol of traditional preparation and of use of each tested plant were obtained from notices of these traditional healers.

The plant materials were extracted first with dichloromethane and then with methanol. The amount of solvent was at least 10 times the volume of plant material. Filtrates were prepared and evaporated to dryness under reduced pressure with a rotary evaporator (Rotavapor®) at 30°C.

Plasmodium falciparum strain FCB (chloroquine-resistant) was grown under standard conditions as previously described (Trager & Jensen 1976). The parasites were synchronized by repeated 5% sorbitol treatment. The plant extracts were dissolved in 100 L of DMSO at an initial concentration of 200 mg/ml and then serially diluted with culture medium before being added to synchronous parasite cultures. The concentration range was 500–0.05 g/ml. Two hundred microliters of synchronized trophozoite suspension

TABLE 1. Plants, local names and parts used in the study

Plant species	Local names	Parts of plant used by traditional healers
Chasalia chartacea	"beras-beras"	Roots are boiled and water used to treat malaria, as cough mixture and after childbirh
Ocimum sanctum	"selasih"	The leaves are pounded to a paste and applied on forehead as febrifuge and as remedy for rashes
Lawsonia inermis	pokok inai	For relief of sore throat, gargle with water in which the leaves have been boiled
Datura metel	"kecubung"	Bathing with water soaked with its leaves help to treat fever and epilepsy
Cinnamomum iners	"kayu manis hutan"	Water boiled with its roots used to treat fever and rheumatism
Carica papaya	"betik", "papaya"	Water in which the leaves have been soaked with its leaves is used to treat fever
Punica granatum	"delima"	Water boiled with its roots and bark help to fever and headache
Tetracera indica	"mempelas"	Water boiled with its roots used to treat fever and lowers hypertension
Ardisia crenata	"mata ayam"	To treat fever and diarrhea, the root is pounded till and eaten
Ageratum conyzoides	"rumput tahi ayam"	Water boiled with its roots and leaves is used to treat high fever and diarrhea

(1.5% final hematocrit in RPMI 1640 + 0.5% Albumax®) was incubated in triplicate with the different concentrations of plant extracts in 96-well flat-bottom plates (NUNC, VWR International, Strasbourg, France). The plates were then placed at 37°C as previously described (Douki et al. 2003) for 42 hours, before being frozen at −20°C for 3 hours to stop parasite growth. Dihydroartemisinin (Sigma–Aldrich) and chloroquine were used as positive and negative controls of parasite growth inhibition in all experiments.

Antiplasmodial activity was analyzed by measuring Plasmodium lactate dehydrogenase (pLDH) activity with a commercial ELISA method as recommended by the manufacturer (ELISA-Malaria anti-gen test; DiaMed AG) (Kaddouri et al. 2006). The ELISA plates were coated with a monoclonal antibody (MAb) against pan-Plasmodium LDH. The absorbance of each well of plate was read with a microplate spectrophotometer (LP400; Bio-Rad) at 450 nm, with a reference wavelength of 620 nm. All experiments were performed at least in triplicate. The results were expressed as the mean IC50 (the drug concentration that reduced parasitaemia to 50%).

The cytotoxicity of the extracts was assessed with MRC-5 human diploid embryonic lung cells, using a tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bro-mide) (Sigma®) colorimetric method based on reagent cleavage by mitochondrial dehydrogenase in viable cells (Mosmann 1983). Briefly, 5000 cells per well were seeded in 96-well microplates in culture medium (DMEM + 10% inactivated SVF+2 mM L-glutamine + penicillin/streptomycin/neomycin (0.5/0.5/1 g/ml)). After 24 hours, the cells were washed and incubated with eight concentrations (from 500 to 0.05 g/ml) of each extract for 7 days at 37°C in 5% CO_2-air. Cytotoxicity was scored as the percentage reduction in absorbance at 540 nm versus untreated control cultures. All experiments were performed at least in triplicate.

The results were expressed as the mean lethal dose 50 (LD50 = the drug concentration that reduced the number of viable cells by 50%). A selectivity index (SI), corresponding to the ratio between the cytotoxic and antiparasitic activities of each plant extract, was calculated as follows:

$$\text{SI Plasmodium} = \frac{\text{LD 50 MRC-5}}{\text{IC 50 Plasmodium}}.$$

Based on WHO guidelines and previous data (Jonville et al. 2008) antiplasmodial activity was classified as follows: high (IC50 <5 g/ml), promising (5 < IC50 < 15 g/ml), moderate (15 < IC50 < 50 g/ml) and inactive (IC50 > 50 g/ml). Only two extracts showed high antiplasmodial activity, while eight showed promising activity. Although methanolic extracts of some plants were more active than the corresponding dichloromethane extracts, the latter tended to be more active overall, and also more selective (Table 2).

Methanolic extract of *Tetracera indica* and dichloromethane extract of *Chasalia chartacera* showed high antiplasmodial activity (IC50 < 1 g/ml) and low cytotoxicity, with selectivity indexes of about 16.43 and 58.25, respectively. Methanolic extract of *Cinnanamomum inners* and dichloromethane extract of *Ocimum sanctum* also showed promising activity (1 < IC50 < 10 g/ml) and low cytotoxicity, with selectivity indexes about 17.4 and 18.48, respectively. Dichloromethane extracts of *Ageratum conyzoides* and *Ardisia crenata* showed moderate activity (10 < IC50 < 40 g/ml), with selectivity indexes about 7.8 and 21.66, respectively. Both extracts of *Carica papaya* had IC50 values of 10–40 g/ml but high cytotoxicity (selectivity indexes < 2.77). The methanolic extracts of *Datura metel, Lawsonia inermis* and *Punica granatum* had moderate antiplasmodial activity (IC50 around 17 to 24 g/ml) but strong cytotoxicity giving a selectivity index of about 0.03 to 0.44.

Although the traditional healers use these plants for the treatment of all sorts of medical problems, some may have no antiplasmodial activity in vitro. It should be noted that we only tested antiplasmodial activity on the asexual erythrocytic stage of *Plasmodium falciparum*, and the

TABLE 2. *In vitro* antiplasmodial activity, cytotoxicity and selectivity index of the selected plant extracts

Plant species	Extract	Antiplasmodial activity (IC 50, g/ml)	Cytotxicity MRC-5	Selectivity Index
Chasalia chartacea	CH_2Cl_2	0.7±0.2	11.5±2.4	16.43
	CH_3OH	NT	NT	ND
Ocimum sanctum	CH_2Cl_2	8.9±2.7	168.5±31.8	18.48
	CH_3OH	410±25	47.2±9.3	0.12
Lawsonia inermis	CH_2Cl_2	12.8±0.4	10.25±3.1	0.15
	CH_3OH	17.1±1.6	44.3±10.3	0.03
Datura metel	CH_2Cl_2	16.7±5.3	0.43±0.2	0.05
	CH_3OH	24.3±2.8	29.3±3.7	0.06
Cinnamomum iners	CH_2Cl_2	27.1±7.2	439.1±7.5	16.20
	CH_3OH	5.5+2.0	95.7+12.3	17.40
Carica papaya	CH_2Cl_2	25.0±4.3	170.5±30.6	1.3
	CH_3OH	15.0±2.5	95.7±12.3	2.77
Punica granatum	CH_2Cl_2	NT	NT	ND
	CH_3OH	16.7±4.9	520±21.7	0.44
Tetracera indica	CH_2Cl_2	13.1±1.9	123.2±10.6	15.20
	CH_3OH	25.0±0.9	46.6±9.5	58.25
Ardisia crenata	CH_2Cl_2	39.6±2.4	75.5±12.9	21.66
	CH_3OH	17.1±2.8	56.5±23.5	3.30
Ageratum conyzoides	CH_2Cl_2	9.95±1.4	63.1±14.9	7.8
	CH_3OH	25.48±5.1	44.8±7.8	1.77
Chloroquine		0.6±0.4	0.4±0.2	ND
Artemisinin		0.04±0.0	ND	ND

NT= Not tested; ND=Not determined; IC_{50}, g/ml = Inhibition concentration 50%+standard concentration; LD_{50}, g/ml=Lethal dose 50%+standard deviation; Selective index= LD_{50}

extracts that we found inactive might possibly inhibit other parasite stages. Alternatively, some plants without in vitro antiplasmodial activity may stimulate the immune response; the example is *Markhamia lutea*, used by traditional healers of Africa, inhibits *Plasmodium berghei* growth in mice but is inactive in vitro (Hakizamungu & Weri 1988).

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