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Phytochemistry of Thai plants and their antiprotozoal activities

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Abstract

The *in vitro* antiprotozoal activities of 12 chloroform extracts from 6 species of Thai plants were evaluated by three strains of protozoa including *Crithidia fasciculata*, *Plasmodium falciparum* chloroquine sensitive strain (3D7) and chloroquine resistant strain (K1). Anticrithidial activity of the chloroform extracts was studies by treatment of cultured promastigotes with various concentrations of the chloroform extracts. Growth inhibition by the sample was measured by microculture tetrazolium (MTT) assay. The antiplasmodial activity was determined by using SYBR Green I against the chloroquine-resistance K1 strain and chloroquine-sensitive strain of *Plasmodium falciparum*. In each assay, the 50% inhibitory concentration (IC_{50}) value for each sample was derived by the drug concentration-response curves.

The results were demonstrated that, the leaf extracts from *Aglaia odorata* (Meliaceae) and *Azadirachta indica* (Meliaceae) showed good active results against all of selected protozoa. The highest anticrithidial activity was found in leaf extract of *Azadirachta indica* (IC_{50} < 7.8125 μg/ml). While the highest antiplasmodial activity 3D7 strain was found in leaf extracts of *Aglaia odorata* and *Azadirachta indica* (IC_{50} < 7.8125 μg/ml). It was shown that the leaf extract of *Azadirachta indica* (IC_{50} 3.846 μg/ml) gave the highest antiplasmodial activity in K1 strain. The plant extracts which showed the most and moderate activities have potential to be developed for antiprotozoal drugs in the future.

Keywords**:** Phytochemistry, Thai plants, antiprotozoal, IC_{50}

Introduction

The use of medicinal plants in the treatment of parasitic diseases is an old practice. Human parasitic infections are serious problems in tropical and subtropical developing country, despite the discover of new antiprotozoal (Mesia *et al.*, 2007).

This present study deals with the *in vitro* evaluation of the antiprotozoal activity of Thai plants against *Crithidia fasciculata,* the chloroquine-sensitive 3D7 strain and the chloroquine - resistant K1 strain of *Plasmodium falciparum*. *Crithidia fasciculate* is a kinetoplastid which occurs in nature as a commensal gut parasite in insects (Kariem *et al*., 1995). This organism is harmless to laboratory workers and it is easy to be cultured in large amounts. Therefore, *Crithidia fasciculata* can be used as a model of flagellated parasites such as, *Trypanosoma* spp., *Leishmania* spp. Malaria is the world's most important tropical disease (Kaur *et al.*, 2009). Resistance to chloroquine has steadily increased especially *Plasmodium falciparum*, so the discovery of new antimalarial drugs is necessary.

The aims of this study were to evaluate *in vitro* the antiprotozoal activities of chloroform extracts from Thai plants, and examine the active substances of these extracts.

Materials and Methods

Plant samples from four families i.e., *Harrisonia perforata* (Simaroubaceae), *Aglaia odorata, Azadirachta indica, Swietenia macrophylla* (Meliaceae), the unknown species cf.

Mitracarpus (Rubiaceae) and *Derris trifoliata* (Fabaceae) were collected during June to October 2009. These plant samples and their biological activities are showed in Table 1

Plant samples: leaf, stem bark, aerial parts and root were dried, grinded and extracted with methanol at room temperature for seven days, filtered, and concentrated. The aqueous residues were extracted with chloroform, and then evaporated to dryness. The chloroform extracts were applied to test for antiprotozoal activities by continuous culture in 96-wells plate.

Table 1: Plant samples and their biological activities

Family	Botanical names	Main constituents	Biological activities
Fabaceae	*Derris trifoliata* Lour.	Alkaloids, flavonoids, tannins, triterpenoids (Khan *et al*., 2006)	- anticancer (Chihiro, 2004) - anticarcinogenic agents (Kennedy, 1998)
Meliaceae	*Aglaia odorata* Lour.	rocaglamides, terpenoids, lignans, alkaloids (Tu, 2007)	- antifungal, herbicide (Tu, 2007) - antileukemia (Hayashi, 2001)
Meliaceae	*Azadirachta indica* A. Juss.	Limonoids, alkaloids, flavonoids, coumarins, tannins (Hout *et al*., 2006)	- antimalarial for chloroquine-resistant strain of *Plasmodium falciparum* (Badani, 1987) - antibacterial and anti-inflammatory (Doraboba, 2004) - herbicide (Nagpal *et al*., 1996)
	Swietenia macrophylla King	triterpenoids, limonoids, flavonoids, tannins (Mootoo, 1999)	Seed: antimalarial (Kadota, 1990; Soediro, 1990)
Rubiaceae	cf. *Mitracarpus* sp.	favonoids, tannins (Frabi *et al*., 2009)	- *Mitacarpus frigidus* : antimicrobial, antileishmanial, antioxidant (Frabi *et al*., 2009) - *Mitacarpus villosus*: antifungal (Irobi and Daramola, 1993)
Simaroubaceae	*Harrisonia perforata* (Blanco) Merr.	Limonoids, quassinoids, chromones (Tanaka *et al*., 1995)	Antimalarial (Nguyen-Pouplin *et al*., 2007)

By Thin layer chromatographic technique (TLC) all chloroform extracts were also examined for alkaloids and terpenoids by spraying with Dragendorff's reagent and anisaldehyde-sulphuric acid reagent respectively.

Results and Discussion

The results from *in vitro* antiprotozoal testing of 12 chloroform extracts are presented in Table 2. The extracts which were considered as the most active against *Crithidia fasciculata* are leaf extracts of *Aglaia odorata* (Meliaceae) and *Azadirachta indica* (Meliaceae). For antimalarial, the extracts which were considered as the most active against 3D7 strain are leaf extracts of *Aglaia odorata* (Meliaceae) and *Azadirachta indica* (Meliaceae) and the most active against K1 strain are leaf extract of *Azadirachta indica* (Meliaceae) and aerial parts of cf. *Mitracarpus* sp.(Rubiaceae).

From the active compound examination, it was found that all of the extracts give positive reaction with Dragendoff's reagent and anisaldehyde-sulphuric acid reagent. Therefore, these demonstrate that all extracts have alkaloids and terpenoids. In previous

report, antiprotozoal activity may be due to the presence of alkaloids, terpenes, flavonoids, or saponins according to these phytochemical groups exhibited in vitro an antiprotozoal activity at the different extents (Phillipson and Wright,1991; Schwikkard *et al*., 2002)

Table 2: Results of plants screened for antiprotozoal activities

Botanical names	Local names	Part	Aniprotozoal activity IC_{50} (μg/ml)		
			Cf	**3D7**	**K1**
Aglaia odorata Lour.	ประยงค์	Leaves Stem bark	9.585 19.25	<7.8125 116.7	93.62 >400
Azadirachta indica A. Juss.	สะเดาอินเดีย	Leaves Stem bark	<7.8125 12.83	<7.8125 19.36	3.846 190.6
Derris trifoliata Lour.	ถอบแถบน้ำ	Leaves Stem bark	448.0 165.2	17.75 32.53	22.98 22.19
Harrisonia perforata (Blanco) Merr.	สีฟันคนทา	Leaves Stem bark	57.93 52.72	nd nd	356.2 109.0
Swietenia macrophylla King	มะฮอกกานีใบใหญ่	Leaves Stem bark	>1000 196.5	>1000 154.8	>1000 89.10
cf. *Mitracarpus* sp.	-	Aerial parts Root	>1000 >100	102.0 nd	<7.8125 >100
Chlroquine	-	-	nd	0.004	0.51
Miltefosine	-	-	5.8 μM	nd	nd

Cf: *Crithidia fasciculata*, **3D7**: the chloroquine-sensitive strain of *Plasmodium falciparum*,
K1: the chloroquine-resistance strain of *Plasmodium falciparum*, **nd**: not determined

IC_{50} < 10 μg/ml = most active, 10 < IC_{50} ≤ 50 μg/ml = high considered moderately active, 50 < IC_{50} ≤ 100 μg/ml = moderately active, 100 < IC_{50} ≤ 500 μg/ml = less active, IC_{50} > 500 μg/ml = none active

Conclusion

This investigation has demonstrated the antiprotozoal property of selected plant extracts. Most of plant extracts have antiprotozoal more than one of the selected protozoa. The extracts from leaves of *Aglaia odorata* (Meliaceae) and leaves of *Azadirachta indica* (Meliaceae) were found to be most active against all the selected protozoa. The plant extracts which show most active and moderately active will be separated by chromatographic technique, purification and structural elucidation of active compounds in the extracts towards development of antiprotozoal drugs.

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