IN VITRO ANTITRYPANOSOMAL ACTIVITY OF MALAYSIAN PLANTS

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NORHAYATI I, GETHA K, MUHD HAFFIZ J, MOHD ILHAM A, LILI SAHIRA H, SITI SYARIFAH MM & MUHD SYAMIL A. 2013. *In vitro* antitrypanosomal activity of Malaysian plants. A total of 119 plant species collected from different localities in Malaysia were screened for *in vitro* antitrypanosomal activity. A hundred and forty methanol extracts prepared from different plant parts were tested on *Trypanosoma brucei brucei* strain BS221. Extracts were also tested for their cytotoxic activities on a mamalian cell line (Vero) to determine their selectivity towards the parasite. For the first time, antitrypanosomal activity was identified in the leaves of *Dyera costulata* and the whole plant of *Cymbopogon nardus* with IC_{50} values of 0.58 ± 0.01 µg mL^{-1} (selectivity index, SI > 169) and 0.31 ± 0.03 µg mL^{-1} (SI > 323) respectively. These plants could be attractive candidates for treatment of sleeping sickness.

Keywords: Methanol extract, sleeping sickness, *Trypanosoma brucei brucei, Dyera costulata, Cymbopogon nardus*

NORHAYATI I, GETHA K, MUHD HAFFIZ J, MOHD ILHAM A, LILI SAHIRA H, SITI SYARIFAH MM & MUHD SYAMIL A. 2013. Aktiviti antitripanosom *in vitro* tumbuhan di Malaysia. Sejumlah 119 spesies tumbuhan yang dikutip dari lokasi berlainan di Malaysia disaring untuk aktiviti antitripanosom *in vitro*. Seratus empat puluh ekstrak metanol yang disediakan daripada bahagian tumbuhan yang berlainan diuji ke atas parasit *Trypanosoma brucei brucei* jenis BS221. Ekstrak juga diuji untuk aktiviti sitotoksik pada satu titisan sel mamalia (Vero) untuk mengenal pasti tahap selektif ekstrak terhadap parasit. Buat kali pertamanya, aktiviti antitripanosom dikenal pasti pada daun *Dyera costulata* dan keseluruhan tumbuhan *Cymbopogon nardus* dengan nilai IC_{50} 0.58 ± 0.01 µg mL^{-1} (indeks selektif, SI > 169) dan 0.31 ± 0.03 µg mL^{-1} (SI > 323) masing-masing. Tumbuhan ini boleh dijadikan calon untuk rawatan penyakit tripanosomiasis.

INTRODUCTION

African trypanosomes are flagellated protozoan parasites responsible for human African trypanosomiasis or sleeping sickness. They are transmitted by the bite of an infected tsetse fly from the genus *Glossina*. The causative agent *Trypanosoma brucei brucei* is closely related to *Trypanosoma b. rhodesiense* (East and South Africa) and *T. b. gambiense* (West and Central Africa). *Trypanosoma brucei brucei* which is not infective on humans due to the tryanolytic factor in human serum (Pays et al. 2006) is very similar to *T. b. rhodesiense* and *T. b. gambiense* on the cellular and metabolic levels and thus ideally suited in *in vitro* experiments. Sleeping sickness was estimated to cause 300,000–500,000 infections in 1998. Control interventions have started since 1999, reducing the number drastically to 7139 reported cases in 2010 (Simarro et al. 2011). Early symptoms of sleeping sickness include malaise and irregular fevers as well as enlarged lymph glands and spleen. These are followed by headache, anaemia, joint pain and swollen tissues. Neurological changes such as sleep disturbances, poor coordination and personality change, if untreated lead to fatality within weeks or months (Sternberg 2004).

Five drugs are currently in use for the treatment of trypanosomiasis, namely, suramin, pentamidine, melarsoprol, eflornithine and nifurtimox. However, the production of some of these drugs is threatened either by increasing price (pentamidine), halted production (eflornithine) or planned cessation of production (nifurtimox, suramin and melarsoprol) and there are no new drugs in

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the pipeline (Van Nieuwenhove 2000). In 2009, a combination therapy of nifurtimox and eflornithine was introduced called NECT. This is currently the best treatment option for second stage *T. b. gambiense* infections (Simarro et al. 2012). There is, therefore, an urgent need to develop new drugs since no vaccines are available.

Plants are an important resource to combat serious diseases. In countries where sleeping sickness occurs, plants have traditionally been used for centuries and are still widely used to treat this illness.

The current study was a collaboration between the Forest Research Institute, Malaysia (FRIM) and the Malaysia Institute of Pharmaceuticals and Nutraceuticals (Ipharm) with the support of the Drugs for Neglected Diseases initiative (DNDi) to study the potentials of Malaysian plants possessing antitrypanosomal activity. The main aim of this study was to screen 119 plant species belonging to 60 families against the trypanosome parasite.

MATERIALS AND METHODS

Plant materials

A total of 119 species comprising 52 forest species and 67 herbal species from 60 families were collected from Sungai Menyala Forest Reserve and Spring Resort, Port Dickson and Berembun Virgin Forest Reserve, Jelebu in Negeri Sembilan Malaysia. Some of the herbal plants were bought from wholesalers in Kepong, Selangor. Plant samples collected from forest reserves were authenticated by botanists at FRIM and voucher specimens were deposited at FRIM.

Plant extraction

Plant parts such as leaves and stem barks were collected, cleaned, cut into small pieces, dried and ground into powder (40–60 mesh). A total of 2 g of samples were immersed overnight in 10 ml methanol in Erlenmeyer flask. The samples were mixed continuously in an orbital shaker at 30 °C, 200 rpm for 24 hours to obtain methanolic extract. After filtering, the methanol filtrate was evaporated at 45 °C by rotary evaporator. About 2 mg of the resulting extracts were aliquoted into a 96-well microtiter plate and kept at -20 °C until assayed. A total of 140 plant extracts were prepared from 119 plant species.

Parasite, cell line and media

Trypanosoma brucei brucei strain BS221 (a derivative of S427 also known as MiTat 1.2/221) was obtained from the Swiss Tropical and Public Health Institute (Swiss TPHI), Basel, Switzerland. Bloodstream form of trypanosomes were cultivated *in vitro* in Balz Minimal Essential Medium (BMEM) containing Minimal Essential Medium (MEM powder which was supplemented with 1 g glucose/L, 25 mM HEPES, 2.2 g $NaHO_2$/L and 10 ml MEM non-essential amino acids/L. An additional 10% (v/v) heat inactivated fetal bovine serum (FBS) was added as well as 0.14% (v/v) mercaptoethanol dilution, 1mM sodium pyruvate and 0.1 mM hypoxanthine (Baltz et al. 1985). The parasites were incubated at 37 °C in humidified atmosphere of 5% CO_2.

The Vero cell lines derived from monkey kidney were cultivated in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 5% (v/v) FBS and antibiotic, 50 IU mL^{-1} penicillin and 50 µg mL^{-1} streptomycin. The cell lines were incubated in humidified atmosphere of 5% CO_2 at 37 °C (Siti Syarifah et al. 2011).

In vitro antitrypanosomal assay

The plant extracts were dissolved in absolute ethanol to produce seven different concentrations ranging from 0.01 to 12.5 µg mL^{-1} and positive control drug pentamidine was dissolved in dimethyl sulfoxide(DMSO). A parasite cell suspension was added to each well of the 96-well microtiter plate to give a final density of 2.0×10^4 parasites/well. The trypanosomes were incubated for 72 hours in the presence of standard drug (pentamidine) or serial plant sample dilution. Wells without plant extracts were included as control with ethanol and DMSO. An amount of 10 µL of fluorescence dye Alamar Blue was added into each well after 72 hours (Räz et al. 1997) and incubated for 4 to 5 hours. The fluorescence

was quantified after incubation of 3–5 hours at excitation wavelength 528 nm and emission wavelength 590 nm to determine the IC_{50} using dose response curve (Otoguro et al. 2008) with fluorescence plate reader.

Antitrypanosomal activities of plant crude extracts were classified into three categories: strongly active ($IC_{50} \leq 1.56$ µg mL^{-1}), moderately active (1.56 µg $mL^{-1} < IC_{50} \leq 12.5$ µg mL^{-1}) and not active ($IC_{50} > 12.5$ µg mL^{-1}). Plant extract that showed selectivity index, SI ≥ 100 was considered to have good selectivity index and was chosen for bioassay guided isolation of active compound.

Cytotoxicity assay

To evaluate the selectivity of the extract samples towards the trypanosome parasites, *in vitro* cytotoxicity test was performed. A total of 90 µL Vero (Monkey kidney) cells were seeded in 96 microtiter plates at 4.0×10^4 cells mL^{-1}. Plant extracts were dissolved in absolute ethanol and 10 µL of six concentrations of plant extracts ranging from 0.1 to 100 µg mL^{-1} were added into the wells. Plates with a final volume of 100 µL/well were incubated as described for antitrypanosomal testing. The 50% inhibitory concentration (IC_{50}) values of extracts were determined from dose response curve after 72-hour incubation at 37 °C in humidified atmosphere of 5% CO_2. Selectivity index corresponding to the ratio between antitrypanosomal and cytotoxicity activities was calculated according to the following formula:

$$SI_{\text{Trypanosoma}} = \frac{IC_{50} \text{ value (Vero cells)}}{IC_{50} \text{ value (}\textit{T. b. brucei}\text{)}}$$

RESULTS AND DISCUSSION

Of the 119 plant species tested, 1.6% showed strong antitrypanosomal activity, 22.7% moderate and 75.6% not active (Table 1). Two plant species showed strong antitrypanosomal activities. The highest antitrypanosomal activities with good selectivity were found in *Dyera costulata* (Apocynaceae) with IC_{50} value of 0.58 ± 0.01 µg mL^{-1} (SI > 169) and whole plant of *Cymbopogon nardus* (Poaceae) with IC_{50} value of 0.31 ± 0.03 µg mL^{-1} (SI > 323).

Dyera costulata is normally found in primary evergreen lowlands or on hills and can be found in Peninsular Malaysia, south of Thailand, Sumatra, Borneo and the intervening islands (Whitmore 1973). The bark and leaves of *D. costula* have been traditionally used for treatment of fever and inflammation (Subhadhirasakul et al. 2003). The chemical constituents of this plant have been reported from leaves such as bisindole alkaloids: ochrolifuanines A, E, F and 18-dehydroochrolifuanines A, E, F (Mirand et al. 1983). Meanwhile β-amyrin, rhamnazin and quercetin-3-O-α-L-rhamnopyranoside were reported from leaves of *D. costulata* and they were shown to have high antioxidative activity (Subhadhirasakul et al. 2003). Leaf extracts of this plant showed antiplasmodial activity and *in vivo* analgesic effect in mice (Reanmongkol et al. 2002, Wong et al. 2011). In the current study, the methanol extracts of *D. costulata* leaves showed strong activity against *T. b. brucei* with IC_{50} value 0.58 ± 0.01 µg mL^{-1} and good selectivity towards the parasite (SI > 169). So far there is no report of *D. costulata* antitrypanosomal activity. Hence bioactivity-guided isolation of active compounds from this plant is needed.

Cymbopogon nardus belonging to the family Poaceae is known for its rich essential oil. The bioactive constituents and bioactivity of *C. nardus* have been reported, including antibacterial (Dorman & Deans 2000), antiviral (Nurul Aini et al. 2006), antifungal (Matasyoh et al. 2011), insect repellent (Bassole et al. 2003), anticancer (Dudai et al. 2005), antimalarial (Tchoumbougnang et al. 2005) and antioxidant (Cheel et al. 2005) activities. Most researchers studying the effect of plants on parasitic infections use aqueous or alcoholic extraction. Purified plant essential oils can also be efficacious in treating or preventing parasitic disease. Essential oil of plants as a potential source of phytochemicals is worth studying for antiparasitic activity (Anthony et al. 2005). The chemical constituents of *C. nardus* include citronella, geraniol and citranellol as major components; other components are

Table 1 Antitrypanosomal and cytotoxicity activities of methanol extracts from 119 Malaysian plant species

Species	Family	Part	IC_{50} ± SEM (µg mL^{-1})		SI
			Antitrypanosomal	Cytotoxicity	
			Trypanosoma brucei brucei	Vero	
Andrographis paniculata	Acanthaceae	Leaf	4.71 ± 0.07	37.68 ± 4.2	8
Acorus calamus	Acoraceae	Rhizome	> 12.5	nd	
Allium sativum	Alliaceae	Leaf	> 12.5	nd	
Buchanania sessilifolia	Anacardiaceae	Leaf	> 12.5	nd	
Anacardium occidentale	Anacardiaceae	Leaf	> 12.5	nd	
Anisophyllea disticha	Anisophylleaceae	Leaf	> 12.5	nd	
Alphonsea maingayi	Annonaceae	Leaf	> 12.5	nd	
		Stem	> 12.5	nd	
Polyalthia cauliflora	Annonaceae	Leaf	5.4 ± 0.27	> 100	> 19
Xylopia malayana	Annonaceae	Leaf	4.71 ± 0.62	> 100	> 21
Xylopia ferruginea	Annonaceae	Leaf	4.81 ± 0.13	> 100	> 21
Goniothalamus macrophyllus	Annonaceae	Leaf	> 12.5	nd	
Cyathocalyx pruniferus	Annonaceae	Leaf	> 12.5	nd	
Centella asiatica	Apiaceae	Leaf	> 12.5	nd	
Oenanthe javanica	Apiaceae	Leaf	> 12.5	nd	
Dyera costulata	Apocynaceae	Leaf	0.58 ± 0.01	> 100	> 169
Alstonia angustiloba	Apocynaceae	Leaf	> 12.5	nd	
Tabernaemontana corymbosa	Apocynaceae	Leaf	> 12.5	> 100	
Kibatalia maingayi	Apocynaceae	Leaf	> 12.5	> 100	
Allamanda catharina	Apocynaceae	Flower	> 12.5	nd	
Amorphophallus paeoniifolius	Araceae	Leaf	> 12.5	nd	
Trevesia burckii	Araliaceae	Leaf	> 12.5	59.19	
Iguanura geonomiformis	Arecaceae	Leaf	> 12.5	nd	
Thottea grandiflora	Aristolochiaceae	Leaf	4.87 ± 0.09	67.47 ± 0.15	14
		Stem	> 12.5	nd	
Chromolaena odorata	Asteraceae	Leaf	> 12.5	nd	
Elephantopus scaber	Asteraceae	Leaf	> 12.5	nd	
Gynura procumbens	Asteraceae	Leaf	> 12.5	nd	
Adenostemma viscosum	Asteraceae	Leaf	> 12.5	nd	
Impatiens balsamina	Balsaminaceae	Leaf	> 12.5	nd	
Durio griffithii	Bombacaceae	Leaf	> 12.5	nd	
Ananas sp.	Bromeliaceae	Fruit	> 12.5	nd	
Dacryodes rostrata	Burseraceae	Leaf	> 12.5	nd	
		Stem	> 12.5	nd	
Pereskia sacharosa	Cactaceae	Leaf	> 12.5	nd	
Cleome gynandra	Capparaceae	Leaf	4.93 ± 0.13	> 100	> 20
Caricca papaya	Caricaceae	Leaf	> 12.5	nd	
Bhesa paniculata	Celastraceae	Leaf	> 12.5	> 100	
Cosmos caudatus	Compositae	Leaf	> 12.5	nd	
Blumea balsamifera	Compositae	Leaf	4.62 ± 0.14	> 100	> 22
Momordica charantia	Cucurbitaceae	Fruit	> 12.5	nd	
Tetracera sp.	Dilleniaceae	Leaf	> 12.5	nd	
Diplazium esculentum	Dryopteridaceae	Leaf	4.32 ± 0.07	> 100	> 23
Diospyros argentea	Ebenaceae	Leaf	> 12.5	nd	
Agrostistachys longifolia	Euphorbiaceae	Leaf	> 12.5	nd	
		Stem	> 12.5	nd	
Aporosa microstachya	Euphorbiaceae	Leaf	> 12.5	nd	
		Stem	> 12.5	nd	
Croton laevifolius	Euphorbiaceae	Leaf	> 12.5	nd	
		Stem	> 12.5	nd	
Croton argyratus	Euphorbiaceae	Leaf	4.85 ± 0.5	54.85 ± 0.2	11
		Stem	> 12.5	nd	
Baccaurea parviflora	Euphorbiaceae	Leaf	> 12.5	nd	
		Stem	> 12.5	nd	

(continued)

Table 1 (continued)

Species	Family	Part	IC_{50} ± SEM (μg mL^{-1})		SI
			Antitrypanosomal	Cytotoxicity	
			Trypanosoma brucei brucei	Vero	
Aporosa prainiana	Euphorbiaceae	Leaf	> 12.5	nd	
		Stem	> 12.5	nd	
Drypetes pendula	Euphorbiaceae	Leaf	> 12.5	nd	
		Stem	> 12.5	nd	
Paracroton pendulus	Euphorbiaceae	Leaf	> 12.5	nd	
Suregada multiflora	Euphorbiaceae	Leaf	> 12.5	nd	
Bridelia stipularis	Euphorbiaceae	Leaf	> 12.5	> 100	
Manihot esculenta	Euphorbiaceae	Leaf	> 12.5	nd	
Mallotus paniculatus	Euphorbiaceae	Leaf	5.14 ± 0.25	95.59 ± 0.09	18
Aleurites moluccana	Euphorbiaceae	Fruit	> 12.5	nd	
Callerya atropurpurea	Fabaceae	Leaf	> 12.5	nd	
		Stem	> 12.5	nd	
Parkia speciosa	Fabaceae	Fruit	4.77 ± 0.13	> 100	> 21
Psophocarpus tetragonolobus	Fabaceae	Fruit	> 12.5	nd	
Pterocarpus indicus	Fabaceae	Leaf	> 12.5	> 100	
Cassia alata	Fabaceae	Leaf	> 12.5	nd	
Mimosa pudica	Fabaceae	Leaf	> 12.5	nd	
Lithocarpus wallichianus	Fagaceae	Leaf	> 12.5	nd	
		Stem	> 12.5	nd	
Castanopsis schefferiana	Fagaceae	Leaf	> 12.5	nd	
Lithocarpus ewyckii	Fagaceae	Leaf	5.36 ± 0.15	54.16	10
Castanopsis inermis	Fagaceae	Leaf	> 12.5	nd	
Quercus infectoria	Fagaceae	Fruit	> 12.5	nd	
Scaevola taccada	Goodeniaceae	Leaf	> 12.5	nd	
Dendrocalamus giganteus	Gramineae	Young leaf	> 12.5	nd	
Calophyllum ferrugineum	Gutiferaceae	Leaf	> 12.5	nd	
		Stem	> 12.5	nd	
Gonocaryum gracile	Icacinaceae	Leaf	> 12.5	nd	
		Stem	> 12.5	nd	
Stemonurus malaccensis	Icacinaceae	Leaf	> 12.5	nd	
Mentha arvensis	Lamiaceae	Leaf	> 12.5	nd	
Orthosiphon aristatus	Lamiaceae	Leaf	> 12.5	nd	
Alseodaphne peduncularis	Lauraceae	Leaf	5.04 ± 0.4	> 100	>20
		Stem	> 12.5	nd	
Beilschmiedia madang	Lauraceae	Leaf	> 12.5	nd	
		Stem	> 12.5	nd	
Cryptocarya infectoria	Lauraceae	Leaf	> 12.5	nd	
		Stem	> 12.5	nd	
Litsea elliptica	Lauraceae	Leaf	> 12.5	nd	
Cinnamomum mollissimum	Lauraceae	Leaf	> 12.5	nd	
Litsea machilifolia	Lauraceae	Leaf	5.13 ± 0.23	18.97	4
Cinnamomum iners	Lauraceae	Leaf	5.02 ± 0.24	> 100	> 20
Leucaena leucocephala	Leguminosae	Fruit	> 12.5	> 100	
Tamarindus indica	Leguminosae	Leaf	> 12.5	nd	
Aloe vera	Liliaceae	Leaf	> 12.5	nd	
Fagrea fagrans	Loganiaceae	Leaf	> 12.5	nd	
Lagerstroemia	Lythraceae	Leaf	> 12.5	nd	
Hibiscus rosa sinensis	Malvaceae	Leaf	4.34 ± 0.01	> 100	> 23
Hibiscus sabdariffa	Malvaceae	Fruit	> 12.5	nd	
Angiopteris evecta	Marattiaceae	Leaf	> 12.5	nd	
Malastoma malabathricum	Melastomataceae	Leaf	4.4 ± 0.23	> 100	> 23
Clidemia hirta	Melastomataceae	Leaf	4.41± 0.13	99.31 ± 0.02	23
Algaia sp.	Meliaceae	Leaf	4.75 ± 0.3	59.17 ± 0.31	12
		Stem	> 12.5	nd	
Aglaia exstipulata	Meliaceae	Leaf	2.7± 1.2	60.79 ± 2.3	23
		Stem	> 12.5	nd	

(continued)

Table 1 (continued)

Species	Family	Part	IC_{50} ± SEM (μg mL^{-1})		SI
			Antitrypanosomal	Cytotoxicity	
			Trypanosoma brucei brucei	Vero	
Xylocarpus granatum	Meliaceae	Leaf	> 12.5	nd	
Azadirachta indica	Meliaceae	Leaf	> 12.5	nd	
Swietenia macrophylla	Meliaceae	Fruit	> 12.5	nd	
Artocarpus heterophyllus	Moraceae	Fruit	> 12.5	> 100	
Aegiceras corniculatum	Myrsinaceae	Leaf	> 12.5	nd	
Ardisia crenata	Myrsinaceae	Leaf	> 12.5	nd	
Baeckea frutescens	Myrtaceae	Leaf	3.94 ± 0.15	63.89 ± 0.05	16
Ochanostachys amentacea	Olacaceae	Leaf	> 12.5	nd	
Chemperea manillana	Opiliaceae	Leaf	> 12.5	> 100	
Piper sarmentosum roxb	Piperaceae	Leaf	> 12.5	nd	
Piper betle	Piperaceae	Leaf	4.61 ± 1.00	76.24 ± 1.8	17
Peperomia pellucida	Piperaceae	Leaf	> 12.5	nd	
Cymbopogon nardus	Poaceae	Whole plant	0.31 ± 0.03	> 100	> 323
Cymbopogon citratus	Poaceae	Rhizome	4.44 ± 0.06	> 100	> 23
Persicaria odorata	Polygonaceae	Leaf	> 12.5	nd	
Morinda elliptica	Rubiaceae	Leaf	> 12.5	nd	
Murraya koenigii	Rutaceae	Leaf	4.38 ± 0.01	> 100	> 23
Murraya paniculata	Rutaceae	Leaf	5.1 ± 0.06	> 100	> 20
Smilax calophylla	Smilacaceae	Leaf	> 12.5	nd	
Sonneratia alba	Sonneratiaceae	Leaf	> 12.5	nd	
Heritiera littoralis	Sterculiaceae	Leaf	> 12.5	nd	
Tacca integrifolia	Taccaceae	Leaf	> 12.5	nd	
Lantana camara	Verbenaceae	Leaf	> 12.5	nd	
Stachytarpheta jamaicensis	Verbenaceae	Leaf	> 12.5	nd	
Rinorea anguifera	Violaceae	Leaf	> 12.5	nd	
		Stem	> 12.5	nd	
Alpina galanga	Zingiberaceae	Rhizome	5.22 ± 0.04	18.19 ± 0.09	3
Zingiber officinale	Zingiberaceae	Rhizome	> 12.5	nd	
Curcuma longa	Zingiberaceae	Rhizome	5.48 ± 1.2	11.71 ± 0.5	2
		Leaf	5.68 ± 0.08	77.9 ± 0.12	14

IC_{50} positive control (pentamidine) = 4.51 ng mL^{-1}; nd = not determined; SI = selectivity index, determined for samples showing strong ($IC_{50} \leq 1.56$ μg mL^{-1}) and moderate ($1.56 < IC_{50} \leq 12.5$ μg mL^{-1}) antitrypanosomal activities

oxygenated sesquiterpenes: elemol, β-eudesmol, γ-eudesmol and citronellyl tiglate (Koba et al. 2009). To our knowledge there is no report of this species on antitrypanosomal activity. Therefore, *C. nardus* is worth studying in bioassay-guided isolation.

The extracts with moderate antitrypanosomal activity such as *Aglaia exstipulata, Baeckea frutescens* and *Piper betle* with IC_{50} values of 2.7 ± 1.2, 3.94 ± 0.15 and 4.61 ± 1.00 μg mL^{-1} respectively are also worth studying. These extracts have a large number of compounds and the active ingredients may show activity in pure form.

The genus *Algaia* is the largest genus in the mahogany family which consists of more than 100 species. Bioactive compounds have been reported from this genus, which show insecticidal (Greger et al. 2001), cytotoxic (Saifah et al. 1993), antifungal (Engelmeier et al. 2000) and anticancer (Mata-Greenwood et al. 2001) activities. Besides *A. exstipulata*, leaves from other *Algaia* species were also found to have antiprotozoal activity (Tasanor et al. 2006). In this study, extracts of *A. exstipulata* showed moderate antitrypanosomal activity with IC_{50} value of 2.7 ± 1.2 μg mL^{-1}. Hence bioassay-guided isolation can be done to obtain the active antitrypanosomal compounds from *A. exstipulata*.

The family *Piperaceae* comprises approximately 5 genera and 1400 species. Some of the species are used as traditional medicine. Various species of *Piper* have antibacterial and

anti-inflammatory activities (Vaghasiya et al. 2007). Phytochemical studies on Piperaceae showed the presence of metabolites from mevalonic acid (monoterpenes and sesquiterpenes), acetic acid/shikimic acid (flavanoid), amides and aristolactams alkaloids (Sengupta & Ray 1987). The methanol extract from the leaf of *P. betle* in this study showed moderate antitrypanosomal activity (IC_{50} = 4.61 ± 1.00 µg mL^{-1}).

Another species with moderate antitrypanosomal activity was *B. frutescens*. *Baeckea frutescens* is a small tree from the family Myrtaceae and is found in Peninsular Malaysia, Sumatra and the coastal areas of southern China and Australia. In Malaysia, it is found both on mountain tops and sandy coasts (Institute for Medical Research 2002). The leaves contain an essential oil and constituents such as sesquiterpenes, chromone C-glycosides, phloroglucinols and flavanones (Fujimoto et al. 1996, Satake et al. 1999). *Baeckea frutescens* was reported to show cytotoxicity (Makino & Fujimoto 1999), anticariogenic (Hwang et al. 2004) and antimalarial (Murningsih et al. 2005) activities. Bioassay-guided isolation from this species is worth further investigation.

In this study, two of the three plant species from the family Zingerberaceae showed moderate activity. A total of 50% of plants from Annonaceae, 40% from Meliliaceae and 30% from Lauraceae showed moderate activity. Thus, these plants can be included as future screening targets for activity against *T. b. brucei* parasites.

CONCLUSION

Two species showed the presence of promising antitrypanosomal compounds with high selectivity towards the parasites *D. costulata* (IC_{50} 0.58 ± 0.01 µg mL^{-1}; SI > 169) and *C. nardus* (IC_{50} 0.31 ± 0.03 µg mL^{-1}; SI > 323).

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