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IN VITRO ANTI-LEISMANIAL ACTIVITY OF MALAYSIAN MEDICINAL AND FOREST PLANT SPECIES

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Leishmaniasis causes major concern around the world as the number of affected patients continues to increase. The available commercial drugs to treat this disease either come with severe side effects or had shown resistance. The current study aims natural products from Malaysian plant species which may be effective against the most prevalent form of leishmania disease, cutaneous leishmaniasis. A total of 106 plant species were collected from two forest reserves in Negeri Sembilan and also purchased from the local market. Methanolic extract samples of plant stem, leaf, seed and/or root were tested for *in vitro* anti-leishmanial activity using promastigote of *Leishmania major* as the test organism. Extracts from three plant species showed strong *in vitro* anti-leishmanial activity: *Iguanura geonomiformis* (IC_{50} = 14.66 ± 0.10 µg mL^{-1}; selectivity index (SI) 10.24, *Anisophyllea disticha* (IC_{50} = 16.53 ± 0.17 µg mL^{-1}; SI = 11.45) and *Swietenia macrophylla* (IC_{50} = 17 ± 0.07 µg mL^{-1}; SI >12). Amphotericin B was used as a positive control drug (IC_{50} = 0.528 µM). Results from this study indicated that seed extracts of *Swietenia macrophylla* showed promising anti-leishmanial activity and warrants further studies to identify the potential bioactive compound as an anti-leishmanial agent.

Keywords: Methanolic extract, Leishmaniasis, promastigote, *Leishmania major, Swietenia macrophylla*

INTRODUCTION

Leishmaniasis, registered in the WHO list of 17 neglected tropical diseases, is a parasitic disease caused by a group of hemoflagellate protozoa of the genus *Leishmania* (Ying et al. 2004, Sadeghi-Nejad et al. 2011). It was estimated that 350 million human populations are susceptible and 12 million are affected by leishmaniasis in about 88 countries, were 80% are developing countries and another 15% are among the poorest regions in the world (Desjeux 2004, Davis & Kedzierski 2005, Ahua et al. 2007).

There are three forms of leishmaniasis, namely the visceral leishmaniasis (VL), cutaneous leishmaniasis (CL) and mucocutaneaous leishmaniasis (ML) (Reithinger et al. 2007). The CL form of leishmaniasis is reported as the most prevalent of the three with about 1.5 million cases yearly and is widespread in countries, namely Afghanistan, Iraq, Saudi Arabia, Iran and parts of South American continent (Desjeux 2004, Varela et al. 2009). The disease is prevalent by the appearance of skin ulcers targeting mainly uncovered body parts i.e. face, arms and legs alike which normally starts off as swelling at the point bitten by the sandfly (Piscopa & Mallia 2006).

To date no vaccines are available for the disease. Over the years, a number of clinical drugs were developed and among the drugs are pentavalent antimonials, amphotericin B and paramomycin which are often associated with toxicity, severe side effects or drug resistance (Amyra et al. 2016). Natural products from plants are an alternative treatment for leishmaniasis, and the research is further supported by WHO (Weniger et al. 2001, Rocha et al. 2005).

Malaysia is recognised for its importance among the mega biodiversity countries in the world. The enormous biodiversity of flora and fauna, ranging from known and unknown

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medicinal and traditionally claimed properties awaits to be explored further for their drug benefits towards the well-being of human. Thus, in this study initiatives were taken to screen selected forest and medicinal plant species for potential *in vitro* anti-leishmanial activity. Collaboration between Forest Research Institute Malaysia (FRIM), Malaysian Institute of Pharmaceuticals and Nutraceuticals (IPHARM) and Drugs for Neglected Diseases initiatives (DNDi) had led to the establishment of *in vitro* anti-leishmanial assay through technology transfer activities from the London School of Hygiene and Tropical Medicine. Extracts from different plant species samples were screened using an extracellular promastigote assay model to evaluate their inhibitory activity against the parasite *Leishmania major*.

The current study also aimed at contributing towards the compilation of an in-house natural products library for Malaysian plant species with activity against the leishmania parasite.

MATERIALS AND METHODS

Parasite strains

The *L. major (MHOM/SA/85/JISH118)*, obtained from the London School of Hygiene and Tropical Medicine, United Kingdom, was used in the following procedure. The inoculum of promastigotes of *Leishmania* was injected subcutaneously into the shaven rump of female SPF BALB/C mice. Parasite suspension containing 0.2 mL of 1×10^7 promastigotes was injected using 25 G × 5/8" syringe and the lesion was allowed to develop. The mice were sacrificed after 8 days of post inoculation and the lesion containing amastigotes was exercised using a sharp scissors. The dermis from the lesion was scraped off and placed in Scheneider's media supplemented with antibiotics. The tissue sample was homogenised and centrifuged at 4 °C, 800 rpm for 10 minutes and the resultant supernatant was re-centrifuged at 3100 rpm for 15 minutes. The recovered pellet containing amastigotes was resuspended in Scheneider's complete media and incubated at 26 °C to obtain the low passage promastigote stage of *L. major*. The transformed promastigote parasites were used in the subsequent assay (Yardley & Simon 1999).

Plant materials

Plant samples consisting of 64 medicinal and 42 forest plant species 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 medicinal plants were also purchased from the local market in Kepong, Selangor. Plants collected from forest reserves were authenticated by FRIM's botanists and the voucher specimens were deposited at Biomolecules Research Laboratory, FRIM for future reference.

Preparation of plant extracts

Plant parts (stem, leaf and roots) for crude extract preparation were cleaned, cut into small pieces, dried and ground into powder using a blender. About 2 mg of powdered plant material was soaked in 10 ml methanol solvent and was shaken for 24 hours at 30 °C, 200 rpm on an orbital shaker. After filtration, the methanol filtrate was evaporated in a rotary evaporator at 45 °C. The solvent-free extract was stored at -20 °C for further analysis. A total of 120 methanolic extracts of plant samples were prepared in this study.

In vitro anti-leishmanial assay

A modified procedure from Kayser & Abreu (2001) was used in this study. Briefly, logarithmic phase of *L. major* promastigotes ($1 \times 10^4 \times 100^{-1}$ µL) were seeded in each well of a 96 well microtiter plates. A total of 100 µL of the standard drug or extracts in ethanol, prepared in a series of 3 fold dilution (starting stock solution for Amphotericin B: 2 µM and plant extracts: 100 µg mL^{-1}) were added to the wells and the plates were incubated for 48 hours at 28 °C. Amphotericin B and ethanol were used as positive and negative control respectively. Parasite viability was quantified using alamar blue assay (Raz et al. 1997). A total of 12.5 mg resazurin was dissolved in 100 ml double distilled water to prepare resazurin solution, and 10 µL was then added to each well and further incubated for 2 hours in 28 °C incubator. The fluorometric reaction was measured using an excitation and emission wavelength at 536 and 588 nm. The activity was calculated as percentage of growth inhibition using equation (1):

$$\text{Growth inhibition (\%)} = [(A_t - A_b) / (A_c - A_b) \times 100] \quad (1)$$

where A_t = absorbance of treated well, A_b = absorbance of blank and A_c = absorbance of control (untreated well).

Cytotoxicity assay

Human normal liver cell line (WRL-68) was used in the assay. Cells were cultured in RPMI complete media at 37 °C under humidified atmospheric conditions of 5% CO_2. Cells (1×10^4 cells well^{-1}) were seeded in a 96-wells microtiter plate and allowed to adhere to the bottom of the well for 24 hours. The old media was removed and replaced with fresh media, containing positive and negative control and plant extracts with an active and moderate anti-leishmanial activity. The plates were further incubated for 48 hours, and finally the cells viability was quantified using alamar blue assay, as described above. Cytotoxicity was expressed as the concentration of extracts needed to inhibit growth of 50% of the cell population in comparison to cells growing in the absence of the extracts. The selective index (SI) was calculated based on the following equation (2):

$$SI = IC_{50}\text{ (cytotoxicity)} \times IC_{50}\text{ (anti-leishmanial activity)}^{-1} \quad (2)$$

RESULTS AND DISCUSSION

To identify a suitable candidate as an anti-leishmanial agent, plants collected from a selected location in Peninsular of Malaysia were tested using an extracellular promastigote assay model. The model provides a fast screening method when a large number of test samples are involved with an acceptable level of sensitivity (Ahua et al. 2007). A total of 120 plant extracts from 106 plants species were tested for their inhibitory activity against the parasite. The scientific and family names, as well as the parts of the plants studied, are given in Table 1 together with the anti-leishmanial activity and cytotoxicity of the extracts. It is common occurrence for plants with targeted bioactivity to exert certain level of cytotoxicity (Abd-Latif et al. 2015). If plant extracts have remarkable bioactivity and minimal cytotoxicity, the resulting high SI would be the criteria to select suitable candidates as anti-leishmanial agents (Lili Sahira et al. 2013). Cytotoxicity assay was performed using alamar blue assay, a simple one-step procedure with consistent and repeatable results (Raz et al. 1997). Cytotoxicity and SI were determined only for extracts that showed active or moderate anti-leishmanial activity. From the study, we found three plant extracts (*Iguanura geonomiformis, Anisophyllea disticha* and *Swietenia macrophylla*) with strong anti-leishmanial activity ($IC_{50} \leq 20$ µg mL^{-1}) while 11 extracts showed moderate activity (20 µg mL^{-1} < $IC_{50} \leq 50$ µg mL^{-1}) and 106 extracts were found to be inactive ($IC_{50} > 50$ µg mL^{-1}) against the parasite *L. major*. Amphotericin B, as a standard drug, had IC_{50} 0.528 µM.

Swietenia macrophylla (Meliaceae) is a big leaf mahagony tree which produces fruits pointing upwards, thus the common name 'sky fruit' among the local. It is a large tropical tree with a height reaching more than 30 m, and widely distributed around the world including America, India, China, Malaysia and Indonesia (Goh & Abdul Kadir 2011, Moghadamtousi et al. 2013). This amazing tree has a wide range of application from traditional medicinal to pharmacological activity, and also in the timber industry. The woods are sought for in furniture making, boat industry and music equipment (Falah et al. 2008). The leaves are used as a dyeing agent (Eid et al. 2013), the bark is used in tanning industry and also has wound healing properties (Radhamani et al. 2009). The fruit was reported to have been used as a pain killer, to treat high blood pressure and diabetic by the Malay folk (Goh & Kadir 2011). Bourdy et al. (2000) reported on leishmaniasis, whereby the aborigines mashed the seeds of *S. macrophylla* and applied to the external infected surfaces.

The crude ethanol extract of mahogany seeds has shown potent anti-tumor, anti-mutagenic and anti-cancer activities (Guevara et al. 1996). The petroleum ether extract of *S. macrophylla* seeds has anti-diarrhoeal effect (Maiti et al. 2007), while the methanol extract has anti-diabetic, anti-bacterial and anti-fungal activities (Dewanjee et al. 2009, Goun et al. 2003). Anti-inflammatory activity ($IC_{50} \leq 35.7$ µM) was reported from six tetranortriterpenes compounds (6-O-acetyl-3'-demethylswietephragmin E, 3,6-O,O-diacetylswietenolide, 3-O-tigloylswietenolide, 3-O-tigloyl-6-O-acetylswietenolide, swietemahonon E and 6-O-acetylswietemahonon G) (Chen et al. 2010).

Table 1 Anti-leishmania activity, cytotoxicity and SI value for 106 Malaysian medicinal and forest species (methanolic extract)

Family	Species	Part	Anti-leishmania		Cytotoxicity (μg ml^{-1})	SI
			IC_{50} (μg ml^{-1})	Activity		
Acanthaceae	*Andrographis paniculata*	Leaf	58.06	Inactive	-	-
Acoraceae	*Acorus calamus*	Rhizome	134.24	Inactive	-	-
Alliaceae	*Allium sativum*	Leaf	> 200	Inactive	-	-
Anacardiaceae	*Anacardium occidentale*	Leaf	> 200	Inactive	-	-
	Buchanania sessifolia	Leaf	> 200	Inactive	-	-
Anisophylleaceae	*Anisophyllea disticha*	Leaf	16.53 ± 0.17	Active	189.24 ± 0.85	11.45
Annonaceae	*Alphonsea maingayi*	Stem	> 200	Inactive	-	-
		Leaf	> 200	Inactive	-	-
	Cyathocalyx pruniferus	Leaf	> 200	Inactive	-	-
	Polyalthia cauliflora	Leaf	163.84	Inactive	-	-
	Xylopia ferruginea	Leaf	192.46	Inactive	-	-
	Xylopia malayana	Leaf	40.79 ± 0.15	Moderate	180 ± 1.25	4.41
Apiaceae	*Centella asiatica*	Leaf	162.4	Inactive	-	-
	Oenanthe javanica	Leaf	> 200	Inactive	-	-
Apocynaceae	*Allamanda catharina*	Flower	> 200	Inactive	-	-
	Alstonia angustiloba	Leaf	> 200	Inactive	-	-
	Dyera costulata	Leaf	> 200	Inactive	-	-
	Kibatalia maingayi	Leaf	82.23	Inactive	-	-
	Tabernaemontana corymbosa	Leaf	113.89	Inactive	-	-
Araceae	*Amorphophallus paeoniifolius*	Leaf	> 200	Inactive	-	-
Arciliaceae	*Trevesia burckii*	Leaf	> 200	Inactive	-	-
Arecaceae	*Iguanura geonomiformis*	Leaf	14.66 ± 0.10	Active	150.18 ± 0.24	10.24
Aristolochiaceae	*Thottea grandiflora*	Stem	68.25	Inactive	-	-
		Leaf	83.63	Inactive	-	-
Asphodelaceae	*Aloe vera linn*	Leaf	> 200	Inactive	-	-
Asteraceae	*Adenostemma viscosum*	Leaf	> 200	Inactive	-	-
	Chromolaena odorata	Leaf	> 200	Inactive	-	-
	Elepharitopus scaber	Leaf	> 200	Inactive	-	-
	Gynura procumbens	Leaf	> 200	Inactive	-	-
Athyriaceae	*Diplazium esculentum*	Leaf	> 200	Inactive	-	-
Balsaminaceae	*Impatiens balsamina*	Leaf	79.81	Inactive	-	-
Bombacaceae	*Durio griffithii*	Leaf	> 200	Inactive	-	-
Bromeliaceae	*Ananas* sp.	Fruit	23.52 ± 0.76	Moderate	> 200	> 8.5
Burseraceae	*Dacryodes rostrata*	Stem	> 200	Inactive	-	-
		Leaf	137.94	Inactive	-	-
Cactaceae	*Pereskia sacharosa*	Leaf	158.15	Inactive	-	-
Celastraceae	*Bhesa paniculata*	Leaf	> 200	Inactive	-	-
Cleomaceae	*Cleome gynandra*	Leaf	> 200	Inactive	-	-
Compositae	*Blumea balsamifera*	Leaf	> 200	Inactive	-	-
	Cosmos caudatus	Leaf	> 200	Inactive	-	-
Cucurbitaceae	*Momordica charantia*	Fruit	161.24	Inactive	-	-
Dilleniaceae	*Tetracera* sp.	Leaf	146.52	Inactive	-	-

Family	Species	Part	Anti-leishmania		Cytotoxicity (µg ml^{-1})	SI
			IC_{50} (µg ml^{-1})	Activity		
Euphorbiaceae	*Agrostistchys longifolia*	Stem	55.33	Inactive	-	-
	Aleurites moluccana	Fruit	> 200	Inactive	-	-
	Aporosa microstachya	Stem	35.91 ± 0.56	Moderate	> 200	> 5.57
		Leaf	> 200	Inactive	-	-
	Aporosa prainiana	Stem	> 200	Inactive	-	-
		Leaf	> 200	Inactive	-	-
	Baccaurea parviflora	Stem	48.5 ± 0.71	Moderate	89.21 ± 2.3	1.83
		Leaf	93.07	Inactive	-	-
	Bridelia stipularis	Leaf	49.97 ± 0.89	Moderate	198.45 ± 0.74	1.97
	Croton argyratus	Stem	33.44 ± 0.28	Moderate	49.23 ± 1.7	1.47
	Croton laevifolius	Stem	> 200	Inactive	-	-
	Drypetes pendula	Stem	115.91	Inactive	-	-
		Leaf	> 200	Inactive	-	-
Fabaceae	*Mannihot esculenta*	Leaf	> 200	Inactive	-	-
	Cassia alata	Leaf	> 200	Inactive	-	-
	Mimosa pudica	Leaf	> 200	Inactive	-	-
	Psophocarpus tetragonolobus	Fruit	> 200	Inactive	-	-
	Pterocarpus indicus	Leaf	> 200	Inactive	-	-
Fagaceae	*Castanopsis inermis*	Leaf	> 200	Inactive	-	-
	Castanopsis schefferiana	Leaf	181.53	Inactive	-	-
	Lithocarpus ewyckii	Leaf	130.29	Inactive	-	-
	Lithocarpus wallichianus	Stem	> 200	Inactive	-	-
		Leaf	94.98	Inactive	-	-
	Quercus infectoria	Fruit	> 200	Inactive	-	-
Gramineae	*Dendrocalamus giganteus*	Leaf	> 200	Inactive	-	-
Icacinaceae	*Gonocaryum gracile*	Leaf	> 200	Inactive	-	-
Lamiaceae	*Mentha arvensis*	Leaf	> 200	Inactive	-	-
	Orthosiphon aristatus	Leaf	> 200	Inactive	-	-
Lauraceae	*Alseodaphne peduncularis*	Stem	78.13	Inactive	-	-
	Beilschmiedia madang	Stem	23.24 ±1.6	Moderate	39.16 ± 2.3	1.68
		Leaf	> 200	Inactive		
	Cinnamomum ineris	Leaf	> 200	Inactive	-	-
	Cinnamomum mollissimum	Leaf	> 200	Inactive	-	-
	Cryptocarya infectoria	Stem	> 200	Inactive	-	-
		Leaf	> 200	Inactive	-	-
	Litsea elliptica	Leaf	> 200	Inactive	-	-
Leguminosae	*Callerya atropurpurea*	Stem	28.14 ± 0.2	Moderate	21.89 ± 0.96	0.78
		Leaf	135.08	Inactive	-	-
	Lencaena leucocephala	Fruit	> 200	Inactive	-	-
	Tamarindus indica	Leaf	21.14 ± 0.27	Moderate	> 200	> 9.46
Loganiaceae	*Fagrea fagrans*	Leaf	143.51	Inactive	-	-
Lythraceae	*Lagerstroemia*	Leaf	197.37	Inactive	-	-
	Lowsonia inersis	Leaf	> 200	Inactive	-	-
Malvaceae	*Hibiscus rosa sabdariffa*	Fruit	> 200	Inactive	-	-
	Hisbiscus rosa sinensis	Leaf	153.1	Inactive	-	-
Marattiaceae	*Angiopteris evecta*	Leaf	> 200	Inactive	-	-

Family	Species	Part	Anti-leishmania		Cytotoxicity (μg ml^{-1})	SI
			IC_{50} (μg ml^{-1})	Activity		
Melastomataceae	*Clidemia hirta*	Leaf	> 200	Inactive	-	-
	Malastoma malabathricum	Leaf	187.73	Inactive	-	-
Meliaceae	*Aglaia exstipulata*	Stem	> 200	Inactive	-	-
	Algaia sp.	Stem	> 200	Inactive	-	-
		Leaf	> 200	Inactive	-	-
	Azadirachta indica	Leaf	108.05	Inactive	-	-
	Swietenia macrophylla	Seed	17 ± 0.07	Active	> 200	> 12
	Xylocarpus granatum	Leaf	> 200	Inactive	-	-
Moraceae	*Artocarpus heterophyllus*	Fruit	166.48	Inactive	-	-
Myrsinaceae	*Aegiceras corniculatum*	Leaf	> 200	Inactive	-	-
	Ardisia crenata	Leaf	> 200	Inactive	-	-
Myrtaceae	*Baeckea frutescens*	Leaf	54.59	Inactive	-	-
Olacaceae	*Ochanostachys amentacea*	Leaf	> 200	Inactive	-	-
Opiliaceae	*Chemperea manillana*	Leaf	> 200	Inactive	-	-
Piperaceae	*Peperomia pellucida*	Leaf	> 200	Inactive	-	-
	Piper betle	Leaf	64.91	Inactive	-	-
	Piper sarmentosum	Leaf	> 200	Inactive	-	-
Poaceae	*Cymbopogon citrates*	Rhizome	121.48	Inactive	-	-
	Cymbopogon nardus	Whole plant	111.8	Inactive	-	-
Polygonaceae	*Persicaria odorata*	Leaf	> 200	Inactive	-	-
Rutaceae	*Murraya koenigii*	Leaf	57.97	Inactive	-	-
	Murraya paniculata	Leaf	> 200	Inactive	-	-
Smilacaceae	*Smilax calophylla*	Leaf	51.05	Inactive	-	-
Sterculiaceae	*Heritiera littoralis*	Leaf	47.7 ± 0.28	Moderate	45.67 ± 0.54	0.96
Taccaceae	*Tacca integrifolia*	Leaf	155.27	Inactive	-	-
Verbenaceae	*Lantana camara*	Leaf	> 200	Inactive	-	-
	Stachytarpheta jamaicensis	Leaf	> 200	Inactive	-	-
		Stem	> 200	Inactive	-	-
Violaceae	*Rinorea anguisera*	Leaf	94.05	Inactive	-	-
Zingiberaceae	*Alpina galangal*	Rhizome	> 200	Inactive	-	-
	Curcuma longa	Leaf	20.86 ± 0.09	Moderate	96.23 ± 0.62	4.61

Amphotericin B (IC_{50} = 0.528 μM); anti-leishmanial activity = active ($IC_{50} \leq 20$ μg ml^{-1}) and moderate (20 μg ml^{-1} < $IC_{50} \leq 50$ μg ml^{-1}); n = 3 and data was recorded as ± standard deviation; SI = selectivity index: IC_{50} cytotoxicity x IC_{50} anti-leishmanial^{-1} activity

Phytochemical constituents of the plant were largely of limonoids and its derivatives which contributed to its wide ethnomedicinal benefits (Moghadamtousi et al. 2013). Major compounds from class of limonoids had been isolated and identified such as swietenine, swietenolide, swietenolide diacetate and their derivatives (Mootoo et al. 1999, Falah et al. 2008). Camacho et al. (2003) reported anti-leishmanial activity of methanolic extract of the *S. macrophylla* bark and leaves against *Leishmania donovani* promastigotes with an IC_{50} = 14.12 ± 0.92 and 9.18 ± 0.18 μg mL^{-1}, and SI = 6.17 and 2.61 respectively. Preliminary anti-Leishmania study on *Pseudocedrela kotschyi* (Meliaceae) using intracellular model of *L. major* had shown a significant activity when exposed to 35 μg/mL of dichloromethane root extract (Hay et al. 2007). *Azadirachta indica* (Meliaceae) crude methanolic extract displayed potential anti-leishmanial agent against *L. major* promastigotes

with LC_{50} = 10.21 µg mL^{-1} (Khalid et al. 2005). The methanolic extracts from the seeds of *S. macrophylla* showed promising anti-leishmanial activity (IC_{50} 17 ± 0.07 µg mL^{-1} and SI > 12).

Anisophyllea disticha (Anisophylleaceae), also locally known as kayu pacat or raja berangkat, grows as a shrub or small tree with a height reaching to about 8 m and commonly found in Malaysia, Thailand, Indonesia and Brunei. Traditionally, the plant has been used as walking sticks, and as therapeutic in libido stimulants and anti-aging therapies (Ong 2006). Extracts from the leaf of *A. disticha* showed strong anti-leishmanial activity with IC_{50} = 16.53 ± 0.17 µg mL^{-1} and SI = 11.45. Nevertheless, reports were found on anti-diabetic, anti-fungal and anti-bacterial of *A. laurina,* as well of its traditional use in relieving toothache, oral care and analgesic property (Kargbo et al. 2015).

Anti-leishmanial activity was also high in the leaf extracts of *Iguanura geonomiformis* (Arecaceae) with IC_{50} = 14.66 ± 0.10 µg mL^{-1} and SI = 10.24. This plant, commonly known as pinang burong, found in the rainforest of Peninsular of Malaysia, was reported to have been used in healing and aborigine's ceremony (Ong et al. 2012). The leaf is used as a wrapper and the fruit for skin irritation and cough relieve (Beng-Jin 2005). *Phoenix dactylifera* (Arecaceae) was reported as a very potent anti-oxidant and anti-mutagenic (Vayavil 2002), attributing to its rich constituent of phenolics, carotenoids and anthocyanins (El Sohaimy et al. 2015). The plants used in this study was also tested against *Trypanosoma brucei brucei*, a sleeping sickness caused by tsetse fly, and the results were negative for *S. macrophylla, A. disticha* and *I. geonomiformis* in inhibiting the *Leishmania* parasite's activity (Norhayati et al. 2013).

Few reports are were available on Meliaceae of *Cipadessa fruticose*. Leite et al. (2010) reported trypanocidal activity from isolated limonoids while Ambrozin et al. (2005) suggested a novel drug for leishmaniasis as seen by the significant inhibition of the extracts on enzyme adenine phosphoribosyltransferase (APRT) from *Leishmania*. No reports were found on anti-leishmanial from other species (Anisophylleaceae and Arecaceae) which were found active in this study.

Further studies should be carried out on the active plants through bio-guided fractionation, to isolate the bioactive compounds responsible for the anti- leishmanial activity.

CONCLUSIONS

The study showed that the methanolic extracts of three plant species had strong anti-leishmania activity: *Iguanura geonomiformis* (IC_{50} =14.66 ± 0.10 µg mL^{-1}; SI 10.24, *Anisophyllea disticha* (IC_{50} = 16.53 ± 0.17 µg mL^{-1}; SI = 11.45) and *Swietenia macrophylla* (IC_{50} = 17 ± 0.07 µg mL^{-1}; SI >12).

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