BIOACTIVITY OF LEAVES OF *MACARANGA* SPECIES IN TROPICAL PEAT SWAMP AND NON-PEAT SWAMP ENVIRONMENTS

TY Lim, YY Lim* & CM Yule

School of Science, Monash University Sunway Campus, Bandar Sunway, 46150 Petaling Jaya, Selangor Darul Ehsan, Malaysia

Received January 2013

LIM TY, LIM YY & YULE CM. 2014. Bioactivity of leaves of *Macaranga* species in tropical peat swamp and non-peat swamp environments. Very few studies have been reported on the chemical properties of peat swamp plants compared with their non-peat swamp counterparts. In this study, the total phenolic content, antioxidant activity and antibacterial activity of fresh *Macaranga gigantea, M. hosei, M. hypoleuca, M. kingii, M. pruinosa* and *M. triloba* leaf extracts from four tropical peat swamp forests and three non-peat swamp forests were measured and compared. Total phenolic content was measured by the Folin–Ciocalteu assay. Antioxidant activity was determined using the 1,1-diphenyl-2-picrylhydrazyl free radical scavenging and ferric reducing power assays, while disc-diffusion technique was used to determine the antibacterial properties. Reversed-phase-high-performance liquid chromatography methods were used to identify and quantify the concentrations of phenolics. Antioxidant properties of leaf extracts from peat swamp forest species were 42 to 60% higher than those of the same plant species from non-peat swamp forest. However, no significant differences were observed for samples collected from within the same environmental conditions. No significant differences were found in antibacterial activities between peat swamp and non-peat swamp samples. The results suggest that trees in peat swamp forests are producing higher levels of phenolic compounds in response to extreme conditions.

Keywords: Phenolic compounds, antioxidant activity, antibacterial activity, HPLC analysis

LIM TY, LIM YY & YULE CM. 2014. Bioaktiviti daun *Macaranga* di persekitaran paya gambut tropika dan bukan paya gambut. Kajian terhadap ciri-ciri kimia tumbuh-tumbuhan paya gambut adalah sedikit berbanding kajian ke atas tumbuh-tumbuhan bukan paya gambut. Dalam kajian ini, jumlah kandungan fenol, aktiviti antioksidan dan aktiviti antibakteria bagi ekstrak daun segar *Macaranga gigantea, M. hosei, M. hypoleuca, M. kingii, M. pruinosa* dan *M. triloba* dari empat hutan paya gambut tropika dan tiga hutan bukan paya gambut disukat dan dibandingkan. Jumlah kandungan fenol disukat menggunakan asai Folin–Ciocalteu. Aktiviti antioksidan disukat menggunakan asai pemusnah radikal bebas 1,1-difenil-2-pikrilhidrazil dan asai penurunan kuasa ferik. Teknik peresapan cakera digunakan untuk menentukan ciri antibakteria. Kaedah fasa balikan kromatografi cecair prestasi tinggi digunakan untuk mengecam dan menentukan kepekatan fenol. Ciri antioksidan ekstrak daun spesies pokok hutan paya gambut ialah 42% hingga 60% lebih tinggi daripada spesies yang sama dari hutan bukan paya gambut. Bagaimanapun, tiada perbezaan signifikan diperhatikan untuk sampel daripada keadaan persekitaran yang sama. Tiada perbezaan signifikan dicerap antara aktiviti antibakteria sampel paya gambut dengan sampel bukan paya gambut. Keputusan mencadangkan yang pokok hutan paya gambut menghasilkan sebatian fenol pada aras yang lebih tinggi sebagai gerak balas terhadap keadaan yang amat teruk.

INTRODUCTION

Tropical peat swamp forests of South-East Asia are waterlogged, seasonally flooded forests where the rate of organic matter accumulation is higher than that of decomposition. Leaves build up as peat deposits up to 20 m deep with high acidity (pH 2.9 to 3.5) and low nutrient availability (Yule & Gomez 2009, Yule 2010). These forests differ from most tropical rainforests where warmth and high humidity aid rapid leaf litter breakdown by fungi and bacteria. As a response to extreme conditions and low nutrient levels, peat swamp plants have developed various chemical and physical defences for survival (Balasundram et al. 2006, Treutter 2006, Yule 2010). For example, they tend to be armoured with spikes and thorns and have tough, leathery leaves that are high in toxic secondary compounds such as resins, aromatic oils and latex (Treutter 2006).

*lim.yau.yan@monash.edu

Studies focusing on chemical properties of plants subjected to adverse environmental conditions have shown that the conditions stimulate the production of certain compounds, particularly phenols and flavonoids (Treutter 2006). Conditions such as altitudinal variation (Spitaler et al. 2008, Wong et al. 2009), herbivory (Lempa et al. 2004, Treutter 2006), ultraviolet B (UVB) exposure and geological environments (Hashiba et al. 2006) have been shown to cause variations in polyphenols of the samples studied. Polyphenolic contents were found to be higher in five species of cultivated herbs compared with their wild counterparts (Spina et al. 2008), while levels of phenols and flavonoids were higher in various yam cultivars grown in lower pH conditions (Chen et al. 2008).

Macaranga is a large genus of over 60 species of Old World trees classified in the family Euphorbiaceae and the only genus in the subtribe Macaranginae. These plants are noted for being recolonisers and are native to tropical Africa, Madagascar, South-East Asia, Australia and the Pacific region (Davies 1998). There are approximately 40 species which are native to Malaysia and they recolonise almost any disturbed terrestrial habitat (Siregar & Sambas 2000), including both peat swamp and non-peat swamp environments. Traditionally, the bioactivity of many *Macaranga* species has been harnessed in folk medicine in tropical regions. For example, the sap of young shoots of *M. gigantea* and *M. triloba* is used to treat fungal infections and the leaf decoction, stomachaches (Grosvenor et al. 1995). *Macaranga hypoleuca* has been used as febrifuge, expectorant and anti-spasmodic (Eswani et al. 2010).

We have previously studied the antioxidant and antibacterial activities of leaves of several *Macaranga* species from three non-peat swamp sites (two secondary forests and one hill dipterocarp forest). No variation in total phenolic content (TPC) was found between trees from the three sites (Lim et al. 2009). In order to study the possible environmental effects on secondary metabolites, this paper compares the TPC antioxidant property and antibacterial activity of the leaves of six *Macaranga* species, namely, *M. gigantea, M. hosei, M. hypoleuca, M. kingii, M. pruinosa* and *M. triloba* from tropical peat swamp and non-peat swamp forests in Malaysia.

MATERIALS AND METHODS

Samples

Mature leaves of *M. gigantea, M. hosei, M. hypoleuca, M. kingii, M. pruinosa* and *M. triloba* were collected from trees in four peat swamp forests (PSF) and four nearby non-peat swamp (NPS) sites. The sites were in north Selangor (PSF: 3° 39' N, 101° 19' E, 4 m above sea level (asl); NPS: 3° 44' N, 101° 26' E, 3 m asl), Kuantan (PSF: 3° 53' N, 103° 22' E, 3 m asl; NPS: 3° 44' N, 103° 22' E, 3 m asl), north Kuala Langat (PSF: 2° 54' N, 101° 35' E, 2 m asl; NPS: 2° 39' N, 101° 48' E, 2 m asl) and Pekan (PSF: 3° 48' N, 103° 38' E, 3 m asl; NPS: 3° 18' N, 103° 32' E, 3 m asl). Samples were rinsed with distilled water, air dried and extracted on the same day. Voucher specimens were deposited at the herbarium of the Monash University Sunway campus.

Preparation of extracts

For TPC and antioxidant tests, 1 g of fresh samples was crushed mechanically into powder in liquid nitrogen. The crushed samples were extracted with 50 mL of 100% methanol by shaking the suspension on an orbital shaker for 1 hour and then filtering through vacuum filtration. The extracts were stored at -20 °C until further use.

For evaluation of antibacterial activity, approximately 100 g of fresh samples were freeze dried, crushed into powder and extracted with 500 mL of 100% methanol. The extracts were filtered via filtration under reduced pressure and the solvent removed by rotary evaporator at 30 °C. The crude extract was stored at -20 °C until further use.

Total phenolic content

The TPC of extracts was determined using the Folin–Ciocalteu assay (Kahkonen et al. 1999). Samples (300 µL, triplicate) were introduced into test tubes followed by 1.5 mL of Folin–Ciocalteu's reagent (10 × dilution) and 1.2 ml of 7.5% sodium carbonate. Tubes were allowed to stand in the dark for 30 min before absorbance at 765 nm was measured. Total phenolic content was expressed as gallic acid equivalent (GAE) in mg GAE/100 g material. The calibration equation

was y = 0.0111x – 0.0148 (r^2 = 0.9998) where y = absorbance and x = concentration of gallic acid in mg L^{-1}.

Sample preparation for high performance liquid chromatography analysis

Samples were treated to remove chlorophyll, fatty compounds and other non-polar compounds from the crude extracts. Crude extracts were dissolved as much as possible in 1 mL of 30% methanol (sonicated). Then, 1 mL of hexane was added to each fraction, vortexed and allowed to stand for 1 min. Fractions were centrifuged for 30 s. The hexane layer (top layer) which contained fatty compounds was removed and the entire process was repeated twice. The aqueous layer was filtered (pore size 0.45 µm) prior to injection into the high performance liquid chromatograph (HPLC).

Analytical chromatographic analysis was performed using HPLC. The instrument consisted of quaternary vacuum degasser pump and diode array detector. Samples were injected through a Rheodyne manual injector valve fitted with 20 µL sample loop. The column consisted of a phenyl-bound silica column (100 × 4.6 mm; 5 µm particle size).

Gradient mode was used in this analysis, involving two solvents: (1) 100% methanol and (2) water, acidified to approximately pH 2.5 with 0.1% trifluoroacetic acid. The elution profile was 40% mobile phase 1 to 60% mobile phase 2 in a linear gradient from 0 to 20 min. The flow rate was set at 1 mL min^{-1}. The detection wavelengths used were 210, 245, 280 and 365 nm with reference wavelength set at 700 nm.

Quantification of specific phenolic compounds

The identification of specific phenolic compounds was based on (1) retention time, (2) spiking with authentic standard and (3) comparing the absorbance spectra with standard compounds. The concentration of each compound was determined based on the construction of a standard curve (measured at 245 nm) for each phenolic compound identified. A total of four phenolic compounds were identified and four different standard curves were constructed. The standard curves for each compound were (1) ferulic acid: y = 6783.4x (r^2 = 0.9983), (2) *p*-coumaric acid: y = 7383.4x (r^2 = 0.9876), (3) quercetin: y = 28173.4x (r^2 = 0.9887) and (4) taxifolin: y = 8874.3x (r^2 = 0.9987) where y = peak area (mAU) and x = concentration (mg mL^{-1}). The content of each compound was expressed as mg/100 g of fresh leaves.

Antioxidant activities

Antioxidant activities of extracts measured included free radical scavenging activity and ferric reducing power (FRP). Methods used were as described in Lim et al. (2009).

In free radical scavenging activity measurement, 1 mL of extract with different dilutions was added to 2 mL of 0.145 mM 1,1-diphenyl-2-picrylhydrazyl (DPPH). All tubes were incubated for 30 min and the absorbance measured at 517 nm. DPPH free radical scavenging ability was calculated as IC_{50} and expressed as ascorbic acid equivalent antioxidant activity (AEAC) in mg ascorbic acid/100 g as follows:

$$AEAC = (IC_{50\ (ascorbic\ acid)} / IC_{50\ (sample)}) \times 10^5$$

The IC_{50} of ascorbic acid used for calculation was 0.00387 mg mL^{-1} (Chan et al. 2007).

In FRP assay, various dilutions of the extract solution (1 mL) were added to 2.5 mL of 0.2 M pH 6.6 potassium phosphate buffer and 2.5 mL 1% potassium ferricyanide. The mixture was incubated for 20 min at 50 °C after which 2.5 mL of 10% trichloroacetic acid were added. The mixture was separated into aliquots of 2.5 mL and mixed with 2.5 mL of deionised water. Then, 0.5 mL of 0.1% w/v $FeCl_3$ was added to each tube and allowed to stand for 30 min. Absorbance for each tube was measured at 700 nm. The FRP was expressed as GAE in mg g^{-1} of samples used (calibration equation: y = 16.767x; r^2 = 0.9974 where y = absorbance and x = concentration of gallic acid in mg mL^{-1}).

Antibacterial activity

The disc-diffusion method described by Chung et al. (2004) was used to screen for antibacterial activity. Agar cultures of Gram-positive bacteria (*Bacillus cereus* ATCC14579, *Micrococcus luteus* ATCC4698 and *Staphylococcus aureus* ATCC 33591) and Gram-negative bacteria (*Escherichia coli* ATCC25922 and *Klebsiella pneumonia* ATCC10031)

were prepared. Suspensions of bacteria (100 µL) were spread evenly onto 20 mL Mueller–Hinton agar preset in Petri dishes. Paper discs were impregnated with 1 mg of plant extract dissolved in 100 µL solvent and transferred onto the inoculated agar. Streptomycin-susceptibility discs (10 µg) and methanol-impregnated discs were used as positive and negative controls respectively. After incubation overnight at 37 °C, inhibition zones were measured and recorded as mean diameter (mm). Antibacterial activity was expressed as inhibition percentage of streptomycin. Minimal inhibition dose was expressed as mass/disc needed to show growth inhibition.

Statistical analysis

Student's t-test was performed. For comparison between several sets of data and identifying the levels of significance, analysis of variance (ANOVA) was carried out using Tukey's test. Differences at $p < 0.05$ were considered as significant.

RESULTS

Total phenolic content

Comparison within the same species showed that samples from PSF possessed TPC values which were significantly higher than NPS samples (Table 1). No significant difference was observed between samples collected from different sites within PS or NPS forests. Average TPC values from all four PSF and NPS sites showed that *M. triloba* (PSF: 4456 mg GAE/100 g; NPS: 3364 mg GAE/100 g) had the highest values, followed by *M. pruinosa* (PSF: 3475 mg GAE/100 g; NPS: 2564 mg GAE/100 g) and *M. kingii* (PSF: 3598 mg GAE/100 g; NPS: 2541 mg GAE/100 g). The remaining species were in the order *M. gigantea* (PSF: 3077 mg GAE/100 g; NPS: 2384 mg GAE/100 g) > *M. hosei* (PSF: 2520 mg GAE/100 g; NPS: 1552 mg GAE/100 g) > *M. hypoleuca* (PSF: 2598 mg GAE/100 g; NPS: 1487 mg GAE/100 g).

HPLC analysis

Based on HPLC analysis, quercetin and taxifolin were found to be consistently higher in PSF for both *M. pruinosa* and *M. triloba* (Table 2). *p*-Coumaric acid and ferulic acid were also higher in PSF for *M. gigantea*.

Antioxidant activities

Table 3 summarises the free radical scavenging activity (expressed as AEAC) and FRP of peat swamp and non-peat swamp *Macaranga* species. In all cases, peat swamp samples showed significantly

Table 1 Total phenolic contents of six *Macaranga* species from peat swamp forest (PSF) and non-peat swamp (NPS) sites

Species	Sample	Total phenolic content (mg GAE/ 100 g fresh weight)			
		KLN	NS	P	K
Macaranga gigantea	PSF	2957 ± 120 a	3126 ± 112 a	3312 ± 163 a	2913 ± 186 a
	NPS	2423 ± 70.6 b	2571 ± 118 b	2217 ± 192 b	2324 ± 120 b
Macaranga hosei	PSF	2617 ± 120 a	–	–	2422 ± 145 a
	NPS	1626 ± 120 b	1727 ± 172	1422 ± 153	1432 ± 116 b
Macaranga hypoleuca	PSF	2632 ± 182 a	–	–	2563 ± 192 a
	NPS	1623 ± 164 b	1466 ± 153	1397 ± 182	1461 ± 172 b
Macaranga kingii	PSF	3632 ± 182 a	–	–	3563 ± 192 a
	NPS	2627 ± 164 b	2481 ± 153	2397 ± 182	2661 ± 172 b
Macaranga pruinosa	PSF	3352 ± 124 a	3663 ± 174 a	3545 ± 123 a	3342 ± 154 a
	NPS	2534 ± 123 b	2612 ± 157 b	2453 ± 182 b	2657 ± 131 b
Macaranga triloba	PSF	4282 ± 172 a	4534 ± 192 a	4434 ± 152 a	4572 ± 182 a
	NPS	3127 ± 226 b	3341 ± 149 b	3497 ± 156 b	3489 ± 178 b

Values are based on five samples determined in triplicate per run; data are presented as means ± SD, SD = standard deviation; for each species, values followed by the same letter are not significantly different at $p < 0.05$ as measured by Tukey's test; KLN = Kuala Langat north, NS = north Selangor, P = Pekan, K = Kuantan; GAE = gallic acid equivalent

Table 2 Comparison of selected major phenolic compounds in six *Macaranga* species from both peat swamp forest (PSF) and non-peat swamp (NPS)

Species	Phenolic compound	Concentration (mg/100 g fresh weight)	
		PSF	NPS
Macaranga gigantea	*p*-Coumaric acid	400 ± 81 a	311 ± 32 b
	Ferulic acid	313 ± 36 a	103 ± 25 b
Macaranga hosei	Ferulic acid	323 ± 26 a	95 ± 12 b
	Quercetin	154 ± 51	Not detected
Macaranga hypoleuca	*p*-Coumaric acid	367 ± 35	Not detected
	Taxifolin	423 ± 53 a	264 ± 47 b
Macaranga kingii	*p*-Coumaric acid	523 ± 76	Not detected
	Ferulic acid	458 ± 155 a	142 ± 64 b
	Taxifolin	319 ± 34	Not detected
Macaranga pruinosa	*p*-Coumaric acid	673 ± 67	Not detected
	Quercetin	1432 ± 144 a	393 ± 166 b
	Taxifolin	453 ± 48 a	325 ± 76 a
Macaranga triloba	*p*-Coumaric acid	223 ± 31 a	112 ± 23 b
	Quercetin	912 ± 104 a	453 ± 92 b
	Taxifolin	332 ± 58 a	116 ± 64 b

All values are based on three samples; values are presented as means ± SD for each compound within a species, SD = standard deviation; values followed by the same letter are not significantly different at $p < 0.05$ as measured by Tukey's test; site: North Selangor

Table 3 TPC, AEAC and FRP variation of peat swamp forest (PSF) and non-peat swamp (NPS) samples according to species

Species	Sample	Average TPC (mg GAE/100 g)	AEAC (mg AA/100 g)	FRP (mg GAE/ g)
Macaranga gigantea	PSF	3076 ± 181 a	3279 ± 91 a	17.8 ± 1.2 a
	NPS	2383 ± 151 b	2249 ± 129 b	11.9 ± 2.5 b
Macaranga hosei	PSF	2520 ± 113 a	2379 ± 101 a	13.2 ± 1.1 a
	NPS	1551 ± 149 b	1443 ± 89 b	8.9 ± 0.5 b
Macaranga hypoleuca	PSF	2597 ± 187 a	2553 ± 122 a	11.8 ± 1.3 a
	NPS	1485 ± 168 b	1478 ± 96 b	8.9 ± 0.7 b
Macaranga kingii	PSF	3598 ± 115 a	3611 ± 91 a	19.1 ± 1.2 a
	NPS	2541 ± 124 b	2345 ± 127 b	10.2 ± 2.5 b
Macaranga pruinosa	PSF	3475 ± 156 a	3481 ± 128 a	21.5 ± 3.7 a
	NPS	2564 ± 89.7 b	2336 ± 112 b	15.0 ± 0.7 b
Macaranga triloba	PSF	4455 ± 129 a	4573 ± 239 a	28.8 ± 1.5 a
	NPS	3366 ± 173 b	2876 ± 172 b	19.2 ± 2.1 b

Values are based on nine samples; all assays are determined in triplicate per run and data are presented as means ± SD, SD = standard error; for each species in a column, values followed by the same letter are not significantly different at $p < 0.05$ as measured by the Tukey's test; TPC = total phenolic content; AEAC = ascorbic acid equivalent antioxidant activity; FRP = ferric-reducing power; GAE = gallic acid equivalent

higher AEAC and FRP values than non-peat swamp. *Macaranga triloba* showed the highest AEAC and FRP values, followed by *M. kingii* ≈ *M. pruinosa* ≈ *M. gigantea* and *M. hosei* ≈ *M. hypoleuca*. The free radical scavenging activity and FRP correlated well with the trend in TPC, indicating that these activities were mainly due to phenolic compounds in the extracts. This trend was observed for both peat swamp and non-peat swamp samples. Generally, TPC, AEAC and FRP values were 42 to 60% higher in peat swamp samples.

Antibacterial activity

Inhibition was only observed for Gram-positive species (*B. cereus, M. luteus* and *S. aureus*), which were moderately inhibited by all *Macaranga* extracts (Table 4). No activity was observed for Gram-negative species.

PSF and NPS samples showed similar bacterial inhibition activity. The crude extracts of *M. gigantea, M. pruinosa* and *M. triloba* were most effective against *B. cereus* and *M. luteus* with an estimated minimum inhibition dosage (MID) values of 5 µg/disc. The MID values of all three extracts towards *S. aureus* were similar with MID values of 10 µg/disc.

DISCUSSION

Various compositions of aqueous methanol can be used to extract phenolic compounds from leaves. In this work, 100% methanol was chosen based on a study by Lim et al. (2009). Generally, leaf samples are extracted with 70% methanol and above as the majority (≥ 80%) of the polyphenolic and antioxidative constituents will be successfully extracted in the first extraction. Furthermore, a high concentration of methanol can inhibit the activity of polyphenol oxidases, which destroy polyphenolic compounds in leaves due to injury or destruction of the leaf (Robards 2003).

The leaves of all six *Macaranga* species exhibited high TPC values that were significantly higher in PSF compared with NPS (Table 1). These values were comparable with those reported in the leaves of *Elingera elatior, E. rubrostriata, E. littoralis* and *E. fulgens* which ranged from 2540 to 3550 mg GAE/100 g (Chan et al. 2007). Some polyphenols, in particular flavonoids such as quercetin, play an important role in plant protection (Treutter 2006). The particularly high TPC values in PSF plants are likely be related to the extreme environmental conditions in the peat swamp forest (low nutrients, low pH and waterlogging). As an important adaptive strategy, it would be beneficial for plants to invest in the production of phenolics for defence against environmental damage from factors such as herbivory, microbial pathogens and UV rays by retaining the existing leaves as far as possible rather than growing new leaves.

The trends observed for individual polyphenolic acids mirrored those for the TPC. Quercetin and taxifolin were consistently higher in PSF for both *M. pruinosa* and *M. triloba*. *p*-Coumaric acid and ferulic acid were also higher in PSF for *M. gigantea* (Table 2). These compounds have been reported to be produced

Table 4 Comparison of antibacterial activity of *Macaranga* species against Gram-positive and Gram-negative bacteria

Species	Sample	*Bacillus cereus*	*Micrococcus luteus*	*Staphylococcus aureus*
		% inhibition at 1 mg/disc (MID) strength		
Macaranga gigantea	PSF	53.0 (5) ++	52.0 (5) ++	50.0 (10) ++
	NPS	53.0 (5) ++	57.0 (5) ++	51.0 (10) ++
Macaranga hosei	PSF	43.0 (5) +	56.0 (10) ++	42.0 (5) +
	NPS	35.0 (5) +	42.0 (10) +	37.0 (5) +
Macaranga hypoleuca	PSF	49.0 (5) +	61.0 (10) ++	41.0 (5) +
	NPS	41.0 (5) +	54.0 (10) ++	35.0 (5) +
Macaranga kingii	PSF	63.0 (5) ++	74.0 (10) ++	62.0 (5) ++
	NPS	51.0 (5) ++	61.0 (10) ++	53.0 (5) ++
Macaranga pruinosa	PSF	60.0 (5) ++	70.0 (5) +++	57.0 (10) ++
	NPS	63.0 (5) ++	69.0 (5) ++	63.0 (10) ++
Macaranga triloba	PSF	62.0 (5) ++	79.0 (5) +++	64.0 (10) ++
	NPS	60.0 (5) ++	77.0 (5) +++	62.0 (10) ++
Streptomycin inhibition zone (mm)		18.3	19.6	19.3
No activity was observed for Gram-negative species				

Mean diameter of zone of inhibition is expressed as % of streptomycin inhibition zone; figures in parentheses are MID values in mg/disc; MID = minimum inhibition dosage; antibacterial activity is categorised as strong (+++) for inhibition p ≥ 70%, moderate (++) for inhibition 50% < p < 70% or weak (+) for inhibition < 50%; PSF = peat swamp forest, NPS = non-peat swamp

in plants as anti-herbivory agents (Mulder & Breure 2003, Schijlen et al. 2004, Treutter 2006) and lipid peroxidation inhibitors to protect the lipid bilayer of the plant cell wall against free radicals and UVB rays (Hashiba et al. 2006, Treutter 2006).

As recolonisers, most *Macaranga* species are often found growing in areas where there is direct exposure to sunlight (UV radiation) due to the low, sparse canopy (Davies 1998) and this can be expected to induce production of phenols. Larger ginger (*Etlingera* and *Alpinia* spp.) plants growing in exposed forest sites have been reported to possess higher antioxidant capability than smaller (*Zingiber, Curcuma, Boesenbergia, Kaempferia* spp.) plants that normally grow under the canopy (Chan et al. 2008). Leaves of vegetables which are exposed to sunlight have been reported to contain higher concentrations of flavones and flavonols (Spina et al. 2008). However, in this study, UV was unlikely to cause the induction of higher levels of phenolics in peat swamp species since samples of all plants were collected from open areas where leaves were accessible.

Phenols, in particular flavonoids, can act as strong antioxidants. In this study, antioxidant activity in terms of free radical scavenging activity and ferric reducing power of peat swamp plants was significantly greater than non-peat swamp *Macaranga* (Table 3). In contrast, antibacterial activity showed no difference between PSF and NPS samples. Inhibition was only observed for Gram-positive species (*B. cereus, M. luteus* and *S. aureus*) which were moderately inhibited by all *Macaranga* extracts from all sites. Gram-negative bacteria have an outer membrane, consisting of lipoprotein and lipopolysaccharide, which selectively inhibits penetration of phenolics into the underlying structures (Chopra & Greenwood 2001). If our hypothesis that *Macaranga* species produce phenols as an anti-herbivory and anti-pathogen response is correct, then this may explain why they have no defence against Gram-negative bacteria.

CONCLUSIONS

In general, peat swamp species showed greater amounts of polyphenols and higher antioxidant properties than their non-peat swamp counterparts in response to extreme environment. All methanolic *Macaranga* leaf extracts showed high antioxidant properties with the highest values in *M. triloba*. No significant differences were observed for antibacterial activities between peat swamp and non-peat swamp *Macaranga* species.

ACKNOWLEDGEMENT

The authors wish to thank Monash University Sunway Campus for financial support.

REFERENCES

Balasundram N, Kalyana S & Samman S. 2006. Phenolic compounds in plants and agri-industrial by-products: antioxidant activity, occurrence and potential uses. *Food Chemistry* 99: 191–203.

Chan ECW, Lim YY & Omar M. 2007. Antioxidant and antibacterial activity of leaves of *Etlingera* species (Zingiberaceae) in Peninsular Malaysia. *Food Chemistry* 104: 1586–1593.

Chan ECW, Lim YY, Wong LF, Lianto FS, Wong SK, Lim KK, Joe CE & Lim TY. 2008. Antioxidant and tyrosinase inhibition properties of leaves and rhizomes of ginger species. *Food Chemistry* 109: 477–483.

Chen Y, Kao W & Lin K. 2008. Effects of pH on the total phenolic compound, antioxidative ability and stability of dioscorin of various yam cultivars. *Food Chemistry* 107: 250–257.

Chopra I & Greenwood D. 2001. *Antibacterial Agents: Basis of Action*. John Wiley & Sons Ltd, Chichester.

Chung PY, Chung LY, Ngeow YF, Goh SH & Imiyabir Z. 2004. Antimicrobial activities of Malaysian plant species. *Pharmaceutical Biology* 42: 292–300.

Davies JS. 1998. Photosynthesis of nine pioneer *Macaranga* species from Borneo in relation to life history. *Ecology* 79: 2292–2308.

Eswani N, Kudus KA, Nazre M, Noor AGA & Ali M. 2010. Medicinal plant diversity and vegetation analysis of logged overhill forest of Tekai Tembeling Forest Reserve, Jerantut, Pahang. *Journal of Agricultural Science* 2: 189–210.

Grosvenor PW, Gothard PK, Mcwilliam NC, Supriono A & Gray DO. 1995. Medicinal plants from Riau Province, Sumatra, Indonesia. *Journal of Ethnopharmacology* 45: 75–95.

Hashiba K, Iwashina T & Matsumoto S. 2006. Variation in the quality and quantity of flavonoids in the leaves of coastal and inland *Campanula punctata*. *Biochemical Systematics and Ecology* 34: 854–861.

Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS & Heinonen M. 1999. Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry* 47: 3954–3962.

Lempa K, Agrawal AA, Salminen J, Turunen T, Ossipov V, Ossipova S, Haukioja E & Pihlaja K. 2004. Rapid herbivore-induced changes in mountain birch phenolics and nutritive compounds and their effects on performance of the major defoliator, *Epirrita autumnata*. *Journal of Chemical Ecology* 30: 303–321.

Lim TY, Lim YY & Yule CM. 2009. Evaluation of antioxidants, antibacterial and anti-tyrosinase activities of four *Macaranga* species. *Food Chemistry* 114: 594–599.

Mulder C & Breure AM. 2003. Plant biodiversity and environmental stress. Pp 501–525 in Market BA, Breure AM & Zechmeister HG (eds) *Bioindicators and Biomonitors*. Elsevier Science Ltd, London.

Robards K. 2003. Strategies for the determination of bioactive phenols in plants, fruits and vegetables. *Journal of Chromatography A* 1000: 657–691.

Schijlen EGWM, de Vos CHR, Van Tunen AJ & Bovy AG. 2004. Modification of flavonoid biosynthesis in crop plants. *Phytochemistry* 65: 2631–2648.

Siregar M & Sambas EN. 2000. Floristic composition of peat swamp forest in Mensemat-Sambas, West Kalimantan. Pp 153–164 in *Proceedings of the International Symposium on Tropical Peatlands*. Volume 3. 22–23 November 1999, Bogor.

Spina M, Cuccioloni M, Sparapani L, Acciarri S, Eleuteri AM, Fioretti E & Angeletti M. 2008. Comparative evaluation of flavonoid content in assessing quality of wild and cultivated vegetables for human consumption. *Journal of Science of Food and Agriculture* 88: 294–304.

Spitaler R, Winkle A, Lins I, Yanar S, Stuppner H & Zidorn C. 2008. Altitudinal variation of phenolic contents in flowering heads of *Arnica montana* cv. ARBO: a 3-year comparison. *Journal of Chemical Ecology* 34: 369–375.

Treutter D. 2006. Significance of flavonoids in plant resistance: a review. *Environmental Chemistry Letters* 4: 147–157.

Wong SK, Lim YY & Chan EWC. 2009. Antioxidant properties of *Hisbiscus* species variation, altitudinal change, coastal influence and floral colour change. *Journal of Tropical Forest Science* 21: 307–315.

Yule CM. 2010. Loss of biodiversity and ecosystem functioning in Indo-Malayan peat swamp forests. *Biodiversity and Conservation* 19: 393–409.

Yule CM & Gomez LN. 2009. Leaf litter decomposition in a tropical peat swamp forest in Peninsular Malaysia. *Wetlands Ecology Management* 17: 231–241.