2011 3rd International Conference on Chemical, Biological and Environmental Engineering
IPCBEE vol.20 (2011) © (2011) IACSIT Press, Singapore

Antioxidant Properties of Some Malaysian Ferns

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Abstract. Malaysia is home to a diverse range of ferns, many of which have been used as traditional medicines in the treatment of various ailments or for general healthcare. The aim of this study was to explore new and natural sources of antioxidant amongst some ferns in Malaysia. Fifteen fern species were screened. Total phenolic content (TPC) was measured using the Folin-Ciocalteau method. Antioxidant properties were determined via the DPPH radical scavenging, ferric reducing power (FRP) and β–carotene bleaching (BCB) assays. Results showed five ferns with very high total phenolic content of above 2000 mg GAE/100 g fresh leaves. These ferns exhibited strong antioxidant activity based on the DPPH radical scavenging activity, ferric ion reducing power and inhibition of lipid peroxidation. The ferns with strong antioxidant properties were *Cyathea latebrosa, Cibotium barometez, Drynaria quercifolia, Blechnum orientale* and *Dicranopteris linearis.*

Keywords: ferns, antioxidant, DPPH radical scavenging, ferric reducing, ion chelating, lipid peroxidation

1. Introduction

Epidemiological studies and a substantial body of evidence have linked the production of free radicals with the occurrence of cardiovascular diseases, carcinogenesis, rheumatoid arthritis and denegerative processes associated with aging. Antioxidants aid in the prevention by scavenging the excess free radicals, hence preventing the formation of reactive oxygen species in the body [1]. The use of synthetic antioxidants such as butylated hydroxytoluene, butylated hydroxyanisole, tert-butylhydroquinone and propyl gallate has been negatively perceived by consumers due to safety and health effects [2]. Hence, there is an increasing interest in the search of natural antioxidants from plant sources.

It is well known that many botanicals possess natural antioxidants with high antioxidant activity [2-4] and investigations on these were initiated based on their uses in traditional folkloric medicines. Ferns belong to a group of non-flowering plants known as *Pteridophytes.* It is estimated that there are 1136 fern species in Malaysia [5], many of which have been used as traditional medicines in the treatment of various ailments or for general healthcare [6-8]. Despite the large number of ferns, knowledge on the antioxidant properties of these botanicals is still scarce.

In this study, we report the antioxidant properties of 15 fern species found in Malaysia. Majority of these ferns have been utilized traditionally to provide cures for many ailments. Total phenolic content was determined and antioxidant activities were measured based on DPPH radical scavenging, reducing power and β–carotene bleaching assay.

2. Materials and methods

2.1. Ferns collection and extract preparation

Cyathea latebrosa (Wall. ex Hook) Copel*, Dicranopteris linearis* (Burm.) and *Pteris vittata* L. were obtained from Mount Kiara Park, Kuala Lumpur. *Cibotium barometz* (L.) J. Sm., *Drynaria quercifolia* (L.) J. Sm.*, Blechnum orientale* L.*, Adiantum raddianum* C. Presl.*, Diplazium esculentum* (Retz.) Sw., *Pityrogramma calomelanos* (L.) Link*, Lygodium circinnatum* (Burm.f.) Swartz and *Microsorum punctatum*

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(L.) Copel Cv. were collected from Putrajaya Botanical Garden. *Nephrolepis biserrata* (Sw.) Schott, *Pteris venulosa* Bl. and *Pyrossia numularifolia* (Sw.) Ching were collected from private gardens. *Acrostichum aureum* L. was collected from the Pantai Jeram mangrove swamp in Selangor. The identity of the species were confirmed by plant taxonomist Anthonysamy S., formerly from University Putra Malaysia and currently a consultant with the landscape consulting firm, Aroma Tropic Limited, Kuala Lumpur. Voucher specimens of the ferns were deposited in the herbarium of Monash University Sunway Campus.

Fresh leaves (about 1 g) were pulverized in liquid nitrogen and extracted with 50.0 mL of methanol continuously for an hour at room temperature in a rotary orbital shaker. The extract was filtered under reduced pressure and stored at -20 °C to be used within one week.

2.2. Total phenolic content (TPC)

TPC in extracts was determined according to the Folin-Ciocalteu procedure [9]. The extract (0.3 mL, in triplicate) was mixed with 1.5 mL of 10% Folin-Ciocalteau's reagent and 1.2 mL of 7.5% (w/v) sodium carbonate. The mixture was kept in the dark for 30 min before measuring the absorbance at 765 nm. The gallic acid standard curve used was y = 0.01078x (R^2=0.9996) where y is absorbance at 765 nm and x is the concentration of gallic acid in mg/L. TPC was expressed as mg gallic acid equivalent (GAE)/100g leaves.

2.3. Antioxidant assays

The DPPH radical scavenging assay employed is as previously described [10]. Various dilutions of the extract (1.0 mL, in triplicate) were added to 2.0 mL of DPPH (5.9 mg/100 mL methanol). The mixture was left in the dark for 30 min before reading the absorbance at 517 nm with methanol as blank. The control consisted of methanol in place of extract. Radical scavenging activity was expressed as a percentage and calculated using the formula: %Scavenging = $(A_{control} - A_{sample})/A_{control}$ x 100. Result was presented as IC_{50}, the concentration of extract required to scavenge 50% of the DPPH radical and also in terms of ascorbic acid equivalent antioxidant capacities (AEAC) which was calculated as follows: AEAC (mg AA/100g) = IC_{50} (ascorbic acid)/IC_{50} sample x 10^5. IC_{50} (ascorbic acid) was determined to be 0.00387 mg/mL.

The Ferric Reducing Power (FRP) assay was carried out according to the procedure described previously [2]. Various dilutions of the extract (1.0 mL , in triplicate) were added to 2.5 mL of 0.2 M phosphate buffer pH 6 and 2.5 mL of potassium ferricyanide (1% w/v). The mixture was incubated for 20 min at 50°C, after which 2.5 mL of 10% trichloroacetic acid was added. An aliquot of 2.5 mL of each mixture was diluted twice with deionised water, before adding 0.5 mL of 0.1% (w/v) $FeCl_3$. Absorbance was measured at 700 nm after 30 min. A calibration curve was constructed using gallic acid. Results were expressed as mg gallic acid equivalent (GAE)/100 g leaves.

In the Beta-carotene bleaching (BCB) assay, a stock solution of β-carotene/linoleic acid was initially prepared by dissolving 5 mg of β-carotene in 50 mL of chloroform [2]. An aliquot of the β-carotene solution (3 mL) was added to 40 mg of linoleic acid and 400 mg of Tween 40. The chloroform was evaporated off using nitrogen gas. Aerated distilled water (100 mL) was added to the mixture. An initial absorbance at 470 nm and 700 nm was immediately recorded. Aliquots of β-carotene/linoleic acid emulsion (3 mL) were mixed with 10 µL, 50 µL and 100 µL of extract. The test and control (containing water in place of sample) tubes were capped and incubated at 50°C. The absorbance of the emulsion at 470 nm and 700 nm was determined after 60 min. All determinations were performed in triplicate. The antioxidant activity was calculated using the formula: Degradation rate (DR) of β-carotene = $Ln(A_{initial}/A_{sample})/60$. Antioxidant activity (%AOA) = $[(DR_{control} - DR_{sample})/DR_{control}]$ x 100.

3. Results and Discussion

3.1. Total phenolic content (TPC)

Phenolic compounds are the most widespread secondary metabolites in plants and have received much attention as potential natural antioxidants and many other health benefits [2, 3, 4, 10]. Figure 1 displays the total phenolic contents in the methanolic extracts of leaves of the ferns investigated. Based on the values of TPC, the ferns were divided into four categories: (a) ferns with very high TPC i.e. > 2000 mg GAE/100 g

leaves: *Cyathea latebrosa*, *Cibotium barometz*, *Drynaria quercifolia*, *Dicranopteris linearis* and *Blechnum orientale,* (b) ferns with high TPC i.e. 1000 – 1999 mg GAE/100 g leaves: *Adiantum raddianum* and *Pteris vittata,* (c) ferns with moderate TPC i.e. 500 – 999 mg GAE/100 g leaves: *Acrostichum aureum*, *Nephrolepis biserrata, Diplazium esculentum*, *Pityrogramma calomelanos*, *Lygodium circinnatum* and *Pyrossia nummularifolia* and (d) ferns with low TPC i.e. < 500 mg GAE/100 g leaves: *Pteris venulosa* and *Microsorum punctatum*.

Phenolic compounds are generated by plants in response to environmental stress. It has been reported that light stimulates the synthesis of flavonoids, especially anthocyanins and flavones via phenylalanine ammonia lyase (PAL) [11] and phenolics are thought to provide a means of protection against UV-B damage and subsequent cell death by protecting DNA from dimerization and breakage [12]. Therefore plants in high-mountain areas which are exposed to a number of stress factors such as low air temperature, decreased partial O_2 pressure, increased UV radiation and unfavorable water regime have generally increased accumulation of antioxidants such as flavonoids [13]. This partially explains the exceptionally high TPC in the ferns with very high TPC (*C. latebrosa*, *C. barometz*, *D. quercifolia*, *B. orientale* and *D. linearis*) and high TPC (*A. raddianum* and *P. vittata*), as they can grow well in habitats of exposed sunlight and at slopes or mountain regions with altitudes up to 1500 – 1700 m.. Salinity is the primary environmental stress factor for a mangrove plant such as *A. aureum* and hence high TPC was expected for this fern. However, *A. aureum* showed moderate TPC (945 mg GAE) which could be due to the shade-tolerant ability of this fern. It has been reported that shades help to reduce the rate of evaporation hence relieving the salt stress encountered by *A. aureum* [14]. For an epiphyte such as *P. nummularifolia*, climbing on tree trunk provides an environment with lower extremes in temperature and sun illumination.

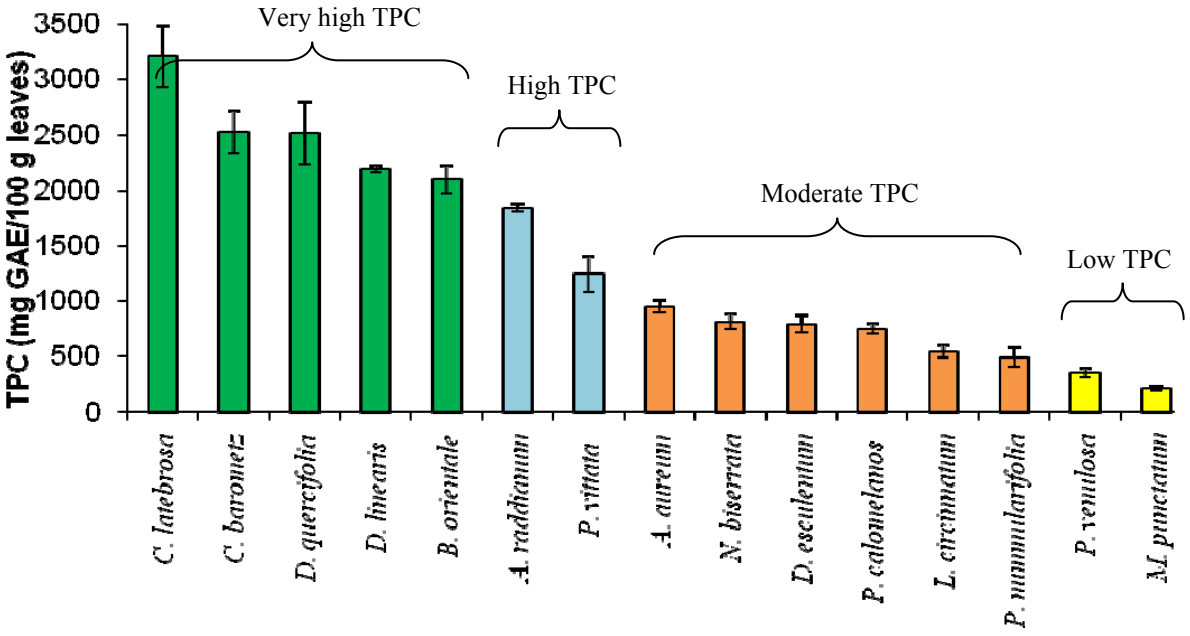

Fig 1: Total phenolic content (TPC) of methanolic leave extracts from fifteen fern species. Data are mean ± S.D of 3 samplings collected randomly in 3 batches from the same source (n=9). Triplicate measurements were conducted in each assay.

3.2. DPPH scavenging, ferric reducing power (FRP) and beta-carotene bleaching (BCB)

In the evaluation of the antioxidant activity, DPPH scavenging activity, ferric reducing power (FRP) and BCB methods have been widely used [2, 3, 4, 10]. In DPPH scavenging assay, the antioxidant activity was measured by the decrease in absorbance as the DPPH radical received an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule [2]. The FRP method measured the ability of an antioxidant to donate electron to Fe (III) resulting in the reducing of Fe^{3+}/ferricyanide complex to Fe^{2+} complex, which could be monitored at 700 nm. Generally, the order of the DPPH scavenging and FRP activities displayed by the ferns were similar to that of TPC i.e. ferns with very high TPC (AEAC 2821 – 3200 mg AA/100g and FRP 963 – 1466 mg GAE/100g) > ferns with high TPC (AEAC 1438 – 1645 mg

AA/100g and FRP 578 – 842 mg GAE/100g) > ferns with moderate TPC (AEAC 225 - 740 mg AA/100g and FRP 201 - 578 mg GAE/100g) > ferns with low TPC (AEAC 157 - 197 mg AA/100g and FRP 121-176 mg GAE/100g) (Table 1).

Table 1: DPPH scavenging activity (expressed in IC_{50} and AEAC) and ferric reducing power (FRP) of the methanolic extracts of fifteen fern species

Fern	Parts taken	DPPH scavenging activity IC_{50} (mg/ml)	DPPH scavenging activity AEAC (mg AA/100 g)	FRP (mg GAE/100g)
Ferns with very high TPC				
C. latebrosa	leaves	0.13 ± 0.03	3111 ± 722	1417 ± 268
C. barometz	leaves	0.12 ± 0.01	3200 ± 336	1466 ± 81
D. quercifolia	leaves	0.14 ± 0.03	2835 ± 448	1091 ± 107
B. orientale	leaves	0.14 ± 0.03	2821 ± 705	1089 ±157
D. linearis	leaves	0.14 ± 0.02	2866 ± 433	963 ± 63
Ferns with high TPC				
A. raddianum	leaves	0.27 ± 0.02	1438 ± 110	842 ± 46
P. vittata	leaves	0.29 ± 0.03	1645 ± 499	578 ± 97
Ferns with moderate TPC				
A. aureum	leaves	0.54 ± 0.04	724 ± 59	524 ± 20
D. esculentum	leaves	0.57 ± 0.12	700 ± 132	578 ± 49
P. calomelanos	leaves	1.20 ± 0.18	329 ± 58	364 ± 27
P. nummularifolia	leaves	1.47 ± 0.08	264 ± 15	201 ± 13
N. biserrata	leaves	0.53 ± 0.05	740 ± 71	422 ± 46
L. circinnatum	leaves	1.73 ± 0.12	225 ± 16	220 ± 9
Ferns with low TPC:				
P. venulosa	leaves	1.99 ± 0.28	197 ± 29	176 ± 12
M. punctatum	leaves	2.51 ± 0.42	157 ± 25	121 ± 26

Data are mean ± S.D of 3 samplings collected randomly in 2 batches from the same source (n=6). Triplicate measurements were conducted in each assay. TPC, total phenolic content; AEAC, ascorbic acid equivalent antioxidant capacity; FRP, ferric ion reducing power.

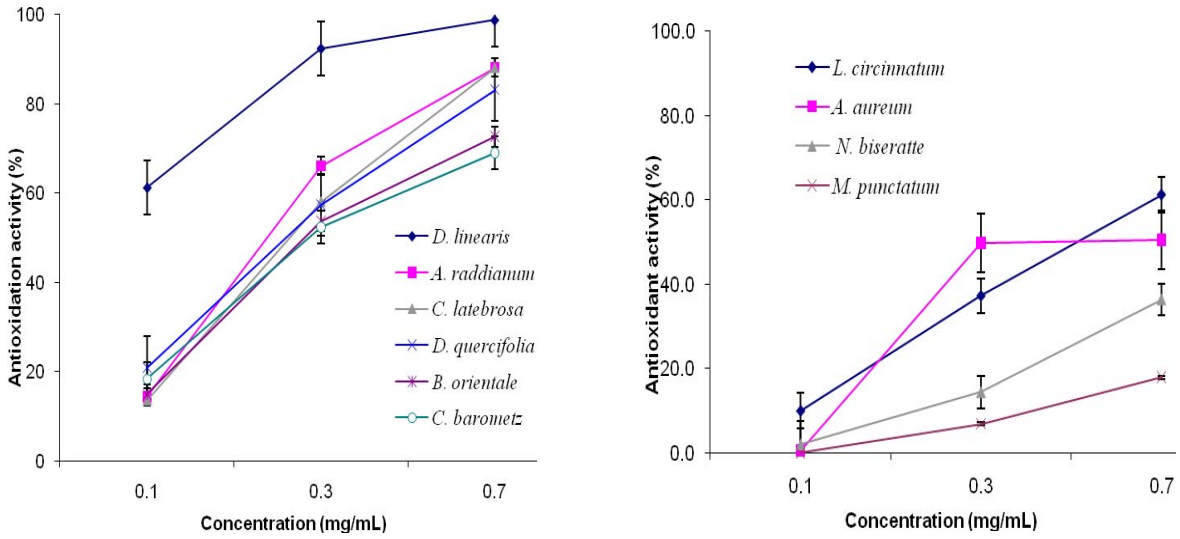

Fig. 2: β-carotene bleaching (BCB) activities of six fern species with high to very high TPC (left) and of four fern species with low to moderate TPC (right).

BCB method measured the ability of an antioxidant to inhibit lipid peroxidation. It can be observed from Fig. 2 that the BCB antioxidant activity increased with increasing concentration of the extract used. *D.*

linearis exhibited the highest BCB antioxidant activity amongst the ferns tested, with 61% at 0.1 mg/mL and extended to 99% at 0.7 mg/mL. The other ferns of high and very high TPC namely *A. raddianum*, *C. latebrosa*, *D. quercifolia*, *B. orientale* and *C. barometz* also showed strong activity of 64-88% at 0.7 mg/mL. These results implied that the potential antioxidant capabilities in *C. latebrosa*, *C. barometz*, *D. quercifolia*, *B. orientale* and *D. linearis* were attributed to the phenolic compounds in these fern species.

In conclusion, the methanol extracts of *C. latebrosa*, *C. barometez*, *D. quercifolia*, *B. orientale* and *D. linearis* showed very high total phenolic content and are potent antioxidants. Further investigative work has been carried out on *B. orientale* and is reported elsewhere [15].

4. Acknowledgements

The authors would like to thank the administrators of Putrajaya Botanical Garden, Kuala Lumpur for the kind contribution of ferns. This project was conducted at Monash University Sunway campus. The financial assistance from the university is deeply appreciated.

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