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Antioxidant Properties and Total Phenolic Content in Different Development Stages of *Barringtonia racemosa* and *Barringtonia spicata* Leaves

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Abstract

Barringtonia racemosa and *Barringtonia spicata* are moderate sized trees that can be found in Southeast Asia. They are medicinal plants that have been used in traditional practice to treat itching, high blood pressure, and as a depurative, by using their leaves. Due to the healing properties of the leaves, it is important to determine the antioxidant properties and total phenolic content from the different developmental stages of the leaves. It was assumed that the young leaves have a higher activity than mature ones. The total phenolic content (TPC) of the plant extract was determined alongside the free radical scavenging ability using a 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay, and also the capacity of the extracts to inhibit lipid peroxidation by using a ferric thiocyanate (FTC) and thiobarbituric (TBA) assay. The highest TPC was observed in the methanolic extracts of the stage 1 leaves of *B. racemosa* (0.34 mg GAE/g DW) and *B. spicata* (0.19 mg GAE/g DW). The highest scavenging activity was shown in the methanolic extract from the stage 3 leaf of *B. racemosa*, with IC_{50} at 33.33 µg/ml. In *B. spicata*, it was shown in stage 4, at 42.33 µg/ml, as compared to the chloroform extraction. All extracts actively inhibited lipid peroxidation with an amount of more than 88 % inhibition. The healing properties of *Barringtonia* could come from their high antioxidant activities. More detailed study will be required to identify which of the active compounds are present in the leaves that act as antioxidant compounds.

Keywords: *Barringtonia*, free radical scavengers, lipid peroxidation, total phenolic contents

Introduction

Normally, the human body maintains a balance between the amount of antioxidants and oxidant initiators by simultaneously producing both, through metabolic processes. When imbalances of these two compounds are present, an oxidative stress can happen [1]. It is a deleterious process that can damage the structure of cells, including lipids, proteins and DNA [2]. Damaged cells have been implicated in several diseases, including aging, cancer, diabetes, cardiovascular disease, inflammation, and decline in the function of the immune system [3,4]. Natural antioxidants that are present in plants, like phenolics, play important roles in inhibiting free radicals and oxidative chain reactions within tissues and membranes [5,6]. The total phenolic content is also a good indicator of antioxidant properties, and studies have reported a high correlation between antioxidant capacity and total phenolic content [7].

Barringtonia racemosa and *Barringtonia spicata* leaves are widely used to treat ailments besides being consumed as a traditional vegetable amongst the people of Southeast Asia [8]. The *B. racemosa* leaves are elliptic with serrated margins, and the leaf bases are crenate, with the opposite leaf arrangement. The flowers are white to pale pink, and hang in racemes from the branches [9]. The fruits are reddish brown, ovoid, slightly four-angled, and crowned by the remains of the persistent calyx [10]. The leaves of

B. spicata are elliptic, with serrate-crenulated margins with the apex rounded, and the base narrowed into a short petiole. The flowers are dark scarlet and pendulous, and the fruits are ovoid, acutely angled to almost globular, and are four or eight winged [11]. Based on the descriptions above, these two species can be distinguished by their flower and fruit characteristics. According to [12], alcohol and water extracts of *B. racemosa* bark are toxic to aphis. The ethanol and chloroform extract of *B. racemosa* roots showed antibacterial activity [13]. The bark has been claimed to be specifically beneficial for gastric ulcers [14]. Chloroform extract of *B. racemosa* leaves also showed anti-inflammatory activities [15].

Most of the studies in evaluation of the antioxidant activity of leaf extract did not mention whether it was taken from an old or young leaf. The biological activity of the leaf can sometimes differ based on the developmental stage; hence, it can give a variety of results [16]. Furthermore, young leaves are consumed as a salad by some communities [10]. The type of solvent used in the extraction method could also affect the results [15,17,18]. Consequently, it is important to study the amount of phenols and the antioxidant potential of different leaf development stages, to find out whether they differ based on their stages, and also to compare the antioxidant activity from the methanol and chloroform extracts. The antioxidant activity of the leaves was evaluated by their ability to scavenge free radicals using a DPPH assay, and the capacity of extracts to inhibit lipid peroxidation was evaluated by ferric thiocyanate (FTC) and thiobarbituric (TBA) assay. We hypothesized that different stages of leaves will have different antioxidant properties.

Materials and methods

Plant materials

The leaves were obtained from the farm of the Malaysian Agricultural Research and Development Institute (MARDI)'s experimental station in Jerangau, Terengganu. The young leaves were divided into five development stages: stage one refers to the first leaf from the shoot tip, followed by second leaf until the fifth leaf stage (**Figure 1**).

Figure 1 Young leaves of *B. racemosa*; S1: Stage 1 of leaf; S2: Stage 2 of leaf; S3: Stage 3 of leaf; S4: Stage 4 of leaf; S5: Stage 5 of leaf.

Extraction of extracts

The dried leaves (± 5 g) were macerated with 500 ml of methanol (Fisher, USA) at room temperature for three days. The solvent was then filtered and evaporated in a rotary evaporator (Buchi, Switzerland) under vacuum at 40 °C. The crude extract was stored and protected from light and kept in a refrigerator at

4 °C until used for assays. The above procedure was repeated using chloroform (Fisher, USA) as a solvent. These crude extracts were dissolved in methanol and used for the assessment of antioxidant activity.

Total phenolic content (TPC) assay

The amounts of total phenolics in the methanol and chloroform extracts were determined using Folin-Ciocalteu's reagent (Sigma-Aldrich, USA) according to the method of [19] with some modifications. The extracts (0.5 ml) and gallic acid (Sigma-Aldrich, USA) (as standard in various concentrations) were mixed with 2.5 ml of Folin-Ciocalteu's reagent (diluted 1:10) and 2 ml of 7.5 % of sodium carbonate in test tubes. The mixture was incubated at room temperature for 90 min, and absorbance was measured at wavelength 765 nm with a UV-Vis spectrophotometer (Shimadzu Corporation, Japan). The amount of total phenolic compounds was calculated as mg of gallic acid equivalents (GAE) from the calibration curve and expressed as mg gallic acid equivalent/g dry weight of the plant material. The data were presented as the average of triplicate analyses.

Free radical scavenging ability onto 1,1-diphenyl-2-picrylhydrazyl (DPPH)

The scavenging activity of extracts against DPPH was evaluated spectrophotometrically by a slightly modified method of [20] with adaptation on the micro-plate reader. The reaction mixtures consisted of 100 µl of 0.3 mM DPPH (Sigma-Aldrich, USA), and 50 µl of leaf extracts and standards were incubated for 30 min. The remaining DPPH was measured for their absorbance value by micro-plate reader (Infinite M 200, Tecan, Switzerland) at 517 nm. The data were presented as the average of triplicate analyses. The concentrations of each sample reaction for 50 % scavenging of DPPH free radicals (IC_{50}) were determined graphically by plotting the inhibition percentage of DPPH free radicals. The inhibition of DPPH free radicals in percentage (I %) was calculated as given below;

$$I\ \% = [(Abs_{control}/Abs_{sample})/Abs_{control}] \times 100 \quad (1)$$

Ferric Thiocyanate (FTC) Assay

The assay was conducted in a linoleic acid emulsion to determine the amount of peroxide at the initial stage of lipid peroxidation. As described by [21], a mixture of 4 mg of sample was placed in 4 ml of ethanol (Merck, Germany); 4.1 ml of 2.52 % (v/v) of linoleic acid (Sigma-Aldrich, USA) in ethanol, 8 ml of 0.05 M phosphate buffer (pH 7.0), and 3.9 ml of water was placed in a vial and then placed in an oven at 40 °C in the dark. The same reaction medium, without any additive of the sample, was used as a control.

To 0.1 ml of this solution, 9.7 ml of 75 % (v/v) of ethanol and 0.1 ml 30 % (w/v) of ammonium thiocyanate (Sigma-Aldrich, USA) were added. Exactly 3 min after the addition of 0.1 ml of 0.02 M ferrous chloride (Sigma-Aldrich, USA) in 3.5 % (v/v) of hydrochloric acid (Fisher, USA) to the reaction mixture, the absorbance was measured at 500 nm with a UV-Vis spectrophotometer (Shimadzu Corporation, Japan) every 24 h, until the absorbance of the control reached maximum. The inhibition percent of linoleic acid peroxidation was calculated the same way as the DPPH assay. The data were presented as the average of triplicate analyses.

Thiobarbituric acid (TBA) assay

The method according to [21] was applied to determine the formation of malonaldehyde. One ml of the sample solution was prepared and incubated, as in the FTC method. A volume of 2 ml of 20 % (w/v) of trichloroacetic acid (Sigma-Aldrich, USA) and 2 ml TBA aqueous solution were added to 1 ml of sample. The mixture was then boiled for 10 min. After cooling at room temperature, it was centrifuged at 3,000 rpm for 20 min (Refrigerated Centrifuge, Sigma), and the absorbance of the supernatant was measured at a wavelength of 532 nm. Antioxidant activity was determined based on the absorbance on the final day of the FTC assay. The inhibition percentage of linoleic acid peroxidation was calculated the same way as the DPPH assay. The data were presented as the average of triplicate analyses.

Results

Total phenolic compound assay

Plant phenolics are the major group of compounds acting as primary antioxidants or free radical scavengers and, therefore, it was reasonable to determine the total phenolic compounds in the plant extract [22]. The highest levels of total phenolic compounds were detected in stage 1 of leaves for both species in methanol extract (**Table 1**). The decline level of the total phenolic compounds started from stage 2 to stage 5, indicating the beginning of leaf senescence. In methanolic extracts of *B. racemosa*, the stage 1 leaf gave the highest total phenolic content ,which is 0.34 mg/g DW, followed by stage 2 (0.22 mg/g DW) and stage 3 (0.11 mg/g DW). Phenolic content remained unchanged in stage 4 and 5 leaves, with the amount of phenolic compounds at 0.08 mg/g DW. An almost similar trend was observed in *B. spicata* methanol extract, where stage 1 was the highest TPC value with 0.19 mg/g DW, followed by the same TPC levels in stage 3 and stage 4 (0.15 mg/g DW). The phenolic content declined in stage 2 (0.10 mg/g DW), and the lowest amount of phenolic compounds was observed in stage 5 (0.07 mg/g DW).

The amount of total phenolic compounds was found to be very low in chloroform extracts. The total phenolic compounds for *B. racemosa* in the stage 5 leaf had the highest TPC values (0.018 mg/g DW). The stage 2 leaf gave the second highest, with 0.016 mg/g DW, followed by stage 1 (0.013 mg/g DW), stage 4 (0.010 mg/g DW), and with the lowest value in stage 3 (0.009 mg/g DW). For *B. spicata*, the middle stage of the leaf (3) gave the highest level of total phenolic compounds (0.023 mg/g DW), followed by stage 1 and 4, with the same amount (0.022 mg/g DW). The stage 2 leaf gave 0.02 mg/g DW, and the lowest value was in the stage 5 leaf, which was 0.013 mg/g DW. Generally, these results showed that the total phenolic compounds was higher in the methanol extract which had a solvent polarity higher than chloroform.

Table 1 Total phenolic compounds of *B. racemosa* and *B. spicata* leaves. Results are expressed as mg of gallic acid equivalents (GAE) per g of dried weight (mg/g DW).

Leaf stages	*B. racemosa*		*B. spicata*	
	Methanol	**Chloroform**	**Methanol**	**Chloroform**
Stage 1	$0.34\pm0.01^{c,3}$	$0.013\pm0.01^{a,b,1}$	$0.19\pm0.06^{c,2}$	$0.022\pm0.01^{c,1}$
Stage 2	$0.22\pm0.08^{b,2}$	$0.016\pm0.01^{b,c,1}$	$0.10\pm0.05^{a,b,1}$	$0.020\pm0.01^{b,1}$
Stage 3	$0.11\pm0.01^{a,2}$	$0.009\pm0.01^{a,1}$	$0.15\pm0.01^{b,c,3}$	$0.023\pm0.01^{c,1}$
Stage 4	$0.08\pm0.01^{a,2}$	$0.010\pm0.01^{a,b,1}$	$0.15\pm0.01^{b,c,3}$	$0.022\pm0.01^{c,1}$
Stage 5	$0.08\pm0.00^{a,4}$	$0.018\pm0.01^{c,2}$	$0.07\pm0.00^{a,3}$	$0.013\pm0.01^{a,1}$

Values with different letters in the same column were significantly different ($p < 0.05$) between stages of leaf. Values with different numbers in the same row were significantly different ($p < 0.05$) between species and solvents. Values represent the mean ± SD according to Duncan's Multiple Range Test.

Free radical scavenging ability onto 1,1-diphenyl-2-picrylhydrazyl (DPPH)

In this assay, DPPH acts as free radical, and the capacity of the plant extract to scavenge the DPPH will be a measurement of the antioxidant activity. The result was expressed in inhibition percentage (I %) of the sample to scavenge free radicals, and also half maximal inhibitory concentration (IC_{50}) (**Table 2**). The highest antioxidant activity for *B. racemosa* was shown in the stage 5 leaf from methanol extract, with 93.68 % and, for *B. spicata*, it was observed in stage 1 (92.24 %) from methanol extract. In contrast, chloroform extraction of leaves gave a very low antioxidant activity, which was below 20 % (data not shown). When compared by IC_{50}, the highest scavenging activity was shown by the stage 3 leaf of *B. racemosa* (33.33 µg/ml) and the stage 4 leaf of *B. spicata* (42.33 µg/ml). Some extracts showed higher activity than the standard, which were ascorbic acid (42.0 µg/ml), quercetin (25.33 µg/ml), and BHA (34.67 µg/ml), the figures indicate a high potential scavenging activity of these extracts.

Table 2 Inhibition percentage (I %) and IC_{50} (µg/ml) of free radical radical scavenging activities from DPPH assay in *B. racemosa* and *B. spicata* leaf extracts at the 500 µg/ml.

Samples	*B. racemosa*		*B. spicata*		Quercetin	
	I %	IC_{50}	I %	IC_{50}	I %	IC_{50}
Stage 1	93.14±0.03^{c}	36.00±2.00^{a}	92.24±0.22^{b,c}	44.33±0.58^{a,b}	92.84±0.09	25.33±1.15
Stage 2	92.92±0.07^{b,c}	47.33±0.58^{b}	92.45±0.07^{c,d}	47.67±0.58^{b,c}	**Ascorbic acid**	
Stage 3	92.69±0.15^{a,b}	33.33±4.16^{a}	92.73±0.06^{e}	50.67±2.31^{c}	93.36±0.13	42.00±2.65
Stage 4	92.39±0.39^{a}	35.00±1.73^{a}	91.98±0.25^{a,b}	42.33±2.52^{a}	**BHT**	
Stage 5	93.68±0.29^{d}	35.33±2.31^{a}	91.92±0.11^{a}	60.67±2.31^{d}	92.91±0.74	34.67±0.58

Values represent the mean of triplicate ± SD according to Duncan's Multiple Range Test. Values with different letters in the same column were significantly different ($p < 0.05$).

Ferric thiocyanate (FTC)

The FTC assay was used to measure the amount of peroxide produced at the initial stage of linoleic acid peroxidation. The peroxides react with ferrous chloride ($FeCl_2$) to form a reddish ferric chloride ($FeCl_3$) that will react with ammonium thiocyanate to produce ferric thiocyanate. Low absorbance value corresponds to the high inhibition percentage of lipid peroxidation. In **Figure 2**, the absorbance values of *Barringtonia* extracts were comparatively lower than the no added sample assay (control), but higher than that of BHT. The absorbance values of the control reached the maximum on day 6 of incubation (2.086 Abs), so the percentage inhibition of lipid peroxidation was calculated on day 6 (**Table 3**). As shown in **Table 3**, for *B. racemosa* methanolic extract, the highest percentage of lipid peroxidation inhibition was shown in stage 5 (93.83 %). For chloroform extract, the highest percentage of lipid peroxidation inhibition was shown in stage 1 (92.78 %). Both extracts gave no significant results. It might be because of the developmental stage of leaf from stage 1 till 5 in *B. racemosa* gave almost the same rate of antioxidant activity. The highest percent inhibition also came from the stage 5 leaf in *B. spicata* methanolic extract (94.41 %), and in chloroform extract, the stage 2 leaf gave the highest percent inhibition (94.55 %). Although the results were not significantly different in antioxidant activity between the development stages of the leaf. Generally, each extract showed very strong antioxidant activity, with the percent inhibition ranging from 88.59 to 94.55 %.

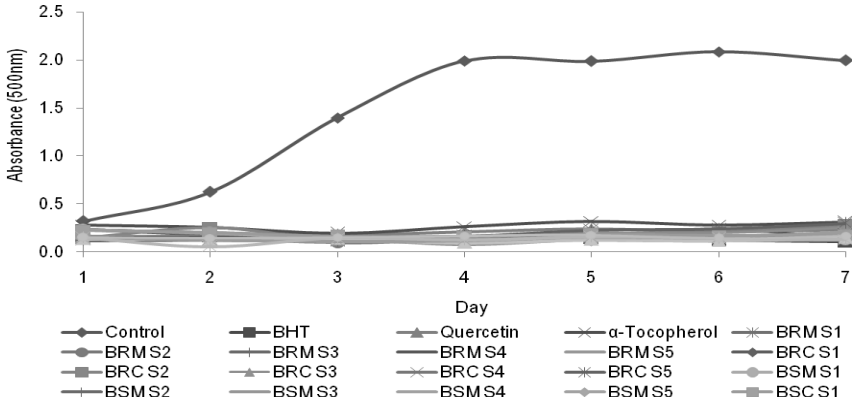

Figure 2 Absorbance value at 500 nm of methanol and chloroform extracts in *B. racemosa* and *B. spicata* leaves as measured by the FTC assay against day of incubation. Values represent the mean of triplicate ± SD according to Duncan's Multiple Range Test. BRM S: *B. racemosa* methanol stage; BRC S: *B. racemosa* chloroform stage; BSM S: *B. spicata* methanol stage; BSC S: *B. spicata* chloroform stage; BHT: butylated hydroxyl toluene.

Table 3 Percentage inhibition of lipid peroxidation as measured by the FTC method.

	B. racemosa		***B. spicata***	
Leaf stage	**Methanol**	**Chloroform**	**Methanol**	**Chloroform**
Stage 1	93.13±0.37	92.78±0.78	93.72±0.41	91.79±0.76
Stage 2	93.34±0.81	89.60±0.75	94.39±0.00	94.55±0.37
Stage 3	93.40±0.12	90.64±0.73	94.18±0.45	92.65±0.31
Stage 4	88.59±0.71	91.63±0.31	93.61±0.75	93.14±0.14
Stage 5	93.83±0.49	88.59±0.49	94.41±0.85	93.27±0.88
Standards	BHT	Quercetin	α-tocopherol	
	94.14±0.26	89.76±0.57	86.66±3.05	

Values represent the mean of triplicate ± SD according to Duncan's Multiple Range Test. I % was calculated from absorbance reading on day 6.

Thiobarbituric acid (TBA) assay

The thiobarbituric acid method was applied to measure the secondary stage of linoleic acid peroxidation, where the peroxide decomposed into malondialdehyde [23]. The malondialdehyde bound TBA to form a red complex, and the relative concentrations were measured spectrophotometrically [15]. Low absorbance values corresponded to the low amount of malondialdehyde produced, which indicated a high inhibition of lipid peroxidation. **Figure 3** shows the absorbance values on day seven of incubation, with the absorbance values of *Barringtonia* extracts comparatively lower than quercetin and α-tocopherol.

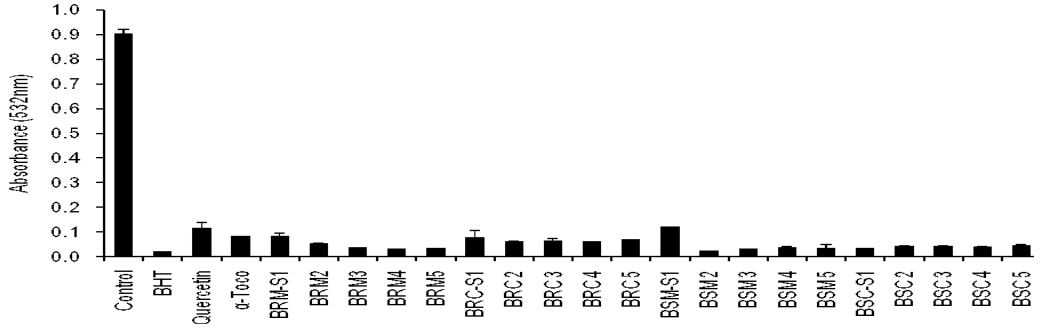

Figure 3 Absorbance value of methanol and chloroform extracts in *B. racemosa* and *B. spicata* leaves as measured by the TBA method. Absorbance values and bars represent the mean ± SD according to Duncan's Multiple Range Test. BRM S: *B. racemosa* methanol stage; BRC S: *B. racemosa* chloroform stage; BSM S: *B. spicata* methanol stage; BSC S: *B. spicata* chloroform stage, BHT: Butylated-hydroxytoluene; α-toco: alpha-tocopherol.

As shown in **Table 4**, the inhibition percentage of lipid peroxidation of most samples were very high, where most samples showed higher than 90 % inhibition. The antioxidant activity of most extracts in this assay was higher than that of quercetin and α-tocopherol. In *B. racemosa* methanolic extracts, the highest antioxidant activity occurred in stage 4 leaves (96.75 %), and for chloroform extracts, in stage 2 (93.50 %). There were no significant differences in the antioxidant activity between leaves in development stage 4 and stage 2 of *B. racemosa*. In *B. spicata,* the stage 2 leaf from methanol extraction showed the highest antioxidant activity (97.56 %), and in chloroform extracts, it was shown by stage 1 leaves (96.51 %). There were no significant differences in the antioxidant activity between leaves in development stage

1 and stage 2 of *B. spicata*. The extraction by chloroform showed markedly higher linoleic peroxidation activity compared to the methanol extraction.

Table 4 Comparison of percentage inhibition of lipid peroxidation as measured by the thiobarbituric acid (TBA) assay.

Leaf Stages	*B. racemosa*		*B. spicata*	
	Methanol	**Chloroform**	**Methanol**	**Chloroform**
Stage 1	91.09±1.83	91.46±0.22	86.75±3.27	96.51±0.28
Stage 2	94.42±0.51	93.50±0.17	97.56±0.55	95.34±0.29
Stage 3	96.23±0.22	93.20±0.23	96.71±1.35	95.38±0.34
Stage 4	96.75±0.17	93.39±0.82	96.19±0.26	95.75±0.34
Stage 5	96.49±0.23	92.50±1.78	96.34±0.22	95.12±0.55
Standards	BHT	Quercetin	α-tocopherol	
	98.00±0.01	87.31±2.72	91.08±0.28	

Values represent the mean of triplicate ± SD according to Duncan's Multiple Range Test.

Discussion

Rapid growth in earlier developmental stages of leaves may be the reason for the high rate of biosynthesis and accumulation of secondary metabolites such as phenolics and flavonoids [16,24-26]. As a result, the highest total phenolic compounds were detected in the stage 1 leaf for both species, and declined with increasing stage of the leaf. Besides that, according to [27,28], different sample extraction techniques will also influence the results of TPC. For example, [29] carried out the extraction of *B. racemosa* leaves by reflux, and it gave a higher total phenolic content compared to the present study. By referring to **Table 1**, methanol extraction gave a higher total of phenolic compounds compared to chloroform. This is because the methanol has the ability to inhibit the reaction of polyphenol oxidase that causes oxidation of phenolics, is easier to evaporate, and gives a higher crude extraction efficiency [30,31].

Previous studies reported that more polar solvents used in sample extraction gave higher antioxidant activity [32,33] because the change in solvent polarity alters its ability to dissolve a selected group of antioxidant compounds and influences the antioxidant activity estimation [34]. The present results from **Tables 2, 3** and **4** show that the highest inhibition scavenging activity was from methanol extraction as compared to chloroform in both *Barringtonia*. The scavenging activity in the *Barringtonia* did not correlate with the amount of total phenolic compounds; in the *B. racemosa* methanolic extracts, the highest activity was observed in the stage 5 leaf, but it possessed the lowest phenolic compounds. The same pattern was also observed in *B. spicata* in the stage 3 leaf. In the FTC and TBA assay, generally, methanolic and chloroform extract of *Barringtonia* showed higher inhibition of linoleic acid peroxidation activity compared to the quercetin and α-tocopherol. High percentages of lipid peroxidation inhibition for all the extracts tested indicate that the *Barringtonia* extracts tested contain antioxidant constituents that could inhibit lipid peroxidation.

Solvents in different polarities are used for extraction of bioactive compounds from plants, and the most common solvent used is methanol. In this study, methanol extract exhibits scavenging and lipid peroxidation inhibition activity, but for chloroform extract only shows scavenging activity. Various extracts and fractions could contain different antioxidant compounds, which demonstrate varying reactivity in the three *in vitro* antioxidant activity assays used in this study. This may be due to the presence of several types of compounds, belonging to different classes, such as more polar thermo stable phenolics in the fractions of methanol extract, oleoresins in hexane extract, or flavonoids in dichloromethane extract, respectively [23]. The same situation was also observed in black tea and mate tea, where extracts using high polarity solvents showed more effective radical scavenger activity then those using less polarity [35]. HPLC analysis found that methanol extract of *B. racemosa* contains gallic

acid and ferrulic acid, a phenolic compound, and four different flavonoids, which are naringin, rutin, luteolin and kaempferol [36]. There was a report of determination of lycopene as the active compound in the *B. racemosa* leaf [15]. The identification of active compounds from previous studies clearly indicates that the *Barringtonia* species is a rich source of phytonutrient.

Conclusions

The highest total phenolic content (TPC) was shown in the methanolic extract of the stage 1 leaves of *B. racemosa* and *B. spicata*. Methanolic extract gave the highest free radical scavenging ability, from the stage 2 leaf of *B. racemosa* and the stage 3 leaf of *B. spicata.* All extracts were active in inhibit lipid peroxidation, with values above 88 %. This paper has partially identified the antioxidant activity in different stages of leaves by the assays, but a more detailed study will be required to identify which of the active compounds are more present in the leaves. A more thorough investigation has to focus on the amount of active compounds and the availability of relevant toxicity data in order to evaluate the medicinal potential, as it is easily accessible as a source of natural antioxidant supplement.

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References

[1] GRMM Haenen, MJTJ Arts, A Bast and MD Coleman. Structure and activity in assessing antioxidant activity *in vitro* and *in vivo*: A critical appraisal illustrated with the flavonoids. *Environ. Toxicol. Phar.* 2006; **21**, 191-8.

[2] H Ebrahimzadeh, E Vatankhah and V Niknam. Activity of antioxidant enzyme during *in vitro* organogenesis in *Crocus sativus*. *Pak. J. Pharm. Sci.* 2010; **54**, 509-14.

[3] Z Mighri, O Boussaada, J Chriaa, R Nabli, S Ammar, D Saidana, MA Mahjoub, I Chraeif and AN Helal. Antimicrobial and antioxidant activities of methanol extracts of *Evax pygmaea* (Asteraceae) growing wild in Tunisia. *World J. Microb. Biot*. 2008; **24**, 1289-96.

[4] R Tsao and Z Deng. Separation procedures for naturally occurring antioxidant phytochemicals. *J.Chromatogr. B*. 2008; **812**, 85-99.

[5] N Ozsoy, A Can, R Yanardag and N Akev. Antioxidant activity of *Smilax excelsa* L. leaf extracts. *Food Chem.* 2008; **110**, 571-83.

[6] RY Nsimba, H Kikuzaki and Y Konishi. Antioxidant activity of various extracts and fractions of *Chenopodium quinoa* and *Amaranthus* spp. seeds. *Food Chem.* 2008; **106**, 760-6.

[7] J Lako, VC Trenerry, M Wahlqvist, N Wattanapenpaiboon, S Sotheeswaraan and R Premier. Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods. *Food Chem.* 2007; **101**, 1727-41.

[8] H Burkill. *A Dictionary of the Economic Products of the Malay Peninsula*. 2nd ed. Ministry of Agriculture and Cooperatives, Kuala Lumpur, 1966, p. 212-4.

[9] TC Whitmore. *Tree Flora of Malaya, a Manual for Foresters*. Vol II. Longman, Kuala Lumpur, 1983.

[10] S Joseph, M Sugumaran and KLW Lee. *Herbs of Malaysia - An Introduction to the Medicinal, Culinary, Aromatic and Cosmetic Use of Herbs*. *In*: KM Wong (ed.). Federal Publications Sdn. Bhd. Kuala Lumpur, 2005, p. 455-8.

[11] TC Whitmore. *Tree Flora of Malaya, a Manual for Foresters*. Vol II. Longman Press, Kuala Lumpur, 1983, p. 324-5.

[12] TJ Thomas, B Panikkar, A Subramoniam, MK Nair and KR Panikkar. Antitumour property and toxicity of in *Barringtonia racemosa* (Roxb.) seed extract in mice. *J. Ethanopharmacol.* 2002; **82**, 223-7.

[13] S Khan, A Jabbar, CM Hasan and MA Rashid. Antibacterial activity of *Barringtonia racemosa*. *Fitoterapia* 2001; **72**, 162-4.
[14] S Deraniyagala, WD Ratnasooriya and CL Goonasekara. Antinociceptive effect and toxicological study of the aqueous bark extract of *Barringtonia racemosa* on rats. *J. Ethanopharmacol.* 2003; **86**, 21-6.
[15] M Behbahani, AM Ali, R Muse and NB Mohd. Antioxidant and anti-inflammatory activities of leaves of *Barringtonia racemosa*. *J. Med. Plants Res.* 2007; **1**, 95-102.
[16] D Bhakta and D Ganjewala. Effect of leaf positions on total phenolics, flavonoids and proanthocyanidins content and antioxidant activities in *Lantana camara* (L). *J. Sci. Res*. 2009; **1**, 363-9.
[17] Y Li, J Gao and S Fei. Characterisation, extraction efficiency, stability and antioxidant activity of phytonutrients in *Angelica keiskei*. *Food Chem.* 2009; **115**, 227-32.
[18] CD Stalikas. *Phenolic Acids and Flavonoids: Occurrence and Analytical Methods*. *In*: RM Uppu (ed.). Free Radicals and Antioxidant Protocols, Methods in Molecular Biology. Humana Press, 1998.
[19] K Slinkard and VL Singleton. Total phenol analyses: Automation and comparison with manual methods. *Am. J. Enol. Vitic.* 1977; **28**, 49-55.
[20] M Habsah, M Amran, MM Mackeen, NH Lajis and H Kikuzaki. Antibacterial and DPPH free radical-scavenging activities of methanolic extracts of *Aaptos* sp. (marine sponges). *Pertanika J. Trop. Agric. Sci*. 2009; **32**, 43-50.
[21] R Ahmad, AM Ali, DA Israf, NH Ismail, K Shaari and NH Lajis. Antioxidant, radical-scavenging, anti-inflammatory, cytotoxic and antibacterial activities of methanolic extracts of some *Hedyotis* species. *Life Sci.* 2005; **76**, 1953-64.
[22] GA Ayoola, AD Folawewo, SA Adesegun and OO Abioro. Phytochemical and antioxidant screening of some plants of Apocynaceae from South West Nigeria. *Afr. J. Plant Sci. Biotech.* 2008; **2**, 124-8.
[23] K Saha, NH Lajis, DA Israf, AS Hamzah, S Khozirah, S Khamis and A Syahida. Evaluation of antioxidant and nitric oxide inhibitory activities of selected Malaysian medicinal plants. *J. Ethnopharmacol*. 2004; **92**, 263-7.
[24] N Wu, K Fu, YJ Fu, YG Zu, FR Chang, Y Chen, XL Liu, Y Kong, W Liu and CB Gu. Antioxidant activities of extracts and main components of pigeonpea (*Cajanus cajan* (L.) Millsp.) leaves. *Molecules* 2009; **14**, 1032-43.
[25] SY Wang and H Lin. Antioxidant activity in fruits and leaves of blackberry, raspberry and strawberry varies with cultivar and developmental stage. *J. Agric. Food Chem*. 2000; **48**, 140-6.
[26] P Achakzai, A Kabir, K Achakzai, A Masood, SA Kayani and RB Tareen. Response of plant parts and age on the distribution of secondary metabolites on plants found in Quetta. *Pak. J. Bot.* 2009; **41**, 2129-35.
[27] N Huda-Faujan, A Noriham, AS Norrakiah and AS Babji. Antioxidant activity of plants methanolic extracts containing phenolic compounds. *Afr. J. Biotechnol.* 2009; **47**, 484-9.
[28] H Nurul-Mariam, M Radzali, R Johari, A Syahida and M Maziah. Antioxidant activities of different aerial parts of putat (*Barringtonia racemosa* L.). *Malaysian J. Biochem. Mol. Biol.* 2008; **16**, 15-9.
[29] K Kamarul-Rahim, AA Azlim-Almey, C Ahmed-Jalal-Khan, I Syed-Zahir, K Mustapha-Suleiman and MR Aisyah. Total phenolic content and primary antioxidant activity of methanolic and ethanolic extracts of aromatic plants leaves. *Int. Food Res. J.* 2010; **17**, 1077-84.
[30] A Moure, JM Cruz, DD Franco, J Sineiro, H Dominguez, MJ Núñez and JC Parajó. Natural antioxidants from residual sources. *Food Chem.* 2001; **72**, 145-71.
[31] Y Baravalia, M Kaneria, Y Vaghasiya, J Parekh and S Chanda. Antioxidant and antibacterial activity of *Diospyros ebenum* (Roxb.) leaf extracts. *Turk J. Biol*. 2009; **33**, 159-64.
[32] SA Dias, FP Cardoso, SMO Santin, WF da Costa, GJ Vidotti, MC de Souza and MH Sarragiotto. Free radical scavenging activity and chemical constituents of *Urvillea ulmaceae*. *Pharm. Biol*. 2009; **47**, 717-20.
[33] K Zhou and L Yu. Antioxidant properties of bran extracts from Trego wheat grown at different locations. *J. Agric. Food Chem*. 2004; **52**, 1112-7.

[34] YS Velioglu, N Turkmen and F Sari. Effects of extraction solvents on concentration and antioxidant activity of black and black mate tea polyphenols determined by ferrous tartrate and Folin-Ciocalteu methods. *Food Chem.* 2006; **99**, 835-41.
[35] NM Hussin, R Muse, S Ahmad, J Ramli, M Mahmood, MR Sulaiman, MYA Shukor, FA Rahman and KNK Aziz. Antifungal activity of extracts and phenolic compounds from *Barringtonia racemosa* L. (Lecythidaceae). *Afr. J. Biotechnol.* 2009; **8**, 2835-42.