Antioxidant Activities of Different Aerial Parts of Putat (*Barringtonia racemosa* L.)

Nurul Mariam H, Radzali M, Johari R, Syahida A and Maziah M

Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor.

Abstract

The antioxidant activities of methanolic, ethanolic and boiling water extracts of *Barringtonia racemosa* leaves, sticks, and barks were studied and their contents of total phenolics, flavonoids and carotenoids were measured. Methanolic extracts of aerial parts of the plant contained relatively higher levels of total phenolics than other extracts (leaf: 16.2 ± 0.02 mg gallic acid equivalent/g freeze dried-weight (FDW) tissue, stick: 29.9 ± 0.02 mg gallic acid equivalent/g FDW tissue, bark: 21.78 ± 0.20 mg gallic acid equivalent/g FDW tissue). The ethanolic extracts in aerial parts gave higher levels of total flavonoid (leaf: 38.55 ± 2.75 mg rutin/ g FDW tissue, stick: 40.72 ± 5.91 mg rutin/g FDW tissue, bark: 68.29 ± 9.63 mg rutin/g FDW tissue). The amounts of β-carotene and lycopene were found higher in methanolic and ethanolic extracts of the leaf (342.2 ± 8.79 μg β-carotene/g FDW tissue, 77.38 ± 4.61μg lycopene/g freeze dried-weight tissue; 356.9 ± 0.93 μg β-carotene/g FDW tissue, 99.3 ± 5.29 μg lycopene/g FDW tissue, respectively). The methanolic and ethanolic extracts in all aerial parts tested exhibited very strong antioxidant properties when compared to butylated hydroxytoluene (BHT), ascorbic acid and α-tocopherol in the free radical scavenging and reducing power assays.

Keywords: *Barringtonia racemosa,* Putat, aerial parts, antioxidant.

Introduction

Locally known as 'Putat Kampung', *Barringtonia racemosa* L. (Family of Lecythidaceae) is an evergreen tree found in East Africa, South East Asia and the Pacific Islands. Ethnomedical survey has shown that the seeds of *B. racemosa* are traditionally used in certain remote villages of Kerala (India) to treat ulcer and cancer [1]. Previous studies have shown that *B. racemosa* possesses significant antifungal activity against plant pathogenic fungi, *Curvlaria sp., Colletotrichum gloeosporioides, Cylindrocladium quinqueseptatum,* and *Rigiodiporus lignosus.* The roots of *B. racemosa* showed antibacterial activity against several Gram positive and Gram negative bacteria [2]. *B. racemosa* is a rich source of phytomedicine. The barks and leaves are used for antidote to snake bites, rat-poisoning and on boils. Its seeds along with other ingredients are used in the preparations for the treatment of itch, piles and thyphoid fever while the bark is claimed to be specific for the treatment of gastric ulcers [3]. Secondary metabolites such as diterpenes, triterpenoids, flavonoids (including polyphenols), steroids and saponins have previously been isolated from *B. racemosa* [2, 3].

B. racemosa has so far not been investigated for its antioxidant properties. Antioxidants are used to preserve food quality mainly by prevention of oxidative deterioration of constituents of lipids [4]. The oxidation process can be prevented effectively by using free radical-scavenging antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate [5]. Vegetables and fruits are rich sources of antioxidants, such as vitamin A, vitamin C, vitamin E, carotenoids, polyphenolic compounds and flavonoids, which prevent the free radical damage, reducing risk of chronic diseases while the consumption of dietary antioxidants from these sources is beneficial in preventing atherosclerosis [6]. The main objective in this study was to determine and evaluate the antioxidant activities in three different aerial parts of *B. racemosa.*

Materials and Methods

Plant materials

Plant materials used in this study include fresh *B. racemosa* leaf, stick, and bark that were obtained from Kampung Kuak Luar, Pengkalan Hulu, Perak Darul Ridzuan, Malaysia. Samples were washed with running tap water and separated before being chopped into pieces. They were freeze-dried for 24 hr between -50 to -54°C and ground into powder.

Extraction of the aerial parts of Barringtonia racemosa

Two methods were used to extract the *B. racemosa* parts. The first method was by Crozier *et al.* [7], where 0.5 grams of the freeze-dried leaves, sticks and bark were extracted with 40 ml absolute methanol and ethanol. Then, 10 ml 6 M HCl was added to each extract. The mixture solution of the extract was hydrolysed by refluxing

Author for correspondence: Radzali M, Department of Biochemistry, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor; Malaysia. Fax: 03-89430913, E-mail: radzali@biotech.upm.edu.my

at 90°C for 2 hr. The hydrolysed extracts were then filtered separately and the filtered extract solution was vacuum-dried in a rotary evaporator at 40°C until all the solvents evaporated. For boiling water extraction, the modified method of Gulcin *et al.* [4] was used. Freeze-dried samples (10 g of leaves, sticks and barks) were extracted with 400 ml boiling water and incubated at 100°C for 15 min while being stirred. The extracts were then filtered separately. The filtrate was vacuum-dried in a rotary evaporator at 40°C to obtain the crude extract. All yields of crude extracts were measured as percentages relative to freeze-dried sample.

Total phenolics assay

The amount of total phenolics in the extract was determined according to the Folin-Ciocalteu's procedure [8]. Five hundred ml (three replicates) of samples and standard phenolics of each concentration were mixed with 2.5 ml of Folin-Ciocalteu's reagent (diluted 1:10, v/v) and 2 ml of 7.5% (v/v) of sodium carbonate in test tubes. Absorbance of all samples was measured at 765 nm after incubation at 30°C for 90 min. Results are expressed as mg of gallic acid equivalents (GAE) per g of freeze dried-weight (FDW) tissue.

Total flavonoids assay

Total flavonoid content was measured by the $AlCl_3$ colorimetric assay according to Marinova *et al.* [9]. An aliquot (1 ml) of extracts or standard solution of rutin was added to 4 ml of distilled water. This is followed by addition of 0.3 ml 5 % (w/v) of $NaNO_2$ and 0.3 ml 10 % (w/v) of $AlCl_3$ after 5 min. At the 6^{th} min, 2 ml 1M NaOH was added and the total volume was made up to 10 ml with distilled water. The absorbance was measured against the prepared reagent blank at 510 nm. Total flavonoid content is expressed as mg rutin per g FDW. Samples were analysed in triplicate.

Total carotenoids assay

Total carotenoid content in terms of β-carotene and lycopene were determined according to the method of Barros *et al.* [6] with a slightly modification. The dried methanolic, ethanolic and boiling water extracts were vigorously shaken with 10 ml of acetone-hexane mixture (4:6, v/v) for 1 min and filtered. Absorbance of the filtrate was measured at 453, 505, 645 and 663 nm. Assays were carried out in triplicate; the results were mean values ± standard deviations and expressed as μg of carotenoids per g FDW of extract.

DPPH radical scavenging assay

The free radical scavenging activity of extracts was measured by 1,1-diphenyl-2-picryl-hydrazyl (DPPH) using the method of Gulcin *et al.* [4]. Briefly, 0.1 mM solution of DPPH in methanol was prepared and 1 ml of this solution was added to 3 ml extracts solution in water at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Absorbance was then measured at 517 nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH radical concentration was calculated using the following equation:

$$\text{DPPH• Scavenging Effect (\%)} = [(A_0 - A_1) / A_0 \times 100]$$

where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the sample.

Reducing power assay

The reductive potential of extracts was determined according to the method of Gulcin *et al.* [4]. Different concentrations of leaves, sticks and barks (20, 40, 60, 80 μg/ml) in 1 ml of distilled water was mixed with phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and $K_3Fe(CN)_6$ (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of trichloroacetic acid (10 %) was added to the mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicated greater reductive potential.

Statistical analysis

Each experiment was performed in triplicate and repeated three times (n=3). Experimental results are expressed as means ± standard deviation (SD) of three parallel measurements.

Results and Discussion

Apparently, methanol was most effective in extracting parts of *B. racemosa* because methanol is more polar than ethanol (Polarity: Methanol = 33.6 and Ethanol = 24.3). The most polar solvent results in a greater yield extract of natural antioxidant compounds because most of them are polar compounds such as flavonoids. These observations are in agreement with that the previous report by Chang *et al.* [10] which showed that solvent with higher polarity are effective for extraction of natural antioxidants.

Phenolic compounds are important due to the ability to serve as antioxidants which are widely found in the secondary products of medicinal plants [11]. Figure 1 shows that the methanolic extracts in all aerial parts studied had greater phenolic content than the ethanolic and boiling water extracts (leaf: 16.21 ± 0.02 mg GAE/g FDW tissue; stick: 29.90 ± 0.02 mg GAE/g FDW tissue; bark: 21.78 ± 0.20 mg GAE/g FDW tissue). However, the ethanolic extracts in all aerial parts tested gave higher levels of total flavonoid (Figure 2; leaf: 38.55 ± 2.75 mg rutin/g FDW tissue, stick: 40.72 ± 5.91 mg rutin/g FDW tissue, bark: 68.29 ± 9.63 mg rutin/g FDW tissue). It is

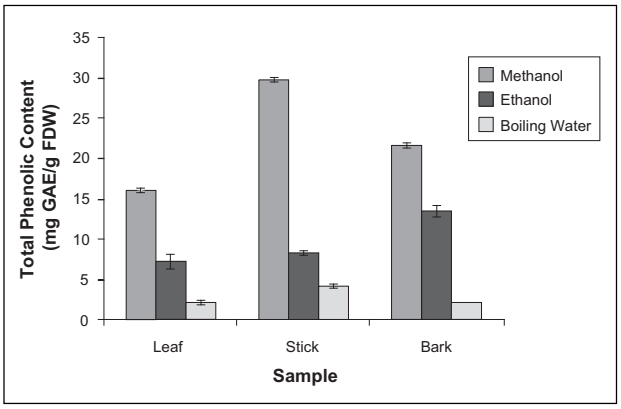

Figure 1: Total phenolic content of *B. racemosa* leaf, stick and bark extracts using methanol, ethanol and boiling water. Bars indicate standard deviation of triplicate determinations (n=3).

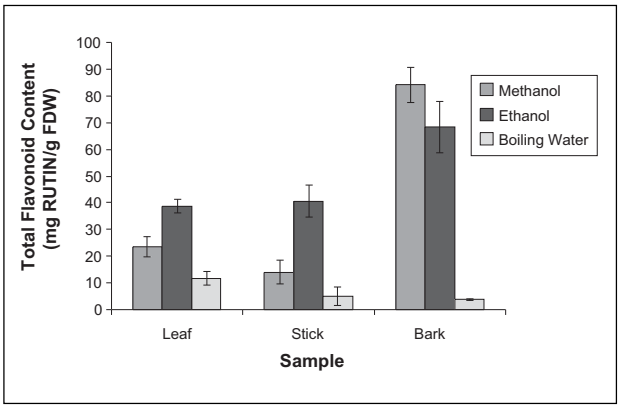

Figure 2: Total flavonoid content of *B. racemosa* leaf, stick and bark extracts using methanol, ethanol and boiling water. Bars indicate standard deviation of triplicate determinations (n=3).

interesting to note that the total flavonoid content was higher than the total phenolic content because flavonoids form the largest group among the natural phenolic compounds, of which several structures are known [6]. The higher phenol and flavonoid content found in the methanolic and ethanolic extracts may account for the better results found for their antioxidant activities compared to the boiling water extracts.

Table 1 shows the β-carotene and lycopene concentrations in the *B. racemosa* extracts. While the total phenolic and total flavonoids were the major antioxidant components mostly found in the *B. racemosa* extracts in all aerial parts, β-carotene and lycopene were found in small amounts (μg). The amounts of both carotenoid compounds were found higher in the methanolic and ethanolic extracts of leaf (342.2 ± 8.79 μg β-carotene/g FDW tissue, 74.1 ± 8.91μg lycopene/g FDW tissue; 356.9 ± 0.93 μg β-carotene/g FDW tissue, 99.3 ± 5.29 μg lycopene/g FDW tissue, respectively). Although the carotenoids were in small quantity, both compounds are potent antioxidants and singlet oxygen quenchers [12]. These compounds are known to take part in protecting animals against damage from free radicals and lycopene has been shown to possess excellent anti-inflammatory or antioxidant properties [13, 14]. The carotenoid content, especially β-carotene, was particularly high in leaves probably due to the universal presence of lutein, violaxantin and neoxanthin in the leaves of higher plants tissue [15].

Table 1: Total carotenoid content of *B. racemosa* leaf, stick and bark extracts using methanol, ethanol and boiling water. Data indicate as mean ± standard deviation of triplicate determinations (n=3).

Extracts			Total carotenoid content (μg β-carotene / g FDW)	(μg lycopene / g FDW)
Methanol	:	Leaf	342.25 ± 8.79	77.38 ± 4.61
		Stick	24.26 ± 1.41	7.53 ± 0.93
		Bark	42.11 ± 0.72	19.57 ± 0.24
Ethanol	:	Leaf	356.91 ± 0.93	99.30 ± 5.29
		Stick	39.70 ± 3.84	12.72 ± 1.17
		Bark	28.37 ± 2.48	20.31 ± 0.74
Boiling water	:	Leaf	19.91 ± 0.64	1.87 ± 0.32
		Stick	7.57 ± 0.15	0.69 ± 0.08
		Bark	10.85 ± 1.16	1.01 ± 0.29

The reduction capability of DPPH radical was determined by the decrease in absorbance at 517 nm induced by antioxidants. BHT, ascorbic acid (vitamin C) and α-tocopherol were used as standards. All the extracts tested were generally able to reduce the stable radical DPPH to yellow-coloured diphenylpicrylhydrazine. The ethanolic extract in all the aerial parts showed stronger DPPH scavenging activity rather than methanolic and boiling water extracts (Figure 3). The DPPH scavenging effect of all extracts and standards on the DPPH radical decreased in the order of: ethanolic extract of bark > ascorbic acid > methanolic extract of bark > ethanolic extract of leaf > BHT > α-tocopherol > ethanolic extract of stick > methanolic extract of leaf > boiling water of

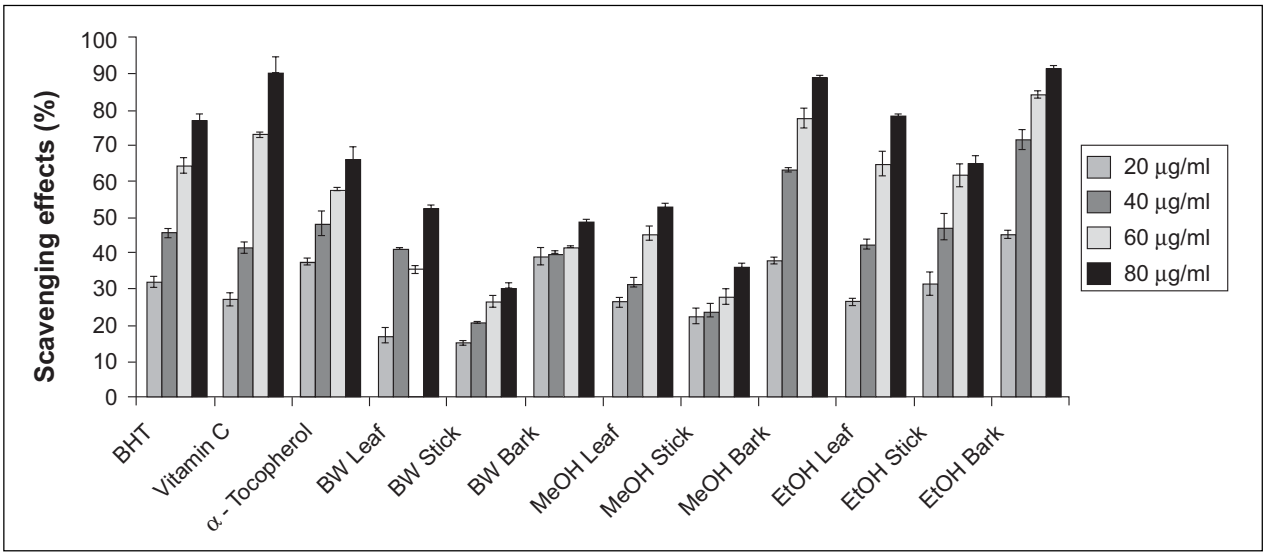

Figure 3: Free radical scavenging activity of methanol (MeOH), ethanol (EtOH) and boiling water (BW) extracts of *B.racemosa* leaf, stick, and bark parts by 1,1-diphenyl-2-picrylhydrazyl radicals (DPPH) (BHT: Butylated hydroxytoluene). Bars indicate standard deviation of triplicate determinations (n=3).

leaf > boiling water of bark > methanolic extract of stick > boiling water of stick and were 91.35%> 90.32%> 89.23%> 78.52%> 77.32%> 66.46%> 64.67%> 52.91%> 52.27%> 49.02%> 36.22% and 30.16% at the concentration of 80 µg/ml, respectively. These results indicated that all extracts of the aerial parts of *B. racemosa* have noticeable effect on scavenging free radical. Free radical scavenging activity also increased with increasing concentration.

Figure 4 shows the reductive capabilities of *B. racemosa* extracts compared to BHT, ascorbic acid, and α-tocopherol. For the measurements of the reductive ability, Fe^{3+} - Fe^{2+} transformation in the presence of *B. racemosa* extracts samples was investigated [4]. The reducing properties are generally associated with the presence of reductones that exert antioxidant action by breaking the free radical chain by donating a hydrogen atom [16]. Like the antioxidant activity, the reductive potential of all extracts in the aerial parts tested (leaf, stick, bark) increased with increasing concentration. All concentrations of *B. racemosa* extracts in leaf, stick and bark parts showed higher activities. Reductive potential of methanolic, ethanolic and boiling water extracts in leaf, stick and bark of *B. racemosa* and standard compounds followed the order: ascorbic acid (vitamin C)

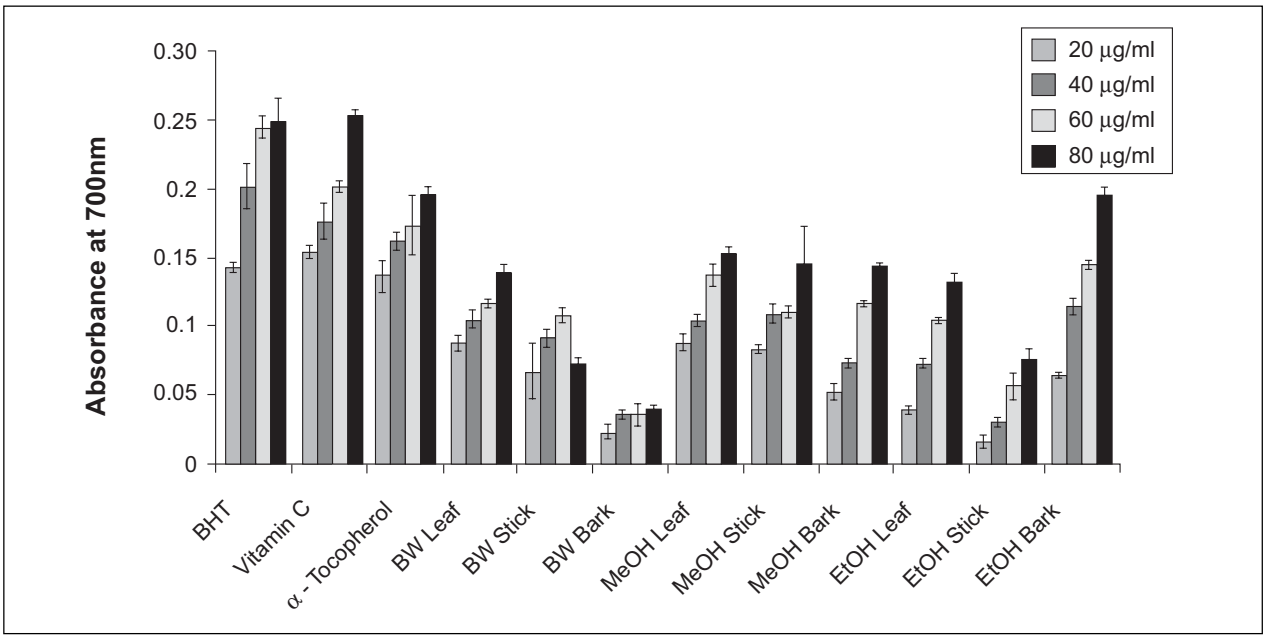

Figure 4: Reductive potential of different concentrations of methanol (MeOH), ethanol (EtOH) and boiling water (BW) extracts of *B.racemosa* leaf, stick, and bark parts, BHT, vitamin C, and α-tocopherol using spectrophotometric detection of the Fe^{3+}-Fe^{2+} transformations (BHT: Butylated hydroxytoluene). Bars indicate standard deviation of triplicate determinations (n=3).

> BHT > ethanolic extract of bark> α-tocopherol> methanolic extract of leaf> methanolic extract of stick> methanolic extract of bark> ethanolic extract of leaf> boiling water extract of leaf> ethanolic extract of stick> boiling water extract of stick> boiling water extract of bark. Compounds with reducing power indicate that they are electron donors, and can reduce the oxidized intermediates of lipid peroxidation processes and they can act as primary and secondary antioxidants [17]. The low phenolic, flavonoid and carotenoid contents as well as the low antioxidant activity detected in the boiling water extracts may be because the antioxidants have been degraded by boiling.

Conclusion

The phenolic, flavonoid and carotenoid compounds appear to be responsible for the antioxidant activities of the extracts of all aerial parts of *B. racemosa* tested in the present study. From these results it is generally clear that the methanolic and ethanolic extracts have powerful antioxidant activities against various antioxidant systems *in vitro,* and this plant can be used as an easily accessible source of natural antioxidants and as a possible food supplement or in pharmaceutical applications.

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