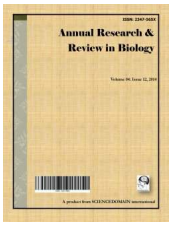

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Nutritional Composition and Antioxidant Activity of Leaves and Rhizomes of Bemban (*Donax grandis*) Grown under Glasshouse Conditions

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Authors' contributions

This work was carried out in collaboration between both authors. Author MHI designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author NAMZ managed the analyses and literature searches of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of this study is to find out the results of proximate analysis, antioxidant activity and mineral composition of leaves and rhizomes of *Donax grandis* (locally known as Bemban), an underexploited herbaceous plant that is usually used to cure shingles and snake bites in Malaysia.
Study Design: The rhizomes of *D. grandis* were purchased from Sungai Lembing, Pahang and were propagated for two months in the glasshouse. The seedlings were then planted in soilless medium containing coco-peat, burnt paddy husk and well composted chicken manure in 5:5:1 (v/v) ratio in 25-cm diameter polyethylene bags.
Place and Duration of Study: Study was conducted at Department of Biology, University of Putra Malaysia between June 2014 and August 2014.

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Methodology: Proximate analysis and antioxidant activity were performed using standard AOAC methods. Total phenolics and flavonoids were determined using Folin-Ciocalteu reagent and mineral contents were determined using atomic absorption spectrometry.
Results: Generally, the leaves of *D. grandis* contained the highest values in terms of proximate analysis, antioxidant activity and minerals compared to the rhizomes. The results indicated that *Donax grandis* was rich in lipids, carbohydrates and total ash. The high amount of total ash (34.21%) suggests a high-value in terms of mineral composition comprising of potassium, sodium, magnesium and calcium as the main elements. The total phenolics, flavonoids, ascorbic acid, DPPH and FRAP values in the leaves were found to be higher (7.12 mg GAE/ g dry weight, 3.21 mg rutin/g dry weight, 1.27 mg/g, 58.2% and 515.21 mmol Fe 2+/g) in comparison to the rhizomes. The DPPH and FRAP showed a strong positive correlation with total phenolics, flavonoids and ascorbic acid indicating that the antioxidant effect might be related to these secondary metabolites.
Conclusion: The present study indicated that *D. grandis* leaves would serve as a rich source of energy, antioxidants as well as micronutrients for human consumption.

Keywords: Donax grandis leaf and rhizome; proximate analysis; antioxidant activity; minerals.

1. INTRODUCTION

Free radicals are regularly and continuously produced as by-products of normal cellular metabolism [1]. The population of free radicals starts increasing due to different exogenous factors and agents that may not be completely neutralized by the built-in antioxidant system. This results in an imbalance between the production and depletion of free radicals in the body; and termed as oxidative stress [2]. Epidemiological studies have proven that oxidative stress can be related to many chronic diseases such as cancer, cardiovascular, Alzheimer's and neurodegenerative disorders [3]. To avoid the hazards associated with oxidative stress, external sources of antioxidants in the form of food supplements is required by the human body [4].

Previously, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have been used as free radical scavengers, but now their use is restricted in dietary items due to reports on their involvement in a number of chronic diseases including cancers and cardiovascular disorders [5]. As a result, plant materials have been identified and documented as promising sources of natural antioxidants [6]. Besides this, antioxidant attributes of these plant materials have been investigated as a function of growing location, species, and cultivation conditions, and noticeable differences have been observed. Now, there is ample evidence regarding variation in proximate composition, phenolics and antioxidant activity with respect to different species of plants [7-9].

The Malaysian herb *Donax grandis* or locally known as Bemban or Tongkat syaitan (Devil staff) is from the family Marantaceae. This plant is usually found in the forest floors along rivers and is usually found in wet places in secondary forests and bamboo thickets. It is widely distributed in Malaysia, Thailand, Singapore, Brunei, Philippine, Papua New Guinea and South-East Asia region. This herb has a hollow stem like bamboos with branching at each segment. The leaves are wide and large, varies between ovate to oval shape with a white flower. The fruits are rounded, smooth and green in color when unripe, and turn to yellow when they ripen. According to Ong [10] it can be propagated by seeds and rhizomes. Although Bemban has long been known in Malaysia, it is underutilized. Fatan [11] reported that *D. Grandis* rhizomes are used traditionally in Malay culture to cure shingles. Medicinally the leaves and root decoctions are used as a bath to cool the body during fever and the juice from the stem has been effectively used against snake bites. Furthermore, the poultices of leaves and stems can also be used as an eye refreshment [12].

Taxonomic variation studies have shown that *D. Grandis* leaves contain high levels of amino acids compared to others monocotyledonous plants [13]. The active compound properties of this plant have not been fully documented; however, previous screening test has shown that saponins are the main active compounds in the plant [14]. Jamaludin et al. [15] had indicated that this plant contained some degree of phenolics, flavonoids, tannins, phytosterols, steroids, terpenoids and alkaloids in the plant parts. However, data on compositional, nutritional and antioxidant potential of this plant are still lacking.

Therefore, the objective of this study was to characterize the composition, nutritional and antioxidant activity of *Donax grandis* plant parts (leaves and rhizomes) to evaluate the potential of this plant as a natural antioxidant and to promote its use for human consumption.

2. MATERIALS AND METHODS

2.1 Preparation of Plant Materials

The rhizomes of *D. grandis* were purchased from Sungai Lembing, Pahang and were propagated for two months in the glasshouse. The seedlings were planted in soilless medium containing coco-peat, burnt paddy husk and well composted chicken manure in 5:5:1 (v/v) ratio in 25-cm diameter polyethylene bags. Day and night temperatures in the greenhouse were maintained at 27–30℃ and 18–21℃, respectively, while the relative humidity was between 50 to 60%. The collected plant samples were washed under running tap water and separated to leaves and rhizomes, rinsed and oven-dried at 40℃ for 48 hour. The dry samples were ground to a fine powder, sifted in a sieve (25 mm) and stored at −15℃ in air-tight containers till further analyses. In the present study, the different plant parts of *D. grandis* (leaves and rhizomes) were evaluated for proximate analysis, antioxidant activity and nutritional composition to determine which plant parts have the higher nutritional value for human consumption. Complete randomized design (CRD) with 20 replication (20 plants) was used during this study.

2.2 Analysis of Proximate Composition

The samples were analyzed for chemical composition using the AOAC procedures [16]. Moisture was analyzed after drying at 105℃ until reaching a constant final mass (AOAC No. 930.04). The crude protein was estimated by the Kjeldahl method (AOAC No. 978.04). The lipid content was determined by extracting a sample with diethyl ether in a Soxhlet apparatus (AOAC No. 920.39) and the ash content was determined by incineration at 460℃ (AOAC No. 920.05). Total carbohydrates were calculated by difference. All samples were analyzed in 20 replications.

2.3 Quantification of Total Phenolics and Flavonoids

The methods of extraction and quantification for total phenolics and flavonoids followed that of Ibrahim et al. [17]. A fixed amount of ground dried tissue samples (0.1 g) was extracted with 80% ethanol (10 mL) in an orbital shaker for 120 min at 50℃. The mixture was subsequently filtered (Whatman™ No.1), and the filtrate was used for the quantification of total phenolics and total flavonoids. Folin-Ciocalteu reagent (diluted 10-fold) was used to determine the total phenolics content of the leaf samples. The sample extract (200 µL) was mixed with Folin-Ciocalteau reagent (1.5 mL) and allowed to stand at 22℃ for 5 min before adding NaNO3 solution (1.5 mL, 60 g L−1). After two hours at 22℃, absorbance was measured at 725 nm. The results were expressed as mg g−1 gallic acid equivalent (mg GAE g−1 dry sample). For total flavonoids determination, a sample (1 mL) was mixed with NaNO3 (0.3 mL) in a test tube covered with aluminium foil, and left for 5 min. Then 10% AlCl3 (0.3 mL) was added followed by addition of 1 M NaOH (2 mL). Later, the absorbance was measured at 510 nm using a spectrophotometer with rutin as a standard (results expressed as mg g−1 rutin dry sample).

2.4 Quantification of Ascorbic Acid

Ascorbic acid content in methanolic extracts of leaf samples was estimated using a titrimetric method [18]. Sample extract (0.3 mL) was added to a titration flask already containing 20 mL mixture of glacial acetic acid (3.0%) and metaphosphoric acid (8.0%). The mixture was then titrated against 2, 6-dichloroindophenol solution (0.025%) until pink color of the solution was sustained for 10 s. The ascorbic acid content was calculated on the basis of a standard curve and was expressed as mg ascorbic acid/g of powdered leaves. The results of three replicate analyses were averaged.

2.5 DPPH Radical Scavenging Assay

The DPPH free radical scavenging activity of each sample was determined according to the method described by Joyeux et al. [19]. A solution of 0.1 mM DPPH in methanol was prepared. The initial absorbance of the DPPH in methanol was measured at 515 nm. An aliquot (40 µL) of an extract was added to 3 mL of methanolic DPPH solution. The change in absorbance at 515 nm was measured after 30 min. The antiradical activity (AA) was determined using the following formula:

$$AA\% = 100 - [(Abs{:}sample - Abs{:}empty\ sample)] \times 100)/Abs{:}control$$

The optic density of the samples, the control and the empty samples were measured in comparison with methanol. One synthetic antioxidant, BHT (butylhydroxytoluene) and α-tocopherol, were used as positive controls. The antioxidant capacity based on the DPPH free radical scavenging ability of the extract was expressed as µmol Trolox equivalent per gram of dried plant material.

2.6 Reducing Ability (Frap Assay)

The ability to reduce ferric ions was measured using modified methods of Ibrahim and Jaafar [20]. An aliquot (200 µL) of the extract with appropriate dilution was added to 3 mL of FRAP reagent (10 parts of 300 mM sodium acetate buffer at pH 3.6, 1 part of 10 mM TPTZ solution and 1 part of 20 mM FeCl36H2O solution) and the reaction mixture was incubated in a water bath at 37℃. The increase in absorbance at 593 nm was measured after 30 min. The antioxidant capacity based on the ability to reduce ferric ions of the extract was expressed as µM Fe(II)/g dry mass and compared with the standards for BHT, ascorbic acid, and α-tocopherol.

2.7 Analysis of Minerals

Plant samples were dried in an oven at 70℃ for 72 h. The oven-dried samples of *D. grandis* were ground and stored in plastic vials. The K, Na, Ca and Mg contents were analyzed using the digestion method [21] and determined using an Atomic Absorption Spectrophotometer (AAS; Perkin Elmer, 5100, USA). The results for mineral contents were expressed as mg/100 g DW.

2.8 Statistical Analysis

Data were analyzed using the analysis of variance procedure in SAS version 17. Means separation test between treatments was performed using Duncan multiple range test and standard error of differences between means was calculated with the assumption that data were normally distributed and equally replicated.

3. RESULTS AND DISCUSSION

3.1 Proximate Analysis

Proximate composition varies depending upon plant variety, agronomic practices, climatic and geological conditions of the area from where plant samples are collected. It is an important indicator and first step during nutritional evaluation of fruits and seeds of plants and crops and it dictates further studies on components which seem more interesting [22]. The mean values of proximate composition of different parts of *D. grandis* indicate that the plant parts are statistically different ($p \leq 0.05$) in the content of total ash, carbohydrates, lipids, proteins and moisture. Total ash content in the leaves was higher (34.21 mg/100 g dry weight) compared to the rhizomes which recorded 16.24 mg/100 g dry weight. The ash content is generally recognized as a measure of functional properties of food [23]. The high ash content in the leaves indicates presence of high amounts of inorganic nutrients in the plant material [24], and this fact was supported by high significant positive correlations ($p \leq 0.05$) between ash content and plant nutrients (Ca, Mg, K, Zn, Fe and Cu; Table 2). The high ash content in leaves compared to the rhizomes showed that the leaves of *D. grandis* contained high inorganic nutrients compared to the rhizomes. The carbohydrate content was also found to be higher in the leaves (37.28 mg/100 g dry weight) compared to the rhizomes (27.12 mg/100 g dry weight), and the carbohydrate content was found to be higher compared to lipids and crude protein content. This implied that the carbohydrates was the highest calorie contributor in this plant as the total lipid and protein contents showed the lowest composition in terms of the total energy produced [25]. The lipid and protein content was found to be statistically higher in the leaves compared to the rhizomes. The present study indicated that the leaves of bemban are a high source of food energy due to the high carbohydrate, lipid and protein contents in the samples collected [26]. The high lipid and protein content in the leaves may be a good indicator that this plant might be a good source of comparable and promising antioxidant activity [27]. The low lipid content in the plant samples suggests that this plant may be useful for individuals on a weight-reducing diet [28]. The moisture content in the leaves and rhizomes was 9.17% and 8.12%, respectively. This indicated that the leaves had higher water content than the rhizomes. High moisture content usually indicates the roughness of leaves as was observed in the study [5].

Table 1. Proximate chemical composition of leaves and rhizomes of *Donax grandis* (g/100 g dry weight; N =20)

	Leaves	Rhizome	P value
Total ash	34.21±1.21[a]	16.24±2.71[b]	0.001
Carbohydrate	37.28±2.11[a]	27.12±3.16[b]	0.054
Lipid	4.21±3.22[a]	3.11±0.89[b]	0.042
Protein	12.71±1.71[a]	11.21±0.51[b]	0.001
Moisture	9.17±0.31[a]	8.12±1.12[b]	0.006

Data are expressed as the mean ± standard deviation; values in the same row having different letters differ significantly (p< 0.05).

Table 2. Pearson correlations between all parameters measured during the study

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1. Ash	1.000																
2.CHO	0.234	1.000															
3. Lipid	0.112	0.761*	1.000														
4. Protein	0.112	0.881*	0.786*	1.000													
5. Moist	0.134	0.334	0.455	0.123	1.000												
6. TP	0.005	0.876*	0.556	0.005	0.124	1.000											
7. TF	0.056	0.912*	0.445	0.009	0.113	0.981*	1.000										
8. AA	0.014	0.876*	0.655*	0.125	0.145	0.876*	0.971*	1.000									
9. DPPH	0.045	0.865*	0.454	**0.213**	0.102	0.879*	0.887*	0.765*	1.000								
10. FRAP	0.013	0.887*	0.345	0.104	0.221	0.912*	0.821*	0.801*	0.871*	1.000							
11. Ca	0.811*	0.221	0.005	0.211	0.104	0.123	0.134	0.023	0.102	0.123	1.000						
12. Mg	0.813*	0.342	0.123	0.145	0.223	0.102	0.236	0.034	0.005	0.231	0.124	1.000					
13. Na	0.731*	0.234	0.234	0.012	0.213	0.224	0.201	0.021	0.213	0.092	0.023	0.321	1.000				
14. K	0.745*	0.346	0.104	0.134	0.002	0.321	0.393	0.012	0.023	0.122	0.003	0.123	0.012	1.000			
15. Fe	0.832*	0.065	0.205	0.311	0.176	0.127	0.125	0.021	0.034	0.091	0.024	0.009	0.123	0.014	1.000		
16. Zn	0.805*	0.123	0.341	0.412	0.376	0.043	0.212	0.034	0.023	0.034	0.123	0.023	0.023	0.125	0.432	1.000	
17. Cu	0.856*	0.145	0.211	0.321	0.134	0.234	0.104	0.014	0.034	0.003	0.012	0.012	0.012	0.342	0.221	0.113	1.000

*Note * and ** significant at p≤0.05 and p≤ 0.01 respectively; CHO= total carbohydrate; Moist = total moisture; TP = total phenolics; TF= total flavonoids; AA = ascorbic acid.*

3.2 Total Phenolics, Flavonoids and Ascorbic Acid

It was found that the total phenolic compounds in the leaves of *D. grandis* was higher (7.12 mg/g GAE dry weight) compared to the rhizomes which recorded 4.12 mg/g GAE dry weight (Fig. 1a). This result was higher than that reported by Jamaludin et al. [15]. The difference in the results might be due to the difference in ontogenical age of the plants harvested. In the current study the plants propagated from rhizomes were six months old, while Jamaludin et al. [15] did not specify the age of the harvested plants. The results obtained showed this plant contained higher total phenolics than L. pumila and some Iranian plants [29,30]. Epidemiological studies have confirmed that diseases prevention and antioxidant activities are related to total phenolics in plants [31]. Total flavonoids content also showed the same trend as total phenolics, where the highest accumulation was observed in the leaves (Fig. 1b). Flavonoids is the largest subgroup of phenolics that constitute about half of the phenolic compounds [32]. Many biological effects including free radical scavenging activity have been reported for flavonoids. This is generally attributed to their structure [33]. In the present study, the flavonoid content in *D. grandis* leaves and rhizomes was higher than in Algerian medicinal plants [34]. Meanwhile, the maximum amount of ascorbic acid was observed in the leaves (1.27 mg/g) and followed by the rhizomes (0.87 mg/g; Fig. 1c). The current results indicated that the leaves contained the highest ascorbic acid content and this ascorbic acid content was higher compared to L. pumila [35]. Ascorbic acid is a non enzymatic natural antioxidant, water soluble and widely used as an alternative to synthetic antioxidants. The increase in production of secondary metabolites (total phenolics, total flavonoids and ascorbic acid) in *D. grandis* might be due to the high level of carbohydrate content in the plants. TNC (d-glucose) is a precursor for the production of total phenolics, flavonoids and ascorbic biosynthesis in plants, and the more the availability of TNC the greater the production of

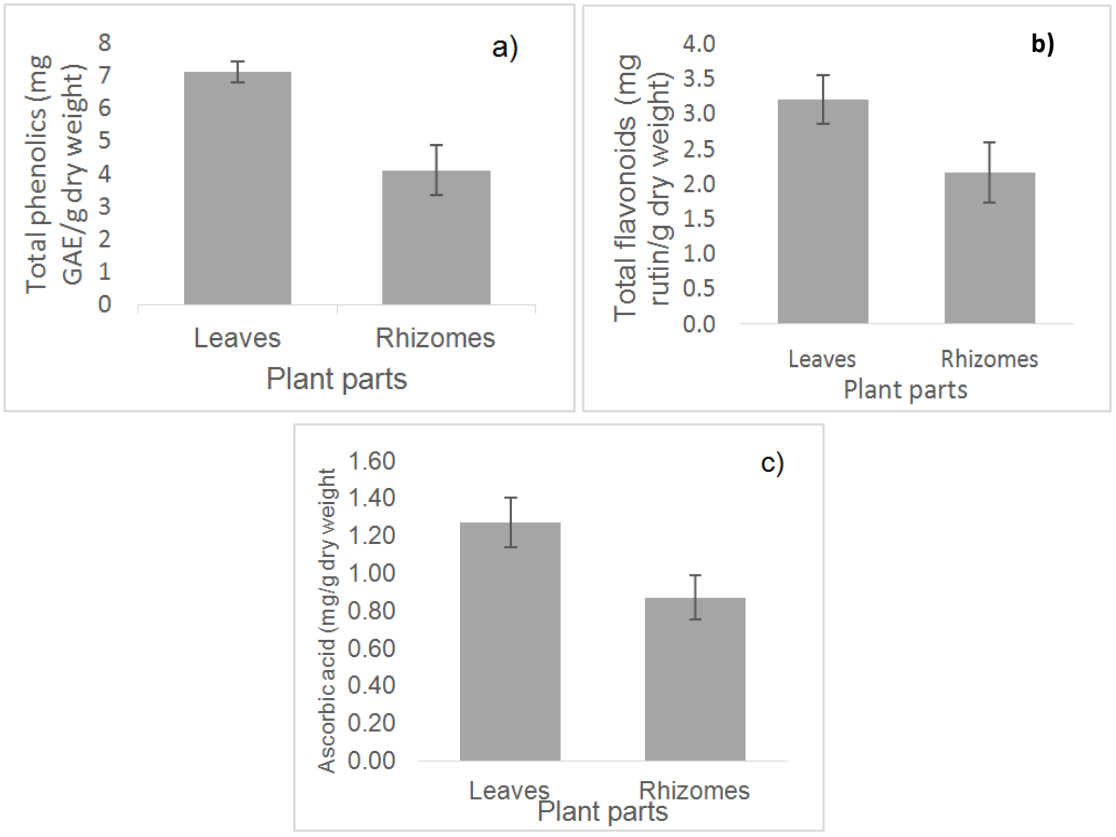

Fig. 1. Total phenolics (a), total flavonoids (b) and ascorbic acid (c) of *D. grandis* under different plant parts. N = 20. bars represent standard error of differences between means

secondary metabolites in the plants [36,37]. The total carbohydrates showed significant positive correlations with total phenolics (r2 = 0.87; p≤0.05), total flavonoids (r2 = 0.962; p≤0.05), and ascorbic acid (r2 = 0.87; p≤0.05), thus justifying the higher production of secondary metabolites in this plant (Table 2).

3.3 DPPH Radical Scavenging Activity

DPPH assay is widely used for evaluation of antioxidant activity of biological samples. The assay worked based on discoloration of DPPH free radical upon reacting with hydrogen donating samples, i.e antioxidants that are present in the plant samples. It must be noted that the DPPH assay principally measures the activity of water-soluble antioxidants [38]. The principle of this method is that in the presence of a molecule consisting of a stable free radical (DPPH), an antioxidant with the ability to donate a hydrogen atom will quench the stable free radical, a process which is associated with a change in the absorption and can be measured spectrophotometrically. The leaves of *D. grandis* showed the highest radical scavenging potential and the least in the rhizomes (Fig. 2a). The DPPH values were found to be much lower than that of grapes [39]. Besides phenolics and flavonoid compounds, other water-soluble antioxidants in the extracts such as vitamin C could also exert an additive effect on the DPPH radical scavenging activity [40]. Studies have shown that a combination of phenolics, flavonoids and ascorbic acid produced a synergistic effect on DPPH radical scavenging activity [41]. In the current study significant positive correlations (p≤0.05) were observed between DPPH and total phenolics (r2 =0.876), total flavonoids (r2= 0.971) and ascorbic acid (r2= 0.873). This indicates that the antioxidant activity in the present samples might be attributable to the total phenolics, flavonoids and vitamin content in the plants.

3.4 Ferric Reducing Antioxidant Potential (FRAP)

The ethanolic extract of dried leaves and rhizomes were subjected to FRAP assay to measure the antioxidant activity. The Ferric Reducing Antioxidant Potential (FRAP) assay measures the total antioxidant power of biological fluids [42]. Total antioxidant power was assessed by the reduction of Fe^{3+} to Fe^{2+}, which occurred rapidly with all reductants with half of the reaction reduction potentials above that of Fe^{3+}/ Fe^{2+} (Fig. 2b). Therefore, the values express the corresponding concentration of electron-donating antioxidants. The ferric reducing ability (FRAP assay) has been widely used in the evaluation of the antioxidant component of dietary polyphenols [43]. The FRAP values in the leaves and rhizomes in the present study was lower compared to kacip fatimah, nectarine, peach and plum [44]. The antioxidant activity is found to be linearly proportionate to the phenolics and flavonoids content [38,44]. Yen and Duh [39] reported that the ferric reducing power of bioactive compounds was associated with antioxidant activity. A strong positive relationship between total phenolics, total flavonoids, and antioxidant activity was reported. A similar trend was observed in the current study where total phenolics and flavonoids displayed significantly positive relationships with FRAP activity (r^2= 0.912 and r^2= 0.821; p ≤0.05; Table 2). Furthermore, FRAP had a significant positive relationship with ascorbic acid (r^2 =0.801; p≤0.05; Table 2) and thus implies that high DPPH and FRAP activity in *D. grandis* extracts might be due to high accumulation of total phenolics, flavonoids and ascorbic acid in the plant [45,46].

3.5 Plant Mineral Composition

Generally, the plant mineral content was statistically higher in leaves than rhizomes of *D. grandis* (p≤0.05; Table 3). The plant was found to have high potassium content followed by Mg, Ca, Na, Fe, Zn and copper. The high potassium content indicates that this plant can be a good potassium source. The abundance of potassium may suggest that this plant can be a good source of diet for those who have problems with hypertension [47]. Intake of potassium would have enhanced brain function, balanced blood pressure, maintain optimal muscle and nerve function, boost metabolism, maintain good heart and kidney health, fluid balance and reduce stress [48]. The higher magnesium content in this plant was important in maintaining the immune system, nerve function and keep the heart beat at a steady rate [49]. The iron content was found to be high in *D. grandis*, which showed that this plant can be a good source for iron. Iron deficiency is a common nutritional problem that affects many people worldwide. Iron deficiency can cause chronic bleeding, infection and menstrual losses in women during reproductive age [50]. The zinc and copper ratio in the leaves and rhizomes of *D. grandis*

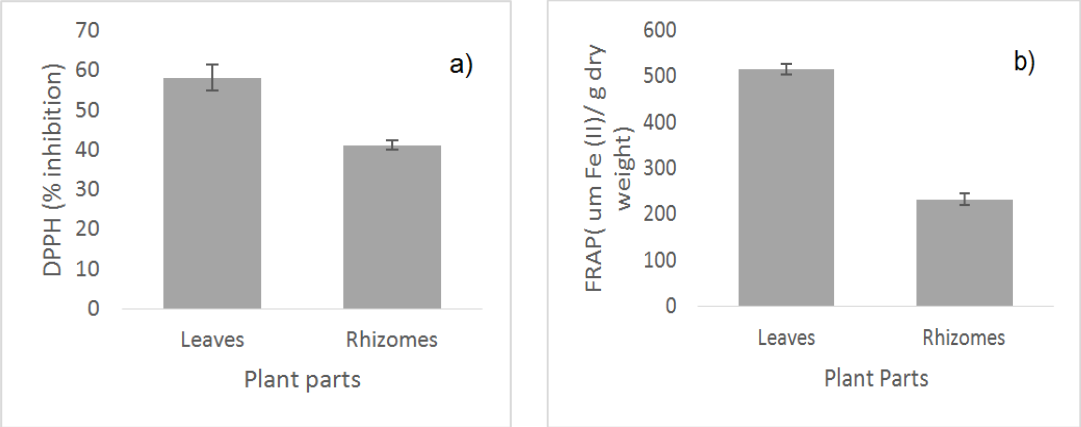

Fig. 2. DPPH (a) and FRAP (b) of *D. grandis* under different plant parts. N = 20. bars represent standard error of differences between means.

Table 3. Plant nutrients in leaves and rhizomes of *Donax grandis* (mg/100 g dry weight; N =20)

	Leaves	Rhizome	P value
Calcium	311.21±12.23[a]	271.21±15.34[b]	0.051
Magnesium	711.21±34.23[a]	612.31±27.14[b]	0.049
Sodium	367.21±3.45[a]	311.01±8.56[b]	0.0012
Potassium	4127.26±123.12[a]	3127.18±421.21[b]	0.0017
Iron	130.21±7.43[a]	97.28±2.34[b]	0.0042
Zinc	78.17±4.43[a]	67.21±5.21[b]	0.002
Copper	15.12±0.21[a]	12.11±0.67[b]	0.003

Data are expressed as the mean ± standard deviation; values in the same row having different letters differ significantly (p< 0.05).

were 5.2 and 5.8 respectively. This was within the recommended rate in human tissues. The recommended zinc and copper ratio in human was 3 - 6. The zinc and copper are needed only in little amounts for some biological functions [51]. Usually the high zinc/copper ratio (>16) in dietary sources have been linked to high risk of cardiovascular disorder [52,53]. This showed that *D. grandis* can be used as a potential food source to control cardiovascular disorder.

4. CONCLUSION

In conclusion, the potential of *D. grandis* as an important source of nutritional, antioxidant and mineral compounds is highlighted in the present study. The present study indicates that *D. grandis* leaves would serve as a rich source of energy, antioxidants, as well as micronutrients for human consumption compared to the rhizomes due to the higher content of energy, antioxidants and minerals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Kakkar AKP. Screening of antioxidant potential of selected barks of Indian medicinal plants by multiple *in vitro* assays. Biomed Environ Sci. 2008;21:24–29.
2. Pihlanto A, Akkanen S, Korhonen HJ. ACE-inhibitory and antioxidant properties of potato (*Solanum tuberosum*). Food Chem. 2008;109:104–12.
3. Singh N, Rajini P. Free radical scavenging activity of an aqueous extract of potato peel. Food Chem. 2004;85:611–16.
4. Adom KK, Liu RH. Antioxidant activity of grains. J Agric Food Chem. 2002;50: 6182–187.
5. Zia-ul-haq M, Iqbal S, Ahmad S, Bhanger MI, Wiczkowski W, Amarowicz R.

Antioxidant potential of desi chickpea varieties commonly consumed in Pakistan. J Food Lipids. 2008;15:326–42.
6. Yang X, Yang L, Zheng H. Hypolipidemic and antioxidant effects of mulberry (*Morus alba* L.) fruit in hyperlipidaemia rats. Food Chem Toxicol. 2010;48:2374–379.
7. Pérez-Gregorio M, Regueiro J, Alonso-González E, Pastrana-Castro L, Simal-Gándara J. Influence of alcoholic fermentation process on antioxidant activity and phenolic levels from mulberries (*Morus nigra* L.). LWT-Food Sci Technol. 2011;44:1793–801.
8. Ercisli S, Orhan E. Some physico-chemical characteristics of black mulberry (*Morus nigra* L.) genotypes from Northeast Anatolia region of Turkey. Sci Hortic. 2008;116:41–46.
9. Memon AA, Luthria DL, Bhanger MI, Pitafi AA. Phenolic acids profiling and antioxidant potential of mulberry (*Morus laevigata* W., *Morus nigra* L., *Morus alba* L.) leaves and fruits grown in Pakistan Pol. J. Food Nutr Sci. 2010;60:25–32.
10. Ong CH. Tumbuhan Liar, Khasiat Ubatan dan Kegunaan Lain. Utusan Publication Sdn. Bhd. 2008;58-59.
11. Fatan HY. Etiologi Penyakit di Kalangan Masyarakat Melayu: Satu Kajian Kes di Mukim Sanggang, Temerloh Pahang, Pulau Pinang: University Science Malaysia. Master Thesis; 1990.
12. Faridah H, Nurulhuda H. The use of Medicinal Plant Species by the Temuan Tribe of Ayer Hitam Forest, Selangor, Peninsular Malaysia. Pertanika J Trop Agri Sci. 1999;22:85-94.
13. Yeoh HH, Wee YC, Watson L. Taxonomic variation in total leaf protein amino acid compositions of monocotyledonous plants. Biochem Systematics and Ecol. 1986;14: 91-96.
14. Rahmani M, Kiew R, Lajis N, Othman R, Toia RF. A Contribution to the phytochemical survey of peninsular Malaysia. Pertanika. 1985;8:347-57.
15. Mohd Daud J, Mohd Hasan HH, Hasyim R, Taher M. Phytochemicals screening and antioxidant activities of Malaysian *Donax grandis* extracts. Euro J Sci Res. 2011;61:572-77.
16. AOAC (Association of Official Analytical Chemists). Official methods of analysis of the association of official analytical chemists, 16th ed.; AOAC: Arlington, VA, USA; 1995.
17. Ibrahim MH, Jaafar HZE, Rahmat A. Zaharah AR. The relationship between phenolics and flavonoid production with total non structural carbohydrate and photosynthetic rate in *Labisia pumila* Benth. under High CO2 and nitrogen fertilization. Molecules. 2011;16:162–74.
18. Association of Official Analytical Chemists (AOAC). Official Method of Analysis, 15th ed.; AOAC: Gaithersburg, MD, USA, 2000; (No. 967.21 Ascorbic acid in vitamin preparations and juices)
19. Joyeux M, Lobstein A, Anton R, Mortier A. Comparative antilipoperoxidant, antinecrotic and scavenging properties of terpenes and biflavones from Ginkgo and some flavonoids. Planta Medica. 1995;61: 126–29.
20. Ibrahim MH, Jaafar HZE. Effects of nitrogen fertilization on synthesis of primary and secondary metabolites in three varieties of kacip fatimah (*Labisia pumila* Blume). Int J Mol Sci 2011;12: 5238–254.
21. Ma T, Zuazaga G. Micro-Kjeldahl determination of nitrogen. A new indicator and an improved rapid method. Ind Eng Chem Anal Ed. 1942;14:280–82.
22. Hussain K, Ismail Z, Sadikun A, Ibrahim P. Proximate and qualitative analysis of different parts of Piper sarmentosum, and quantification of total amides in various extracts. Phcog Res. 2009;1:113–19.
23. Uusiku N, Oelofse A, Duodu KG, Bester MJ, Faber M. Nutritional value of leafy vegetables of sub-Saharan Africa and their potential contribution to human health: A review. J Food Comp Anal. 2010;23:499–09.
24. Hofman PJ, Vuthapanich S, Whiley AW, Klieber A, Simons DH. Tree yield and fruit minerals concentrations influence “Hass” avocado fruit quality. Sci Hort. 2002;92: 113–23.
25. Choi M, Scholl UI, Yue P, Bjorklund P, Zhao B, Nelson-Williams C, Ji W, Cho Y, Pael A, Men CJ. K+ channel mutations in adrenal aldosterone-producing adenomas and hereditary hypertension. Sci. 2011;331:768–72.
26. Mensah JK, Okoli RI, Turay AA, Ogie-Odia EA. Phytochemical analysis of medicinal plants used for the management of hypertension by Esan people of Edo state, Nigeria. Ethnobotanical Leaflets. 2009;13: 1273–287.

27. Gupta S, Jyothi Lakshmi A, Manjunath M, Prakash J. Analysis of nutrient and antinutrient content of underutilized green leafy vegetables. LWT-Food Sci Technol. 2005;38:339–45.
28. Maisuthisakul P, Pasuk S, Ritthiruangdej P. Relationship between antioxidant properties and chemical composition of some Thai plants. J Food Compos Anal. 2008;21:229–40.
29. Zia-Ul-Haq M, Ahmad S, Shad MA, Iqbal S, Qayum M, Ahmad A, Luthria DL, Amarowicz R. Compositional studies of some of lentil cultivars commonly consumed in Pakistan. Pak J Bot. 2011;43: 1563–567.
30. Ibrahim MH, Jaafar HZ, Rahmat A, Rahman ZA. Effects of nitrogen fertilization on synthesis of primary and secondary metabolites in three varieties of Kacip Fatimah (*Labisia pumila* Blume). Int J Mol Sci. 2011;12:5238–254.
31. Bouayed J, Piri K, Rammal H, Dicko A, Desor F, Younos C, Soulimani R. Comparative evaluation of the antioxidant potential of some Iranian medicinal plants. Food Chem. 2007;104:364–68.
32. brahim MH, Jaafar HZE. Primary, secondary metabolites, H2O2, malondialdehyde and photosynthetic responses of *Orthosiphon stimaneus* Benth. to different irradiance levels. Molecules. 2012;17:1159–176.
33. Ibrahim MH, Jaafar HZE. Carbon dioxide fertilization enhanced antioxidant compounds in Malaysian Kacip Fatimah (*Labisia pumila* Blume). Molecules. 2011;16:6068–081.
34. Figueiredo-Gonzalez M, Martinez-Carballo E., Cancho-Grande B, Santiago JL, Martinez MC, Simal-Gandara J. Pattern recognition of three *Vitis vinifera* L. red grapes varieties based on anthocyanin and flavonol profiles, with correlations between their biosynthesis pathways. Food Chem. 2012;130:9–19.
35. Han NM, May CY. Determination of antioxidants in oil palm leaves (*Elaeis guineensis*). Am J App Sci. 2010;7:1243–247.
36. Bouayed J, Piri K, Rammal H, Dicko A, Desor F, Younos C, Soulimani R. Comparative evaluation of the antioxidant potential of some Iranian medicinal plants. Food Chem. 2007;104:364–68.
37. De Tullio MC, Arrigoni O. Hopes, disillusions and more hopes from vitamin. Cell and Mol Life Sci. 2004;61:209–19.
38. Hancock RD, Viola R. Biosynthesis and catabolism of L-ascorbic acid in plants. Critical Rev Plant Sci. 2005;24:167–88.
39. Miliauskas G, Venskutonis PR, Van Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem. 2004;85:231-37.
40. Tamura H, Yamagami A. Antioxidative activity of monoacylated anthocyanins isolated from Muscat bailey A grape. J Agri Food Chem. 1994;42:1612-615.
41. Bravo L. Polyphenols: Chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev. 1998;56:317-33.
42. Jaitak V, Sharma K, Kalia K, Kumar N, Singh HP, Kaul VK, Singh B. Antioxidant activity of *Potentilla fulgens*: An alpine plant of western Himalaya. J Food Compos Anal. 2010;23:142–47.
43. Agnaniet H, Menut C, Bessie JM. Aromatic plants of tropical central Africa. Part XLIX: Chemical composition of essential oils of the leaf and rhizomes of *Aframomum giganteum* from Gabon. Flavour Fragrance. 2004;19:205-09.
44. Gil MI, Thomas-Barberan FT, Hess-Pirce B, Kader AA. Antioxidant capacities, phenolic compounds, carotenoids and vitamin C contents of nectarine, peach and plum cultivars from California. J Agric Food Chem. 2002;50:4976-982.
45. Yen GC, Duh PD. Scavenging effects of methanolic extract of peanut hulls on free-radical and active oxygen species. J Agric Food Chem. 1994;42:629-32.
46. Luximon-Ramma A, Bahorun T, Soobrattee AM, Aruoma OI. Antioxidant activities of phenolic, proanthocyanidin and flavonoid components in extracts of Acacia fistula. J Agric Food Chem. 2005;50:5042–047.
47. Oktay M, Gulcin I, Kufrevioglu OI. Determination of in vitro antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. Lebensm Wiss Technol. 2003;36: 263–71.
48. Mensah JK, Okoli RI, Turay AA, Ogie-Odia EA. Phytochemical analysis of medicinal plants used for the management of hypertension by Esan people of Edo state, Nigeria. Ethnobotanical Leaflets. 2009;13: 1273–287.

49. King JC, Cousins RJ. Zinc. In Modern Nutrition in Health and Disease, 10th ed.; Shils, M.E., Shike, M., Ross, A.C., Caballero, B., Cousins, R.J., Eds.; Lippincott Williams and Wilkins: Baltimore, MD, USA. 2006;71–285.
50. Housecroft CE, Sharpe AG. Inorganic chemistry (3rd ed.). Prentice Hall. ISBN 978-0131755536. 2008;305–06.
51. Osredkar J, Sustar N. Copper and zinc, Biological role and significance of copper/zinc imbalance. J Clinic Toxicol. 2011;S3:001. DOI:10.4172/2161-0495.S3-001.
52. Ma J, Netts NM. Zinc and copper intakes and their major food sources for older adults in the 1994-96 continuing survey of food intakes by individuals (CSFII). J Nutr. 2000;130:2838–843.
53. Klevay LM. The ratio of zinc to copper of diets in the United States. Nutr Rep Int. 1975;11:237–42.

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