

Nutritional compositions, biological activities, and phytochemical contents of the edible bamboo shoot, *Dendrocalamus asper*, from Malaysia

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Abstract

The edible shoots of *Dendrocalamus asper* (family Poaceae) is an underutilised food. The present work was conducted to evaluate the nutritional compositions, biological activities, and phytochemical contents of the shoots of *D. asper* obtained from different regions of Malaysia, Peninsular (DP) and East Malaysia (DS). The nutritional analysis was conducted using the Official Methods of Analysis of the AOAC International. All minerals were quantified using an inductively coupled plasma-mass spectrometer, except for potassium which was measured using a flame atomic absorption spectrometer. Total phenolic content (TPC) was determined using the Folin-Ciocalteu method. Antibacterial and antifungal activities were assayed using a colourimetric broth microdilution method, while antioxidant activity was tested using DPPH radical scavenging activity, ferric-reducing antioxidant power, and cellular antioxidant activity (CAA) assays. Enzyme inhibitory activities were examined using α -amylase and α -glucosidase. Both bamboo shoots (boiled at 100°C for 20 min) were high in moisture (> 93 g/100 g FW), crude protein (> 21 g/100 g DW), and crude fibre contents (> 9 g/100 g DW), but low in fat content (< 4 g/100 g DW). Potassium was the most abundant mineral at 205.67 and 203.83 μ g/100 g DW of bamboo shoots of DP and DS, respectively. The extracts (hexane, ethyl acetate, ethanol, and water) of both shoots showed stronger antifungal activity than antibacterial activity against selected human pathogens. All extracts of DP shoots demonstrated higher CAA in HeLa cells and α -amylase inhibitory activity than that of DS shoots. In contrast, the extracts of DS shoots exhibited stronger inhibition on α -glucosidase and contained higher TPC than that of DP shoots. The *D. asper* shoots obtained from the Peninsular Malaysia and East Malaysia contained different types of secondary metabolites which account for the differences in the biological activities. In conclusion, *D. asper* shoots have potential as a nutritional and functional food.

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Introduction

Bamboo is the fastest growing plant belonging to family Poaceae, and can be utilised from top to bottom. Its freshly erupting culms, known as bamboo shoots, can be consumed fresh, fermented, canned, or pickled (Nirmala *et al.*, 2007). Bamboo shoots contain high amounts of proteins, dietary fibres, minerals, and vitamins, but are low in fat (Choudhury *et al.*, 2012). The shoots are also a rich source of secondary metabolites (phytochemicals), such as flavones, phenolic acids, and steroids (Choudhury *et al.*, 2012). Phytochemicals are known to possess many biological properties, which are beneficial to human health (Etxeberria *et al.*, 2012; Pohl and Kong Thoo Lin, 2018).

Malaysia has approximately 70 species of bamboo with 50 species in Peninsular Malaysia, 30 species in Sabah, and 20 species in Sarawak (Wong, 1989). Bamboo species from the genera *Bambusa*, *Dendrocalamus*, *Gigantochloa*, and *Schizostachyum* are commercially exploited for making furniture, blinds, chopsticks, toothpicks, skewer sticks, handicraft, and incense sticks (Azmy and Abd Razak, 1991). The edible shoots of some of these species, such as *D. asper*, are underutilised as food. The present work was thus conducted to evaluate the nutritional compositions of the shoots of *D. asper* obtained from two different regions of Malaysia, as well as to evaluate the extracts of the two bamboo shoots for phytochemical contents, and antibacterial, antifungal, antioxidant, and enzyme inhibitory activities.

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Materials and methods

Materials

The following chemicals and reagents were used: amphotericin B, chloramphenicol, and gallic acid (purity $\geq 99\%$) (Bio Basic, Canada); foetal bovine serum (FBS) and Roswell Park Memorial Institute (RPMI)-1640 medium (Biowest, USA); acarbose (purity $\geq 95\%$) (Fluka, Germany); ethanol (AR grade), ethyl acetate (AR grade), and L-ascorbic acid (purity $\geq 99\%$) (Fisher Scientific, UK); Mueller-Hinton broth (MHB) (HiMedia, India); 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH), 3,5-dinitrosalicylic acid, disodium hydrogen phosphate anhydrous, Folin-Ciocalteu's phenol reagent, hexane (AR grade), methanol (AR grade), penicillin-streptomycin solution, *p*-nitrophenyl β -D-glucopyranoside, sodium dihydrogen phosphate anhydrous, sodium acetate trihydrate, and sodium carbonate (Merck, Germany); 2,4,6-tripyridyl-s-triazine (TPTZ) (Nacalai Tesque, Japan); multi-elements calibration standard and potassium single element calibration standard (PerkinElmer, USA); 70% nitric acid (RCI Labscan, Thailand); ferric chloride and ferrous sulphate heptahydrate (R & M Marketing, UK); glacial acetic acid (QReC, Thailand); 2,2-diphenyl-1-picrylhydrazyl (DPPH), α -amylase (≥ 10 U/mg) from porcine pancreas type VI-B, α -glucosidase (≥ 10 U/mg) from *Saccharomyces cerevisiae* type I, dichlorodihydrofluorescein diacetate (DCFH-DA), Dulbecco's phosphate-buffered saline (DPBS), Eagle's minimum essential medium (EMEM), griseofulvin, *p*-iodonitrotetrazolium chloride, and quercetin (purity $\geq 95\%$) (Sigma, USA); hydrogen peroxide and soluble starch (System, Malaysia).

The shoots of *D. asper* were obtained from two different regions in Malaysia. One was purchased from a wet market at Malim Nawar, Perak (Peninsular Malaysia) and designated as *D. asper* (Perak) or DP shoots, while the other was obtained from a market in Tambunan, Sabah (East Malaysia) and designated as *D. asper* (Sabah) or DS shoots. The bamboo species was identified by Dr. Wei Lim Goh, a bamboo expert affiliated with the Faculty of Science, Universiti Tunku Abdul Rahman (UTAR), Malaysia. Voucher specimens using the culm sheath of both shoots were prepared, numbered (DP: UTAR/FSC/15/001; DS: UTAR/FSC/16/004), and deposited at the Faculty of Science, UTAR.

Preparation of samples and extracts

The culm sheaths of fresh bamboo shoots were removed. The shoots were cut into cubes (~ 3 cm each side) and boiled at 100°C for 20 min (Rawat *et al.*, 2016).

A portion of the boiled shoots was lyophilised using a freeze-dryer (Martin Christ Alpha, UK) for crude protein, crude fat, crude fibre, and mineral analyses, while the boiled shoots were used for moisture and ash analyses. The boiled shoots were sequentially extracted using hexane, ethyl acetate, ethanol, and distilled water in order to obtain extracts for phytochemical content and bioactivity testing. The maceration was performed in three cycles (one day/cycle) at ambient temperature with agitation (130 rpm) using an orbital shaker. The filtrate for each extract was evaporated to dryness at 40°C using a rotary evaporator (Buchi R205, Switzerland) except for the water extract which was lyophilised. The dry extracts were kept at -20°C prior to analysis.

Nutritional analysis

Moisture, ash, crude protein, crude fat, and crude fibre contents of the bamboo shoots were determined using the Official Methods of Analysis of the AOAC International (AOAC, 1997). The average value was obtained from triplicate readings for each assay.

Minerals content

The K concentration of the bamboo shoots was measured using a flame atomic absorption spectrometer (AAnalyst™ 200, PerkinElmer, USA) with the acetylene gas and compressed air running at 2.5 and 10.0 L/min, respectively. Other minerals (Ca, Mg, Na, Cr, Cu, Fe, Mn, Se, and Zn) were quantified using an inductively coupled plasma-mass spectrometer (NexION® 300, PerkinElmer, USA), as described by Shumo *et al.* (2014) with modifications. The flow rates for the plasma (argon), auxiliary, and nebuliser gases were 16.0, 1.20, and 0.98 L/min, respectively. The analysis of each shoot was performed in triplicate.

Phytochemical content

The extracts were screened for the presence of alkaloids (Dragendorff's test), phenolic acids (ferric chloride test), flavonoids (Shinoda test), saponins (foam test), anthraquinones (hydroxyanthraquinone test), tannins (gelatine test), triterpenoids (Salkowski test), and coumarins (Roopalatha and Nair, 2013). The total phenolic content (TPC) of each extract was quantified based on the Folin-Ciocalteu method (Herald *et al.*, 2012) with modifications. The absorbance value for each sample was recorded at 765 nm using a microplate reader (FLUOstar® Omega, BMG Labtech, Australia). A series of gallic acid solutions (2.5 - 320 $\mu\text{g/mL}$) was used to construct a calibration curve. The linear regression equation for the calibration curve was $y = 0.0065x + 0.0199$ with a regression

coefficient (R^2) of 0.9994. The TPC of each extract was assayed in triplicate and expressed as mg gallic acid equivalent/g sample.

Antimicrobial activity

The antimicrobial potency of the bamboo shoot extracts was evaluated using *p*-iodonitrotetrazolium-based colourimetric broth microdilution method, as previously described by Sit *et al.* (2017). The extracts were evaluated for antibacterial activity against two species of Gram-positive bacteria (*Bacillus cereus* ATCC®11778™ and *Staphylococcus aureus* ATCC®6538™) and four species of Gram-negative bacteria (*Acinetobacter baumannii* ATCC®19606™, *Escherichia coli* ATCC®35218™, *Klebsiella pneumoniae* ATCC®13883™, and *Pseudomonas aeruginosa* ATCC®27853™); for antifungal activity against three species of yeasts (*Candida albicans* ATCC®90028™, *Candida parapsilosis* ATCC®22019™, and *Cryptococcus neoformans* ATCC®90112™), and three species of filamentous fungi (*Aspergillus fumigatus* ATCC®204305™, *Trichophyton interdigitale* ATCC®9533™, and *Trichophyton rubrum* ATCC®28188™). All microbial strains were obtained from the American Type Culture Collection. The stock solution for each extract was prepared at 10 mg/mL in a methanol-water mixture (2:1, v/v) and serially diluted in the assay medium (MHB/RPMI-1640) to achieve a final concentration range of 0.02 to 2.50 mg/mL. Positive (chloramphenicol/amphotericin B/griseofulvin), growth (bacterium/fungus only), sterility (medium only), and negative (extracts only) controls were included in each 96-well microplate. The minimum inhibitory concentration (MIC) and the minimum bactericidal/fungicidal concentration (MBC/MFC) were determined for each of the extracts. This was performed in triplicate.

Antioxidant activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The DPPH radical scavenging activity of the bamboo shoot extracts was determined following a method modified from Yang *et al.* (2011). Six different extract concentrations, i.e. 31.3, 62.5, 125, 250, 500, and 1000 µg/mL were evaluated. DPPH solution without extract served as a control, while extract solution of different concentrations without DPPH served as a sample blank. Ascorbic acid (1.6 - 100 µg/mL) and gallic acid (1.6 - 100 µg/mL) were used as positive controls. This test was conducted in triplicate. The half-maximum inhibitory concentration (IC_{50}) of each extract was determined from the plot of percentage inhibition of DPPH radical scavenging activity *versus* concentration of extract.

Ferric-reducing antioxidant power (FRAP)

The measurement of the reduction of ferric 2,4,6-tripyridyl-s-triazine complex (Fe^{3+} -TPTZ) to its ferrous form (Fe^{2+} -TPTZ) in the presence of an antioxidant was performed following the method of Yang *et al.* (2011) with modifications. Ferrous sulphate heptahydrate solutions (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, and 1.6 mM) were used to construct the calibration curve. The linear regression equation for the calibration curve constructed was $y = 1.9676x + 0.4062$ with R^2 of 0.9944. Each extract was evaluated at 1,000 µg/mL. Extract solution without the addition of the FRAP reagent was used as a sample blank. The actual absorbance value for each sample was then corrected for the respective sample blank's absorbance. The FRAP value of an extract was interpolated from the calibration curve and expressed as mM Fe^{2+} equivalent/g sample. All measurements were performed in triplicate.

Cellular antioxidant activity (CAA)

The CAA assay was performed as described by Wolfe and Liu (2007) with modifications. Briefly, human cervical cancer (HeLa) cells (RCB0007 from the RIKEN BioResource Research Centre, Japan) were seeded at a density of 6×10^4 cells/well in a 96-well, clear bottom, black microplate with 100 µL of EMEM (supplemented with 10% FBS and 1% penicillin-streptomycin). After 24 h of incubation at 37°C and 5% CO_2 , the medium was gently removed, and the cells were washed with 100 µL of DPBS. The cells were then treated with a mixture containing 50 µL of 25 µM DCFH-DA solution and 50 µL of extract (0.63, 1.25, 2.5, and 5 mg/mL)/quercetin (positive control; 31.3 – 2,000 µM) solution, and incubated for 1 h at 37°C and 5% CO_2 . The control wells contained cells treated with 100 µL of DCFH-DA only whereas blank wells contained cells without any treatment. After incubation, the medium was removed, and the cells were washed again with 100 µL of DPBS. Subsequently, 100 µL of AAPH (free radical initiator) was introduced to the cells, and the fluorescence intensity was measured at λ_{ex} of 485 nm and λ_{em} of 520 nm using the microplate reader at 5 min intervals for 60 min. The area under the curve (AUC) for each plot of fluorescence intensity *versus* time was determined, and used to calculate the CAA unit (%) for each extract concentration.

Enzyme inhibitory activity

α -amylase inhibitory activity

The α -amylase inhibition assay was performed following the method of Telagari and Hullatti (2015) with some modifications. Extracts

were evaluated at 31.3, 62.5, 125, 250, 500, and 1,000 $\mu\text{g/mL}$ concentrations. The absorbance of the test mixture was read at 540 nm using the microplate reader. A control (buffer instead of sample extract) and sample blanks at respective concentrations (without addition of enzyme) were included. Acarbose (3.9, 7.8, 15.6, 31.3, 62.5, and 125 $\mu\text{g/mL}$) was used as a positive control. The assay was performed in three independent experiments, and IC_{50} was determined from the plot of percentage inhibition against concentration of extract.

α -glucosidase inhibitory activity

The α -glucosidase inhibition assay was adapted from Telagari and Hullatti (2015) with modifications. Six concentrations (31.3, 62.5, 125, 250, 500, and 1,000 $\mu\text{g/mL}$) of extracts were tested. The absorbance of the released *p*-nitrophenol from the reaction was measured at 405 nm using the microplate reader. A control (buffer instead of sample extract) and sample blanks at respective concentrations (without addition of enzyme) were included. Acarbose (31.3, 62.5, 125, 250, 500, and 1,000 $\mu\text{g/mL}$) was used as a positive control. The assay was performed in three independent experiments, and IC_{50} was determined from the plot of percentage inhibition against concentration of extract.

Statistical analysis

Significance difference of data was determined by Student's *t*-test (for nutritional and mineral analyses) and one-way analysis of variance (ANOVA) followed by *post hoc* tests, either with Tukey's HSD (equal variances assumed) or Dunnett's T3 (equal variances not assumed), for TPC, antioxidant, and enzyme inhibition assays. The statistical analysis was performed using the IBM SPSS Statistics for Windows Version 22.0 software. The significance level was set at $p < 0.05$.

Results and discussion

Nutritional compositions

The DP and DS shoots were boiled at 100°C for 20 min prior to nutritional, minerals, phytochemical contents, and bioactivities analyses. Boiling is the most common method used to process bamboo shoots before safe consumption as it degrades the antinutrient taxiphyllin (a cyanogenic glycoside) present in the shoots (Rawat *et al.*, 2016). The DP shoots had significantly higher ($p < 0.05$) moisture, crude fat, and crude fibre contents, but lower protein content as compared to the DS shoots (Table 1). However, the ash content was similar for both shoots. The shoots of *D. asper*

had high moisture (> 93 g/100 g FW), crude protein (> 21 g/100 g DW), and crude fibre contents (> 9 g/100 g DW), but low in fat content (< 4 g/100 g DW). The moisture, ash, proteins, fat, and dietary fibre contents for the fresh juvenile shoots of *D. asper* from India were 89.40, 0.95, 3.59, 0.40, and 3.54 g/100 g FW, respectively (Nirmala *et al.*, 2007). Pandey and Ojha (2014) reported that the total protein, assessed using Lowry's method, for the boiled shoots of *D. asper* from India was 0.21 g/100 g FW. Both samples of shoots assessed in the present work had high crude fibre content, indicating that they also contain high dietary fibre content, as these two measures are highly correlated (Marlett and Bokram, 1981; Bright-See and McKeown-Eyssen, 1984). The high dietary fibre content in *Phyllostachys nigra* bamboo shoot diet has been shown to improve the bowel function of healthy young women (Park and Jhon, 2009).

Table 1. Nutritional composition of *Dendrocalamus asper* bamboo shoots

	<i>D. asper</i> from Perak (DP)	<i>D. asper</i> from Sabah (DS)
Moisture (g/100 g FW)	94.27 \pm 0.42*	93.15 \pm 0.07*
Ash (g/100 g FW)	0.50 \pm 0.10	0.66 \pm 0.03
Crude protein (g/100 g DW)	21.67 \pm 0.24*	26.43 \pm 0.31*
Crude fat (g/100 g DW)	3.17 \pm 0.15*	2.28 \pm 0.05*
Crude fibre (g/100 g DW)	14.07 \pm 0.15*	9.37 \pm 0.16*
Minerals ($\mu\text{g}/100$ g DW)		
Calcium	10.65 \pm 0.20*	6.49 \pm 0.11*
Magnesium	14.49 \pm 0.34*	18.98 \pm 0.40*
Potassium	205.67 \pm 4.19	203.83 \pm 4.31
Sodium	8.56 \pm 3.46*	52.03 \pm 3.46*
Chromium	0.04 \pm 0.00*	0.44 \pm 0.00*
Copper	0.63 \pm 0.07	0.65 \pm 0.04
Iron	36.10 \pm 0.74*	11.79 \pm 3.93*
Manganese	3.51 \pm 0.08*	2.44 \pm 0.17*
Selenium	2.18 \pm 3.30	2.11 \pm 6.87
Zinc	6.57 \pm 0.26*	12.37 \pm 0.67*

FW: fresh weight; DW: dry weight. Data are means \pm standard deviations of triplicates ($n = 3$). Means with asterisk within similar row are significantly different ($p < 0.05$) by Student's *t*-test.

Mineral composition

Ten human essential minerals were quantified from the shoots of *D. asper*, with potassium being the highest amount in DP and DS shoots. The potassium concentration was not significantly different between

the two shoots. However, the DP shoots contained significantly lower concentrations ($p < 0.05$) of magnesium, sodium, chromium, and zinc than the DS shoots (Table 1). The four most abundant minerals, in decreasing order, were potassium, iron, magnesium, and calcium for DP shoots, and potassium, sodium, magnesium, and zinc for DS shoots. Potassium was found to be the most abundant mineral (0.33 g/100 g FW) in *D. asper* shoots from India, followed by magnesium, sodium, and calcium (Pandey and Ojha, 2014).

Both shoots contained low amounts ($< 4 \mu\text{g}/100 \text{ g DW}$) of chromium, copper, manganese, and selenium. To the best of our knowledge, chromium is reported here for the first time from the shoots of *D. asper*. Chromium serves as a cofactor of insulin (chromodulin) and facilitates the binding of insulin to its receptor (Pechova and Pavlata, 2007). The human daily requirement for chromium is $5 \mu\text{g}/\text{day}$ (Prashanth et al., 2015). Iron, zinc, manganese, copper, and selenium have been reported from the fresh juvenile shoots of *D. asper* from India (Nirmala et al., 2007). These minerals are constituents or cofactors of many critical enzymes involving in the biological functions of body cells (Bender, 2012).

Phytochemical content

Extracts from *D. asper* bamboo shoots were subjected to qualitative screening for eight types of phytochemicals. Table 2 shows that DP and DS shoots contained different types of phytochemicals. Only alkaloids, flavonoids, and tannins were detected from DP shoots, while DS shoots contained phenolic acids, anthraquinones, and coumarins, besides alkaloids and tannins. The TPC of all extracts of DP shoots were 1.2 - 3.0-fold and significantly lower ($p < 0.05$) than that of the DS shoots (Table 2)

Baguistan et al. (2017) reported the presence of cardiac glycosides, flavonoids, saponins, and terpenoids, but noted the absence of tannins and alkaloids in both ethanol and hot water extracts of *D. asper* shoots from the Philippines. In contrast, the present work did not find any saponins and triterpenoids in all extracts of DP and DS shoots. The phytochemical profile of a plant can be influenced by factors such as latitude, longitude, rainfall, temperature, quality of soil, habitats, and agricultural practices (Dhami and Mishra, 2015).

Antimicrobial activity

The extracts of *D. asper* shoots were evaluated for antimicrobial activity against selected human pathogens, consisting of six species of bacteria and six species of fungi. All extracts of DP shoots showed bacteriostatic activity against *B. cereus*,

S. aureus, *A. baumannii*, *E. coli*, and *K. pneumoniae* with a MIC range of 0.63 to 2.50 mg/mL while *P. aeruginosa* was only inhibited by the ethanol extract. By contrast, all extracts of the DS shoots (except hexane extract for *A. baumannii*) were active against all the six bacteria, with a lower MIC range of 0.16 to 2.50 mg/mL. However, extracts from both shoots exhibited limited bactericidal activity against the tested bacteria. None of the extracts were able to kill *A. baumannii* and *E. coli*. Our findings concur with the study by Baguistan et al. (2017) who reported the antibacterial activity of the ethanol and hot water extracts of *D. asper* shoots from the Philippines against *S. aureus* and *E. coli* using disc diffusion method.

The lowest MIC value (0.16 mg/mL) was shown by the water extract of DS shoots against *S. aureus*, suggesting the phenolic acids and tannins that present in the extract (Table 2) may work together for the antibacterial effect against this species. Phenolic acids inhibit the growth of bacteria by altering the membrane permeability of bacterial cells, thus allowing the diffusion of the undissociated acids across the membrane which leads to the acidification of the cytoplasm, and probably inducing cell death (Cueva et al., 2010). Tannins exert antibacterial effect by blocking the essential extracellular microbial enzymes, causing deprivation of the substrates needed for cell proliferation, and inhibition of oxidative phosphorylation, which eventually affects microbial metabolism (Barbieri et al., 2017).

The present work reports the antifungal activity of *D. asper* shoot extracts against human fungal pathogens. To the best of our knowledge, this has not been previously investigated. Extracts of DP and DS shoots showed similar antifungal activity against three species of yeasts (*C. albicans*, *C. parapsilosis*, and *C. neoformans*) and two species of filamentous fungi (*T. interdigitale* and *T. rubrum*) with MIC and MFC ranges of 0.08 - 2.50 and 0.16 - 2.50 mg/mL, and 0.08 - 1.25 and 0.08 - 2.50 mg/mL, respectively. In contrast, the growth of *A. fumigatus* could only be inhibited by the ethyl acetate extract of DS shoots. These results indicate that *D. asper* shoots exhibit more pronounced killing effects on fungi than on bacteria. Based on the phytochemical content results (Table 2), flavonoids and tannins in the extracts of DP shoots, and phenolic acids, anthraquinones, tannins, and coumarins in the extracts of DS shoots are likely to account for the antifungal activity of the shoots. These phytochemicals are important classes of secondary metabolites with antifungal property against *Candida* spp., *Cryptococcus* spp., and *Trichophyton* spp. (Arif et al., 2009; Lopes et al., 2017).

Table 2. Phytochemical constituents and total phenolic content of the extracts of *Dendrocalamus asper* bamboo shoots.

Extract	Phytochemicals										Total Phenolic Content (mg GAE/g sample)
	Alkaloids	Phenolic acids	Flavonoids	Saponins	Anthraquinones	Tannins	Triterpenoids	Coumarins			
<i>D. asper</i> from Perak (DP)											
Hexane	-	-	+	-	-	+	-	-	-	-	10.28 ± 0.19 ^{a*}
Ethyl acetate	+	-	+	-	-	+	-	-	-	-	13.23 ± 0.22 ^{b*}
Ethanol	-	-	+	-	-	+	-	-	-	-	15.96 ± 0.17 ^{c*}
Water	-	-	+	-	-	+	-	-	-	-	15.74 ± 0.15 ^{c*}
<i>D. asper</i> from Sabah (DS)											
Hexane	+	-	-	-	+	-	-	-	-	+	31.23 ± 0.21 ^{a*}
Ethyl acetate	-	-	-	-	+	-	-	-	-	+	34.08 ± 0.32 ^{b*}
Ethanol	-	-	-	-	-	+	-	-	-	-	19.46 ± 0.11 ^{c*}
Water	-	+	-	-	-	+	-	-	-	+	23.79 ± 0.10 ^{d*}

'+' = presence of phytochemical, '-' = absence of phytochemical, GAE = gallic acid equivalent. Means with different superscript letters among the extracts are significantly different ($p < 0.05$) by one-way ANOVA test. Means with asterisk are significantly different ($p < 0.05$) between the DS and DP shoots by Student's *t*-test.

Antioxidant activity

Two chemical-based antioxidant assays, namely DPPH radical scavenging activity and ferric-reducing antioxidant power (FRAP), and a cell-based assay (cellular antioxidant assay; CAA) were employed to examine the antioxidant potential of *D. asper* shoots. The DPPH radical scavenging activity of DP and DS shoots was found to be concentration-dependent. Generally, the ethanol and water extracts of both shoots exhibited higher DPPH radical scavenging capacity than the hexane and ethyl acetate extracts. This observation is further supported by their IC_{50} values, as shown in Table 3. The lowest IC_{50} value for DP shoots was shown by its ethanol extract, while water extract yielded the lowest IC_{50} value for DS shoots.

The FRAP values for the extracts, expressed as mM Fe^{2+} equivalent/g sample, are tabulated in Table 3. The ethanol and water extracts of both shoots showed approximately 2.0 - 3.5-fold higher ferric-reducing ability than the hexane and ethyl acetate extracts. The ethanol extracts of DP and DS shoots had the highest FRAP value.

Based on CAA units, the strength of antioxidant activity of the extracts against peroxy radical-induced oxidation in HeLa cells, in decreasing order, was water, hexane, ethanol, and ethyl acetate extracts for DP shoots, and ethyl acetate, hexane, ethanol, and water extracts for DS shoots. All extracts of DP shoots produced higher CAA units as compared to the extracts of DS shoots (67.3 - 83.6% vs 40.6 - 61.2%). The results indicated that the antioxidant capacity of *D. asper* shoots based on the CAA assay did not correspond to the DPPH radical scavenging activity and FRAP assays. This confirms earlier findings that a high antioxidant value in chemical assays does not contribute for a high CAA value, and *vice versa* (Wolfe and Liu, 2007; Bender *et al.*, 2014). CAA assay is greatly dependent on the physicochemical properties of phytochemicals and their feasibilities of uptake, distribution, and metabolism in a cellular environment, which reflects a more relevant physiological condition (Bender *et al.*, 2014).

The phytochemical analysis (Table 2) indicates that flavonoids and tannins in DP shoots,

Table 3. The half-maximum inhibitory concentration (IC_{50}) values for different assays and the ferric-reducing antioxidant power (FRAP) values of the extracts of *Dendrocalamus asper* bamboo shoots.

Sample	DPPH radical scavenging assay	Ferric-reducing antioxidant power value	α -amylase inhibitory activity	α -glucosidase inhibitory activity
	IC_{50} (μ g/mL)	(mM Fe^{2+} equivalent/g sample)	IC_{50} (μ g/mL)	IC_{50} (μ g/mL)
<i>D. asper</i> from Perak (DP)				
Hexane	-	461.71 \pm 56.61 ^a	8.91 \pm 2.36 ^a	-
Ethyl acetate	-	333.81 \pm 98.86 ^a	16.52 \pm 2.70 ^a	653.27 \pm 125.25 ^a
Ethanol	473.18 \pm 9.22 ^a	1099.55 \pm 14.00 ^b	92.70 \pm 10.75 ^b	76.55 \pm 0.50 ^b
Water	663.93 \pm 140.39 ^a	911.67 \pm 41.11 ^c	52.95 \pm 13.79 ^c	-
<i>D. asper</i> from Sabah (DS)				
Hexane	-	308.90 \pm 18.68 ^a	39.78 \pm 3.29 ^a	618.15 \pm 42.62 ^a
Ethyl acetate	-	392.93 \pm 36.14 ^a	319.29 \pm 10.79 ^b	505.56 \pm 13.46 ^b
Ethanol	356.11 \pm 6.44 ^a	1072.61 \pm 137.07 ^b	25.89 \pm 5.52 ^c	65.88 \pm 0.43 ^c
Water	173.27 \pm 12.01 ^b	909.81 \pm 179.68 ^b	-	432.35 \pm 5.57 ^d
Ascorbic Acid	18.86 \pm 0.69	-	-	-
Gallic Acid	28.51 \pm 1.62	-	-	-
Acarbose	-	-	13.92 \pm 0.42	95.12 \pm 7.50

Data are means \pm standard deviations of triplicates ($n = 3$). '-' = not determined. Means with different superscript letters among the extracts are significantly different ($p < 0.05$) by one-way ANOVA test.

and anthraquinones, coumarins, and tannins in DS shoots are likely to account for the antioxidant activities. The presence of flavonoids in the DP shoots but their absence in the DS shoots may account for the higher CAA values in the DP shoots. Polyphenols such as flavonoids, anthraquinones, and coumarins are potent antioxidants due to the presence of hydroxyl or phenolic hydroxyl groups in their structures which can scavenge radicals by donating their electrons (Yen *et al.*, 2000; Torres *et al.*, 2014; Khanum *et al.*, 2015). Tannins have the ability to chelate metal ions, such as Fe(II), and thus interfere with the Fenton reaction retarding oxidation (Karamac, 2009).

Enzyme inhibitory activity

Extracts of *D. asper* shoots were assessed for antidiabetic potential based on the inhibitory activity on the two carbohydrate hydrolysing enzymes; α -amylase and α -glucosidase. The percentage inhibition curves (Figure 1) illustrated a concentration-dependent inhibitory effect of the shoots on α -amylase. At the highest concentration tested (1,000 $\mu\text{g/mL}$), all the extracts of DP and DS shoots were able to inhibit > 75% of α -amylase activity, except for the water

extract of DS shoot which only showed approximately 50% of inhibition. For DP shoots, the hexane extract showed the strongest inhibitory activity with the lowest IC_{50} value, while for DS shoots, the ethanol extract yielded the lowest IC_{50} value (Table 3).

For α -glucosidase activity, unlike the extracts of DS shoots which showed a concentration-dependent inhibitory effect, the inhibitory effect for hexane extracts of DP shoots was only noticeable when the concentration exceeded 250 $\mu\text{g/mL}$, and for ethyl acetate and water extracts when the concentration was $\geq 500 \mu\text{g/mL}$ (Figure 2). For both shoots, the ethanol extract exhibited the strongest α -glucosidase inhibitory activity with the lowest IC_{50} value; 76.55 $\mu\text{g/mL}$ and 65.88 $\mu\text{g/mL}$ for DP and DS, respectively (Table 3). Both values were even lower than that of acarbose ($\text{IC}_{50} = 95.12 \mu\text{g/mL}$), which was used as the positive control for the assay. The results suggest that alkaloids, flavonoids, and tannins in DP shoots, and alkaloids and tannins in DS shoots are responsible for the strong inhibitory activity on the two carbohydrate hydrolysing enzymes. Many phenolic compounds from plants, especially flavonoids and tannins possess inhibitory effect on α -amylase (Sales *et al.*, 2012; Etxeberria *et al.*, 2012). Similarly, many

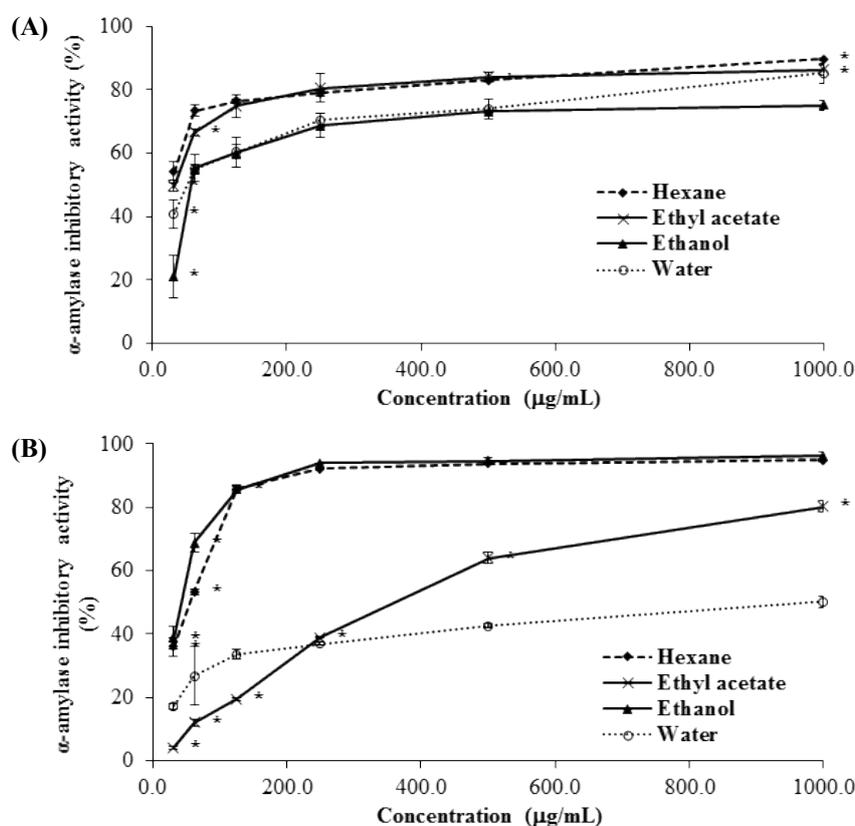


Figure 1. α -amylase inhibitory activity of the extracts of *Dendrocalamus asper* shoots obtained from the states of (A) Perak (DP shoot), and (B) Sabah (DS shoot). Data are means \pm standard deviations of triplicates ($n = 3$). Means with asterisk are significantly different ($p < 0.05$) by one-way ANOVA test.

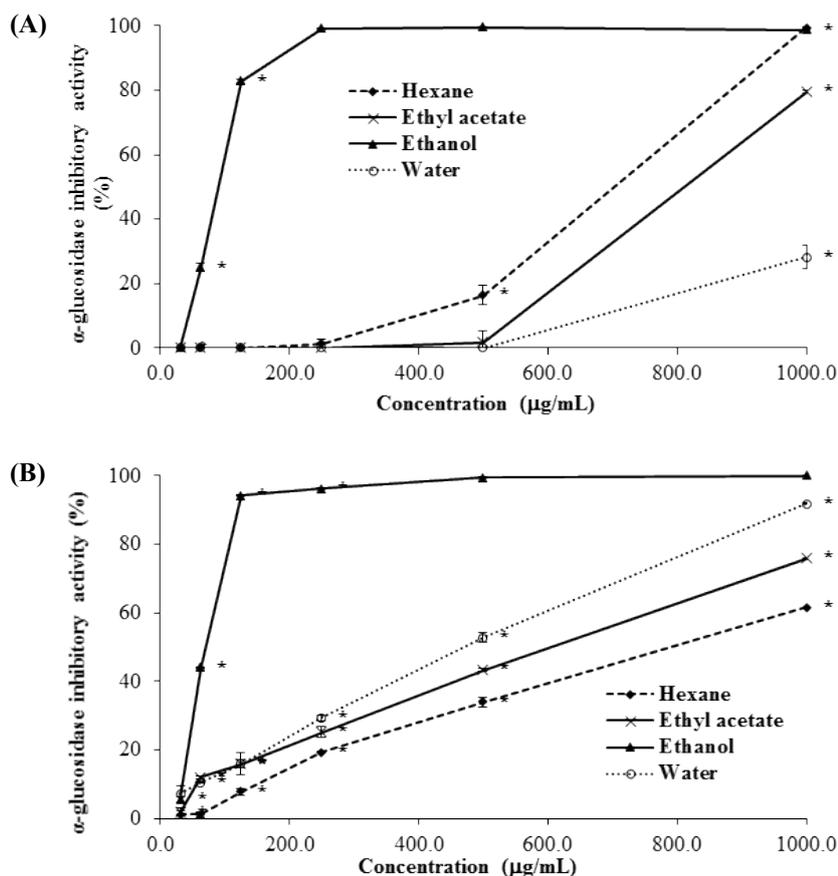


Figure 2. α -glucosidase inhibitory activity of the extracts of *Dendrocalamus asper* shoots obtained from the states of (A) Perak (DP shoot), and (B) Sabah (DS shoot). Data are means \pm standard deviations of triplicates ($n = 3$). Means with asterisk are significantly different ($p < 0.05$) by one-way ANOVA test.

α -glucosidase inhibitors that are phytochemicals, such as flavonoids, alkaloids, terpenoids, anthocyanins, glycosides, and phenolic compounds have been isolated from plants (Kumar *et al.*, 2011).

Conclusion

The potential of *D. asper* shoots as a source for nutrition is highlighted in the present work. *D. asper* shoots obtained from the Peninsular Malaysia (DP) had higher moisture, crude fat, and crude fibre contents, but lower protein and mineral (magnesium, sodium, chromium, and zinc) concentrations than the shoots obtained from the East Malaysia (DS). The extracts of both shoots showed similar antifungal activity, and this was stronger than antibacterial activity. However, the DP shoots demonstrated higher cellular antioxidant activity in HeLa cells and α -amylase inhibitory activity, but lower total phenolic content and inhibitory activity with α -glucosidase than that of DS shoots. The DP and DS shoots obtained from different geographical regions revealed different types of secondary metabolites which might have accounted for the differences in the biological

activities. Nevertheless, the results suggest that *D. asper* can be utilised as a nutritional and functional food.

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