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Native Australian fruits — a novel source of antioxidants for food

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Abstract

Twelve native Australian fruits, finger lime (red and yellow), riberry, brush cherry, Cedar Bay cherry, muntries, Illawarra plum, Burdekin plum, Davidson's plum, Kakadu plum, Molucca raspberry and Tasmanian Pepper, were investigated for their antioxidant capacity and presence of phenolic compounds, anthocyanins and ascorbic acid. The radical scavenging activities of five of the evaluated fruits were significantly higher (3.1 to 5.2-fold in the TEAC assay and 1.2 to 4.2-fold in the PCL assay, respectively) than that of the control blueberry, cv. Biloxi. The total phenolics level (Folin–Ciocalteu assay) in six of the twelve fruits was 2.5 to 3.9-fold of that of blueberry. Kakadu plum was identified as the richest source of ascorbic acid (938-fold of that of control). A high correlation between total phenolics (but not anthocyanins) and antioxidant capacity was observed. The HPLC-DAD/ESI/MS-MS profiles revealed simple anthocyanin composition (one to four individual pigments) with cyanidin as the dominating type. Australian native fruits investigated in this study are shown to be a novel rich source of antioxidant compounds.

Keywords: Native Australian fruits; Antioxidant capacity; Total phenolics; Anthocyanins; Ascorbic acid

Industrial relevance: The search for world unique food ingredients and flavors with enhanced health-beneficial properties is at present one of the key market trends. Botanicals from the regions linked to wellness and natural functionality with exotic fruits called "superfruits", such as acai from Amazonia, are becoming a popular target of health-conscious consumers and industry managers. Sustainability and responsibility for the environment is another important reason which brings a commitment to ethical products. Utilization of local and seasonal fruits will not only enhance the variety of exotic fruits available on international market, but will contribute toward sustainable agriculture. Our research program addresses all these essential issues. In this manuscript we are describing for the first time twelve native Australian fruits as a rich source of antioxidants. We propose these exotic fruits to be considered as a potential source of bioactive phytochemicals for application in health promoting foods.

1. Introduction

To the indigenous people of Australia, the Aboriginals, edible native Australian fruits have served as a source of food and medicine for thousands of years (Roberts, Jone & Smith, 1990). The number of fruiting rainforest edible plants in north-eastern Australia (Queensland) has been reported to access 2400 (Cooper, 2004). The native fruits come with various shapes, size, colours and tastes (Cooper, 2004), and were reported to possess unique nutritious and organoleptic characteristics (Hodgson & Wahlqvist, 1992). Undervalued by the modern Australians over many years, recently the native edible plants (bushfood plants) are becoming popular. Over the last ten years a number of commercially significant crops have been identified and research on their propagation, breeding and cultivation has been undertaken (Ahmed & Johnson, 2000).

At present in Australia there are 77 growers of native fruits and spices who offer 91 primary products (Rogers & Rogers, 2005). Cultivation of selected commercially promising native crops in Australia has already been initiated (e.g. riberry, Illawarra plum, Davidson's plum). Commercial production alone (beside collection from wild) offers about 50 tonnes per annum with a wholesale price of A$30–35/kg (V. Cherikoff, personal communication). Fresh fruits as well as their products are available from the growers, in local supermarkets, restaurants and are sold overseas. To date only limited data on nutritious values of the native fruits have been generated. Selected bushfoods were evaluated predominantly for the presence of protein, fat, carbohydrate, fibre, ash, energy,

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minerals and vitamins (Brand, Rae, McDonnell, Lee, Cherikoff & Truswell, 1983). Some bushfoods were evaluated for their "survival value" (the "value" of a plant or fruit as a source of essentials (e.g. nutrients, vitamins and minerals) to keep someone alive in the bush) by the Commonwealth Defence Science Organisation in Australia (James & Forbes-Evan, 1982).

In recent years consumption of fruits and vegetables has been associated with the prevention of modern life-style related degenerative diseases (Liu, 2003). The presence of dietary polyphenols in fruits and vegetables was found to contribute towards maintaining a good health. Polyphenols are bioactive phytochemicals with strong antioxidant activity (Rice-Evans, 2001; Rice-Evans, Miller & Paganga, 1996; Scalbert, Johnson & Saltmarsh, 2005) and play an active role in the prevention of degenerative diseases such as cancer (Hertog, Bueno de Mesquita, Fehily, Sweetnam, Elwood & Kromhout, 1996; Lambert, Hong, Yang, Liao & Yang, 2005) and cardiovascular diseases (Geleijnse, Launer, Van der Kuip, Hofman & Witteman, 2002; Vita, 2005). Polyphenols were also found to exert neuroprotective (Youdim, Spencer, Schroeter & Rice-Evans, 2002) and anti-diabetic actions (Matsui et al., 2002; Tsuda, Horio, Uchida, Aoki & Osawa, 2003) and may contribute to the reduction of obesity (Tsuda et al., 2003). They protect cell constituents against oxidative damage through scavenging free radicals and thereby avert their deleterious effects on nucleic acids, proteins and lipids (Rice-Evans, 2001; Rice-Evans et al., 1996). Recently direct interactions of dietary plant polyphenols with receptors or enzymes involved in signal transduction have also been reported (Moskaug, Carlsen, Myhrstad & Blomhoff, 2005). Therefore, the incorporation of extracts rich in polyphenols into food could provide health benefits beyond those attributable to basic nutritional functions.

To utilize local resources for the development of novel foods with potential health-protective properties we undertook a systematic study on polyphenolic complexes in selected native Australian fruits. Our preliminary data on seven native Australian fruits indicated that selected fruits possess higher levels of phenolic compounds as well as stronger radical scavenging activities (DPPH assay) and total reducing capacities (FRAP assay) than blueberry (Netzel, Netzel, Tian, Schwartz & Konczak, 2006). Exploring unknown plants as sources of bioactive phytochemicals offers enormous opportunities for the functional food industry. Therefore, our study has been extended and new commercially grown fruits were included. Here we report on antioxidant capacity evaluated in TEAC and PCL assays as well as the levels of total phenolics, anthocyanins, and ascorbic acid in fruit extracts.

2. Materials and methods

2.1. Plant material

Fully ripened fruits of riberries (*Syzygium luehmannii,* Myrtaceae) and brush cherry (*Syzygium australe*, Myrtaceae) were collected from trees growing in suburbs of Sydney, NSW. Muntries (*Kunzea pomifera* F. Muell., Myrtaceae), Illawarra plum (*Podocarpus elatus* R. Br. ex Endl., Podocarpaceae), Burdekin plum (*Pleiogynium timorense* DC. Leenh, Anacardiaceae), Cedar Bay cherry (*Eugenia carissoides* F. Muell., Myrtaceae), Davidson's plum (*Davidsonia pruriens* F. Muell. var. *pruriens*, Davidsoniaceae), Molucca raspberry (*Rubus moluccanus* var. *austropacificus* van Royen, Rosaceae) and finger lime (yellow and red, *Microcitrus australasica*, Rutaceae) were purchased from the Australian Native Foods "Playing with Fire", Teven, NSW. Tasmanian Pepper (*Tasmanian lanceolata* R. Br., Winteraceae) was purchased from the grower Russell Langfield in Kimberley, Tasmania. Kakadu plum (*Terminalia ferdinandiana*, Combretaceae) was obtained from Coradji Pty. Ltd., Annandale, NSW. Blueberries (*Vaccinum spp.* cv. Biloxi) used as a control were obtained from the Blueberry Farm of Australia in Corrindi, NSW. Fresh fruits were snap-frozen in liquid nitrogen, freeze-dried and stored at −20 °C until analyzed.

2.2. Reagents and standards

Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich (Sydney, Australia) or Merck (Darmstadt, Germany) and were of analytical or HPLC grade. Cyanidin 3-glucoside, cyanidin 3-sambubioside, cyanidin 3-rutinoside, and cyanidin 3,5-diglucoside were purchased from Polyphenols Laboratories AS (Hanaveien, Norway). Deionized water was used throughout.

2.3. Sample preparation

The samples were prepared according to Kammerer, Claus, Carle and Schieber (2004) with modifications. Freeze-dried fruits were finely ground. A 200 mg portion of the pulverized fruits were weighed into test tubes and extracted with 3 mL of acidified methanol (80% methanol, 19.9% H_2O and 0.1% HCl, v/v/v) 2 h by stirring under a nitrogen atmosphere to prevent oxidation. The extracts were centrifuged (10 min, 5000 rpm), and the material was re-extracted two times with 3 mL of the organic solvent. Aliquots (9 mL) of the combined supernatants were stored frozen (−20 °C) until analyzed. For anthocyanin identification (LC/ESI/MS-MS), approximately 1 mg of lyophilized tissue was extracted twice with 10 ml of deionized water containing 5% formic acid and vortexed for 2 min. The suspensions were centrifuged at 4000 rpm for 10 min, the supernatants were combined and filtered through a 0.20 μm nylon filter.

2.4. Antioxidant capacity

The antiradical properties of the fruit extracts were determined using the Trolox Equivalent Antioxidant Capacity (TEAC) assay and the Photochemiluminescence (PCL) assay. The TEAC assay is based on the reduction of the ABTS (2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid)) radical cation by antioxidants (Re, Pellegrini, Proteggente, Pannala, Yang & Rice-Evans, 1999) and was adapted with minor modifications (Schlesier, Harwat, Böhm & Bitsch, 2002). The ABTS radical cation was prepared by mixing ABTS stock solution (7 mM in water) with 2.45 mM potassium persulfate. This mixture has to remain for 12–24 h until the reaction is complete and the

absorbance is stable. For the photometric assay 1 ml of the $ABTS^{\bullet+}$ solution and 100 μl of antioxidant solution (sample) were mixed for 45 s and measured immediately after 1 min at 734 nm. The antioxidant capacity of the samples was calculated by determining the decrease in absorbance at different concentrations. Results were expressed as micromoles of Trolox equivalents (TE) per gram of fresh weight (μmol TE/g FW).

In the PCL assay (Popov & Lewin, 1999; Popov & Lewin, 1994) the photochemical generation of free radicals is combined with the sensitive detection by using chemiluminescence. This reaction is induced by optical excitation of a photosensitiser which results in the generation of superoxide radicals $O_2^{\bullet-}$. The free radicals are visualised with a chemiluminescent detection reagent. Luminol works as photosensitiser as well as oxygen radical detection reagent. This reaction takes place in the Photochem®. The antioxidant potential of the samples was evaluated by measuring the lag phase and the results were expressed as micromoles of Trolox equivalents (TE) per gram of fresh weight (μmol TE/g FW).

2.5. Total phenolics

The total phenolic content was determined using the Folin–Ciocalteu assay (Singleton & Rossi, 1965). Diluted fruit extracts were directly assayed at 750 nm with gallic acid serving as a standard. Results were expressed as micromoles of total phenolics (gallic acid equivalents, GAE) per gram of fresh weight (μmol GAE/g FW).

2.6. Ascorbic acid

The centrifuged (5 min, 10.000 rpm) and diluted samples (1:10 with 5% meta-phosphoric acid) were directly injected onto a Phenomenex Luna C18 column protected by a Phenomenex 4.0×3.0 mm i.d. C18 ODS guard column (Phenomenex, Penant Hills, NSW, Australia) and eluted under isocratic conditions with water acidified with sulphuric acid to pH 2.2 following the method of Vazquez-Oderiz, Vazquez-Blanco, Lopez-Hernandez, Simal-Lozano and Romero-Rodriguez (1994). Detection was carried out at 245 nm at a flow rate of 0.8 mL/min. Ascorbic acid (fruit extracts) was identified by comparing the retention time and characteristic UV/Vis spectra with those of synthetic L-ascorbic acid (HPLC system II). It was quantified using a L-ascorbic acid calibration curve and calculated as micromoles per gram of fresh weight (μmol/g FW).

2.7. Identification of anthocyanins (system I: HPLC-DAD/ESI/MS-MS)

Identification of anthocyanins was conducted using a LC-MS system consisting of a Waters 2695 gradient HPLC separation module, an auto-injector, a 996 diode array ultraviolet–visible (UV/Vis) absorbance detector (Waters Corporation, Milford, MA, USA), and a triple quadrupole ion-tunnel mass spectrometer (Quattro Ultima, Micromass Limited, Manchester, UK) equipped with a Z-spray electrospray ionization (ESI) source. Calibration of the mass spectrometer was performed using sodium iodide and caesium iodide. HPLC analysis was performed on a 150×3.0 mm i.d., 3 μ Atlantis dC_{18} column (Waters Corporation, Milford, MA, USA) at 35 °C. The solvent system consisted of a gradient mobile phase from 5% B to 20% B in 25 min, then to 5% B in 5 min. Solvent A was formic acid:water (5/95, v/v) and solvent B was acetonitrile:formic acid (95/5, v/v). The flow rate was set at 0.6 mL/min. UV/Vis spectra of anthocyanins were recorded from 200 to 600 nm using the in-line diode array detector. Cyanidin 3-glucoside, cyanidin 3-sambubioside, cyanidin 3-rutinoside, and cyanidin 3,5-diglucoside were used to tune the mass analyzer for each MS/MS experiment. Standards were also used to further confirm the identities of anthocyanins whenever these compounds were found in the fruit extracts.

For precursor ion analysis experiment, the precursors of all six anthocyanidins, including cyanidin (MW 287), delphinidin (MW 303), malvidin (MW 331), peonidin (MW 301), pelargonidin (MW 271), and petunidin (MW 317), were scanned simultaneously during analysis of all fruit samples. The molecular ions detected during precursor ion scan were further fragmented using product ion to obtain the potential sugar species in the

Table 1
Content of total phenolics, ascorbic acid, and antioxidant capacity (TEAC and PCL) of twelve native Australian fruits and blueberry (cv. Biloxi)

Fruit	Total phenolics μmol GAE/g FW	TEAC μmol TE/g FW	PCL μmol TE/g FW	Ascorbic acid μmol/g FW
Muntries [a]	67.12±4.62	123.82±10.8	49.34±4.29	0.91±0.08
Tasmanian pepper	82.51±5.52	123.18±7.49	129.04±3.90	ND [b]
Molucca raspberry	21.91±0.80	45.09±0.84	4.08±0.23	ND
Davidson's plum	16.75±1.03	36.52±0.93	2.33±0.18	ND
Illawarra plum	68.21±2.30	122.77±4.25	14.61±0.67	ND
Cedar Bay cherry	64.95±4.07	129.53±5.14	51.70±1.89	ND
Burdekin plum [a]	100.50±7.40	191.98±5.85	37.82±1.60	1.29±0.08
Riberry [a]	13.08±0.81	28.07±2.45	8.43±0.30	1.77±0.04
Brush cherry [a]	12.55±0.59	26.95±0.64	3.28±0.19	0.72±0.06
Finger lime (red) [a]	8.65±0.59	13.82±0.96	3.25±0.09	2.32±0.12
Finger lime (yellow) [a]	10.93±0.48	16.24±0.49	5.43±0.12	3.38±0.24
Kakadu plum [a]	74.72±5.23	204.81±1.78	73.90±4.5	71.32±0.39
Blueberry [a]	26.00±0.64	39.45±3.83	30.49±1.96	0.076±0.002

[a] Total phenolic, TEAC, and PCL values of these fruits are corrected for ascorbic acid.
[b] Not detectable.

structure. The UV–Vis spectral data ($A_{Vis\text{-}max}$, $A_{440}/A_{Vis\text{-}max}$, and $A_{280}/A_{Vis\text{-}max}$) were used for additional confirmation of the glycosylation position in the structure (Harborne, 1958).

2.8. Quantification of anthocyanins (system II: HPLC-DAD)

Quantification of anthocyanins in fruit extracts was carried out following the method of Terahara, Konczak-Islam, Nakatni, Yamakawa, Goda and Honda (2000). The HPLC system consisted of two LC-10AD pumps, SPD-M10A diode array detector, CTO-10AS column oven, DGU-12A degasser, SIL-10AD auto-injector and SCL-10A system controller (Shimadzu Co., Kyoto, Japan) equipped with a 250×4.6 mm i.d., 5 μ Luna C18(2) column (Phenomenex). Analytical HPLC was run at 25 °C and monitored at 520 nm. The following solvents in water with a flow rate of 1.0 mL/min were used: A, 1.5% phosphoric acid and B, 1.5% phosphoric acid, 20% acetic acid and 25% acetonitrile. The elution profile was a linear gradient elution for B of 25% to 43% over 30 min in solvent A, followed by 10 min 85% B and then to 25% B in 5 min. Individual anthocyanin compounds were quantified using a cyanidin 3-glucoside calibration curve and were calculated as micromoles of cyanidin 3-glucoside equivalents (CE) per gram of fresh weight (μmol CE/g FW).

2.9. Equipment (antioxidant capacity and total phenolics)

Measurements were performed in disposable cuvettes or microplates or reaction tubes using a spectrophotometer model Uvidec-610 (Jasco, Groß-Umstadt, Germany), a Photochem® device (Analytik Jena AG, Jena, Germany), and a microplate reader model Multiscan RC, version 4 (Labsystems, Finland) operated by the DeltaSoft3 program (Elisa Analysis for the Macintosh with interference for the Multiscan Microplate Readers, BioMetallics, Inc., 1995).

3. Results and discussion

3.1. Antioxidant capacity, total phenolic content and ascorbic acid

Antioxidant capacity as evaluated in the TEAC and PCL assays (Radical Scavenging Activity, RSA) and the levels of

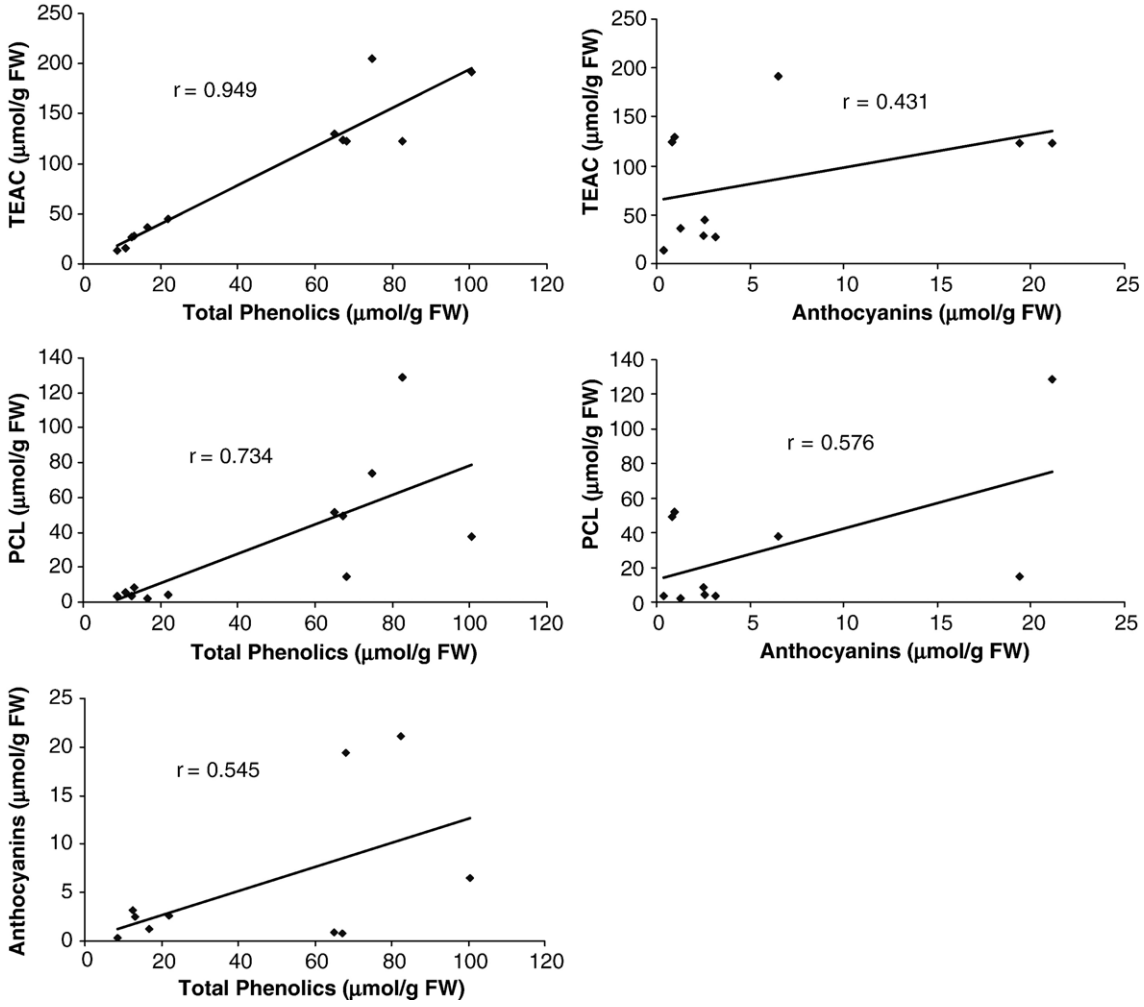

Fig. 1. Relationship between the levels of phenolic compounds and antioxidant capacity for twelve native Australian fruits examined in this study. Total phenolic, TEAC, and PCL values are corrected for ascorbic acid.

total phenolics and ascorbic acid in fruits are shown in Table 1. The total phenolic content ranged from a low of 8.65 μmol GAE/g FW (finger lime, red) to a high of 100.5 μmol GAE/g FW (Burdekin plum). In respect to the total phenolics, Davidson's plum and closely followed Molucca raspberry (16.7 and 21.9 μmol GAE/g FW, respectively) are comparable to blueberries. However, the level of total phenolics in Burdekin plum is 3.9-fold and in muntries, Tasmanian Pepper, Kakadu plum, Illawara plum and Cedar Bay cherry is 2.5 to 3.2-fold of that in blueberries. RSA values ranged from a low of 13.8 μmol TE/g FW for finger lime (red) to a high of 204.8 μmol TE/g FW for the Kakadu plum in the TEAC assay, and from a low of 2.33 μmol TE/g FW for Davidson's plum to a high of 129.0 μmol TE/g FW for Tasmanian Pepper in the PCL assay. Most of the evaluated samples possess higher antioxidant potential than blueberries. On the basis of fresh weight of fruit, the Burdekin plum had the highest total phenolic value (3.9-fold that of the blueberry control), the Kakadu plum the highest TEAC value (5.2-fold that of blueberry), and the Tasmanian Pepper the highest PCL value (4.2-fold that of blueberry). Ascorbic acid was found in amounts of 0.076 (blueberry)–71.3 μmol/g FW (Kakadu plum). The exceptionally high level of ascorbic acid in the Kakadu plum is in agreement with earlier published data (Brand et al., 1983; Brand, Truswell, Lee & Cherikoff, 1982). The values obtained in the TEAC, PCL, and total phenolic assays of blueberry, muntries, Burdekin plum, Kakadu plum, finger lime (red and yellow), brush cherry, and riberry were corrected for ascorbic acid. It should be noted that a significant difference between the corrected and uncorrected values in the TEAC (204.81→221.35 μmol TE/g FW), PCL (73.9→99.60 μmol TE/g FW), and total phenolic (74.72→104.46 μmol GAE/g FW) assays could be observed only in the Kakadu plum. The ascorbic acid effect in the other seven fruits was negligible.

Positive correlations were observed between the TEAC and PCL assays and total phenolics (Fig. 1). Previous studies reported a linear relationship between total phenolic content and antioxidant capacity in berry crops and herbs (Zheng & Wang, 2003; Zheng & Wang, 2001). Our data support these results and indicate that phenolic compounds play a major role as a source of antioxidants in the evaluated native Australian fruits. A variety of phenolic compounds such as flavonoids (anthocyanins, flavan-3-ols, etc.) and phenolic (benzoic and cinnamic) acids are considered to contribute to this activity.

3.2. Anthocyanins in selected native Australian fruits

Anthocyanins in fruit extracts, separated and tentatively identified by HPLC/ESI/MS-MS, are presented in Table 2. A

Table 2
Composition of anthocyanins (%) in ten native Australian fruits and blueberry (cv. Biloxi) tentatively identified by HPLC-ESI/MS-MS

Anthocyanins[a]	MS-MS fragment ions, *m/z*	Native Australian fruits[a]									
		M	TP	MR	DP	IP	CBC	BP	R	BC	FL
C3G	449,287	73.9	27.0	14.3		99.5	100	100	11.9		68.4
C3R	595,449,287		73.0	81.2							
C3S	581,287				60.1						
C3,5dG	611,449,287								88.1		
D3G	465,303	26.1									
D3S	597,303				21.5						
D3,5dG	627,465,303									6.6	
P3G	433,271					0.5					
P3R	579,433,271			4.5							
Peo3G	463,301										31.6
Peo3S	595,301				14.8						
Peo3,5dG	625,463,301									3.8	
Pet3S	611,317				3.6						
Pet3,5dG	641,479,317									13.3	
M3,5dG	655,493,331									76.3	
Total amount (relative) [%][b]		100	100	100	100	100	100	100	100	100	100
Total amount (absolute) [μmol CE/g FW][c]		0.84±0.06	21.13±1.81	2.57±0.02	1.27±0.04	19.39±1.00	0.97±0.03	6.07±0.51	2.52±0.07	3.17±0.06	0.38±0.04

Blueberry	sum of delphinidin 3-galactoside, delphinidin 3-glucoside, cyanidin 3-galactoside, delphinidin 3-arabinoside, cyanidin 3-glucoside, petunidin 3-galactoside, cyanidin 3-arabinoside, petunidin 3-glucoside, peonidin 3-galactoside, petunidin 3-arabinoside, peonidin 3-glucoside, malvidin 3-galactoside, malvidin 3-glucoside, and malvidin 3-arabinoside	Total amount (absolute) [μmol CE/g FW][c]	11.51±0.30

[a] Abbreviations: *Anthocyanins:* cyanidin 3-glucoside (C3G), cyanidin 3-rutinoside (C3R), cyanidin 3-sambubioside (C3S), cyanidin 3,5-diglucoside (C3,5dG), delphinidin 3-glucoside (D3G), delphinidin 3-sambubioside (D3S), delphinidin 3,5-diglucoside (D3,5dG), pelargonidin 3-glucoside (P3G), pelargonidin 3-rutinoside (P3R), peonidin 3-glucoside (Peo3G), peonidin 3-sambubioside (Peo3S), peonidin 3,5-diglucoside (Peo3,5dG), petunidin 3-sambubioside (Pet3S), petunidin 3,5-diglucoside (Pet3,5dG), malvidin 3,5-diglucoside (M3,5dG). *Native Australian fruits:* muntries (M), Tasmanian Pepper (TP), Molucca raspberry (MR), Davidson's plum (DP), Illawarra plum (IP), Cedar Bay cherry (CBC), Burdekin plum (BP), riberry (R), brush cherry (BC), finger lime red (FL).

[b] The relative amount of individual anthocyanins with respect to the total amount (=100%).

[c] Anthocyanins were quantified by HPLC-DAD and total anthocyanin levels (absolute) were expressed in cyanidin 3-glucoside equivalents (CE).

total of fifteen different anthocyanidin glycosides were detected across the ten cultivars (Kakadu plum and finger lime (yellow) contained no anthocyanins). The composition of anthocyanins showed that the predominant sugars, glucose and sambubiose, were combined with cyanidin, delphinidin, malvidin, pelargonidin, peonidin, and petunidin at the C-3 and C-5 position (Table 2 and Fig. 2). Beside glucose and sambubiose, rutinose was identified as the third sugar moiety. The anthocyanin analysis revealed relatively simple anthocyanin profiles in all samples, consisting of one to four individual pigments. The main components of anthocyanin mixtures were predominantly cyanidin-based anthocyanins (Table 2). Cyanidin 3-glucoside was the main anthocyanidin glucoside in muntries, finger lime (red), Illawarra plum, Burdekin plum, and Cedar Bay cherry. Among the four anthocyanin compounds detected in Davidson's plum, cyanidin 3-sambubioside was the main pigment accounting for 60.1% of the total anthocyanin content. The three minor peaks were identified as delphinidin 3-sambubioside (21.5% of total anthocyanin content), peonidin 3-sambubioside (14.8% of total anthocyanin content), and petunidin 3-sambubioside (3.6% of total anthocyanin content), respectively. Malvidin 3,5-diglucoside was the main pigment in brush cherry, and cyanidin 3,5-diglucoside in riberry with 76.3 and 88.1% of total anthocyanins, respectively. The remaining two species, Tasmanian Pepper and Molucca raspberry, both contained cyanidin 3-rutinoside as the primary anthocyanin (73 and 81.2% of total anthocyanin content, respectively) with either low amounts of cyanidin 3-glucoside (27%, Tasmanian Pepper) or small amounts of cyanidin 3-glucoside and pelargonidin 3-rutinoside (14.3 and 4.5%, respectively, Molucca raspberry). The Tasmanian Pepper and the Illawarra plum exhibited by far the highest level of total anthocyanins (sum of all quantifiable anthocyanidin glycosides), whereas the muntries and finger lime (red) showed the least amount (Table 2). Compared to blueberries (cv. Biloxi), the total anthocyanin content of Tasmanian Pepper and Illawarra plum was about 1.8 and 1.7-fold higher, whereas the total anthocyanin content of Burdekin plum, brush cherry, Molucca raspberry, riberry, Davidson's plum, Cedar Bay cherry, muntries, and finger lime (red) was only 52.7, 27.5, 22.3, 21.9, 11.0, 8.4, 7.3, and 3.3% of that in the Biloxi blueberry, respectively. The levels of total anthocyanins in Tasmanian Pepper (21.13 μmol/g FW) and Illawarra plum (19.39 μmol/g FW) were higher than in most well-known berries (including blueberries from different cultivars). Taruscio, Barney and Exon (2003) analyzed anthocyanin levels in nine *Vaccinium* species from either cultivated or undomesticated colonies within the northwestern United States. The concentrations ranged from 0.25 to 8.11 μmol CE/g FW. A content of 0.71–9.53 μmol CE/g FW was found by Zheng and Wang (2003) in cranberry (cv. Ben Lear), lingonberry (cv. Amberland), blueberry (cv. Serra), and chokeberry (wild), respectively. The total anthocyanin contents of strawberry (*Fragaria x ananassa* Duch., cv. Kent), raspberry (*Rubus idaeus* Michx., cv. Nova), highbush (*Vaccinium corymbosum* L., cv. Bluecrop) and lowbush blueberry (*Vaccinium angustifolium* Aiton) ranged between 0.155 [strawberry] and 4.35 [lowbush blueberry] μmol malvidin 3-

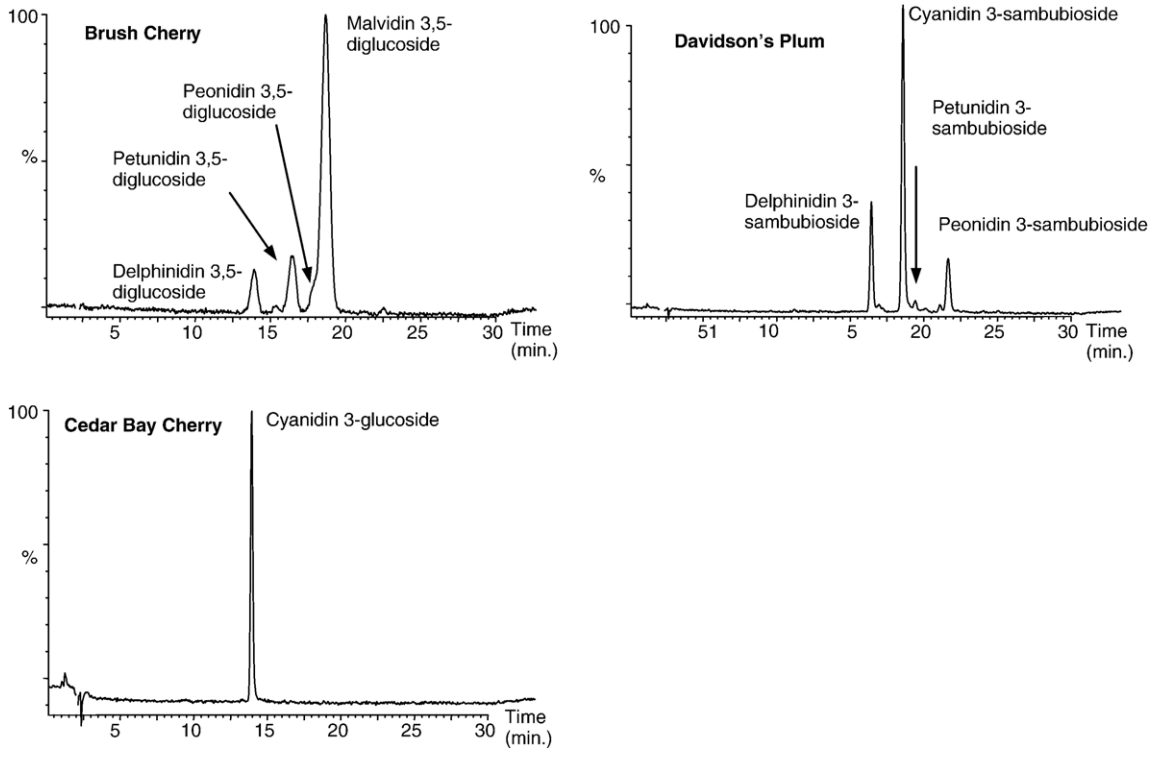

Fig. 2. Reverse-phase HPLC chromatograms of anthocyanins detected at 520 nm from three selected native Australian fruits. Brush cherry (contains four different anthocyanidins with glucose attached to the C-3 and C-5 position), Davidson's plum (contains four different anthocyanidins with sambubiose attached to the C-3 position), and Cedar Bay cherry (contains only cyanidin 3-glucoside).

glucoside equivalents/g FW as reported by Kalt, Forney, Martin and Prior (1999). These levels are about 99.3 and 77.6% lower than the values determined for Tasmanian Pepper and Illawarra plum in the present study. Similarly, in the study by Zheng, Wang, Wang and Zheng (2003) the levels of total anthocyanins in highbush blueberries (*Vaccinium corymbosum* L. ev. Duke) were 80.8 and 79.1% lower than that of Tasmanian Pepper and Illawarra plum, respectively.

The correlation coefficients between the levels of anthocyanins and TEAC, PCL, and total phenolic assays were low (Fig. 1). This result clearly indicates that, contrary to other crops such as berries (Zheng & Wang, 2003), red-fleshed sweetpotato and purple corn (Cevallos-Casals & Cisneros-Zevallos, 2003), other phenolic compounds may play major roles as sources of antioxidant activities in the selected native Australian fruits. Of our interests are especially Cedar Bay cherry, muntries and Burdekin plum. The first two fruits accumulate anthocyanins at very low levels: 0.97 and 0.84 μmol CE/g FW, respectively. These levels are approximately 12–14 times lower than in blueberry. However, total phenolics and radical scavenging activity of these fruits are 2.5-fold and 1.6–3.3-fold those of the control blueberry, respectively. The level of anthocyanins in Burdekin plum is about 50% of that in blueberry and the antioxidant capacity is 1.2-fold and 4.9-fold higher (PCL and TEAC assay, respectively). In order to identify the source of exceptional antioxidant activities of these fruits detailed identification of other phenolic compounds is in progress.

4. Conclusions

In conclusion, our data on 12 native Australian fruits indicate that selected fruits represent rich sources of antioxidants, with significant higher levels of phenolic compounds and stronger radical scavenging activities than blueberries (cv. Biloxi). Therefore, utilizing native Australian fruits as sources of bioactive phytochemicals could offer enormous opportunities for the functional food industry. Studies for the identification of further antioxidant compounds as well as clinical trials for testing the fruits bioactivity *in vivo* are in progress.

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