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Sources of Antioxidant Activity in Australian Native Fruits. Identification and Quantification of Anthocyanins

MICHAEL NETZEL,*,†,‡ GABRIELE NETZEL,†,‡ QINGGUO TIAN,§ STEVEN SCHWARTZ,|| AND IZABELA KONCZAK†

Food Science Australia, Riverside Life Science Center, 11 Julius Avenue, North Ryde, NSW 2113, Australia, Institute of Nutrition, Friedrich-Schiller-University Jena, Dornburgerstrasse 29, D-07743 Jena, Germany, Bioanalytical Laboratory, Pharmaceutical Product Development, Inc., 3230 Deming Way, Suite 190, Middleton, Wisconsin, and Department of Food Science and Technology, The Ohio State University, 2015 Fyffe Road, Columbus, Ohio 43210

Selected native Australian fruits, muntries (*Kunzea pomifera* F. Muell., Myrtaceae), Tasmanian pepper berry (*Tasmanian lanceolata* R. Br., Winteraceae), 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), and Molucca raspberry (*Rubus moluccanus* var. *austropacificus* van Royen, Rosaceae), were evaluated as sources of antioxidants by 2,2-diphenyl-1-picrylhydrazyl and ferric reducing antioxidant power assays and compared with blueberry (*Vaccinum* spp. cv. Biloxi). The total reducing capacity of five fruits was 3.5−5.4-fold higher than that of blueberry, and the radical scavenging activities of muntries and Burdekin plum were 1.5- and 2.6-fold higher, respectively. The total phenolic level by Folin−Ciocalteu assay highly correlated with the antioxidant activity. Therefore, systematic research was undertaken to identify and characterize phenolic complexes. In the present study we report on the levels and composition of anthocyanins. The HPLC-DAD and HPLC/ESI-MS−MS (ESI = electrospray ionization) analyses revealed simple anthocyanin profiles of one to four individual pigments, with cyanidin as the dominating type. This is the first evaluation of selected native Australian fruits aiming at their utilization for the development of novel functional food products.

KEYWORDS: Australian native fruits; antioxidant activity; DPPH; FRAP; total phenolics; anthocyanins; HPLC/ESI-MS−MS

INTRODUCTION

Australian native flora represents a vast resource of attractive edible plants. Cooper (*1*) described 2440 species of fruiting rainforest plants in tropical Queensland. Of these, 500 species extend into New South Wales and 500 into Northern Territory, and up to 300 occur also in Western Australia. Cherikoff and Isaacs (*2*) identified 245 native edible species of plants from rainforest habitats (rainforest bush foods) and 231 from dryland (dryland bush foods). For the Sydney region alone the same authors have reported 208 edible species. To the indigenous people of Australia, the Aborigines, edible native Australian fruits have served as a source of food and medicine for thousands of years (*3*). These fruits were reported to possess unique nutritious and organoleptic characteristics (*4*). Over the past 20 years multiple projects have been undertaken to generate data on the composition of Australian Aboriginal foods and to evaluate their nutritional values. Selected foods have been evaluated predominantly for the presence of protein, fat, carbohydrate, fiber, ash, energy, minerals, and vitamins (*5*). In recent years, native edible plants (bushfood plants) have increased in popularity. A number of commercially significant crops have been identified, and research on their propagation, breeding, and cultivation has been undertaken (*6*). Selected native Australian fruits have already entered commercial production, are available from local growers, in supermarkets, and in restaurants, and are sold overseas. Among the most important native fruits are muntries, Tasmanian pepper berry, Illawarra plum, Burdekin plum, Cedar Bay cherry, Davidson's plum, and Molucca raspberry. Muntries (*Kunzea pomifera* F. Muell., Myrtaceae), also known as emu apples, native cranberries, or munthari, are native to the southern coast of Australia. The fruit is about 1 cm in diameter and green with a tinge of red at maturity. It has a flavor of a spicy apple. Muntries are used in sweet and savory products such as jam, conserves, chutneys, and relishes (*6*, *7*). Tasmanian pepper (*Tasmanian lanceolata* R. Br.) belongs to the Winteraceae family,

* To whom correspondence should be addressed. Phone +61 2 9490 8570. Fax +61 2 9490 8524. E-mail michael.netzel@csiro.au.
† Food Science Australia.
‡ Friedrich-Schiller-University Jena.
§ Pharmaceutical Product Development, Inc.
|| The Ohio State University.

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palaeodicots associated with the humid Antarctic flora of the southern hemisphere. The plant is an attractive shrub up to 5 m high with dark green leaves and distinctive crimson young stems. Both the pepper-flavored berry (5−7 mm diameter) and leaves are sold commercially and used as a condiment, giving an unusual fragrant, spicy taste and a "bushy" rainforest feel (*6*, *8*). Illawarra plum (*Podocarpus elatus* R. Br. ex Endl., Podocarpaceae), known also as a brown pine, is an evergreen rainforest conifer growing along the east coast of Australia. The fruit is composed of two segments: a hard inedible seed about 1 cm wide, and a large, fleshy, purple-black, seedless, grape-like stalk about 2.5 cm in diameter. It has a plumlike taste, with subtle pine and mild resinous flavor, and is used in jams, jellies, sauces, preserves, and muffins, served in wild food restaurants (*9*). Burdekin plum (*Pleiogynium timorense* DC. Leenh, Anacardiaceae), a close relative of mango, is a tropical rainforest tree growing in the northeastern part of Queensland. The fruit is large (4 cm in diameter) and has a large pitted stone which fills 70−80% of the fruit. It is sour and astringent and needs to be held for some days to soften and mellow to increase palatability. It can be eaten raw or is used in wines, jams, and jellies. The fruits were widely eaten by Aborigines (*9*). Davidson's plum (*Davidsonia pruriens* F. Muell. var. *pruriens*, Davidsoniaceae) grows in subtropical rainforests in northern New South Wales and Queensland. The fruit, a purple plum, is 3−5 cm long, sour, and tangy. It is rated among the best of native Australian fruits and used in both, sweet, and savory products, such as jams and conserves, dipping sauces, vinegars, dressings, ice creams, drinks, and stewing. Plantation trials exhibited satisfactory growth with large yields at an early stage (*6*, *9*). Cedar Bay cherry (*Eugenia carissoides* F. Muell., Myrtaceae) is native to rainforest areas of eastern Australia. It grows in many forms and many habitats, although usually as a low bush or small tree which produces red, cherry-size fruit with white, deliciously sweet and juicy flesh of berry- or grape-like flavor (*10*). It is promoted as a cherry substitute for hot humid climates. Molucca raspberry (*Rubus moluccanus* var. *austropacificus* van Royen, Rosaceae) is a native Australian raspberry, indigenous to Vanuatu and northern Australia. The aggregate fruit is red, about 1.2 cm in diameter. It is used for the preparation of juices and desserts.

In the past 10 years, research on the effects of dietary polyphenols has intensified (*11*). Both epidemiological and *in vitro* studies strongly suggest that polyphenols play an active role in the prevention of degenerative diseases such as cancer (*12*, *13*) and cardiovascular diseases (*14*, *15*). Polyphenols were also found to exert neuroprotective (*16*) and antidiabetic (*17*, *18*) actions and reduce obesity (*18*). Polyphenols protect cell constituents against oxidative damage through scavenging free radicals and thereby avert their deleterious effects on nucleic acids, proteins, and lipids in cells (*19*). Recently direct interactions of dietary plant polyphenols with receptors or enzymes involved in signal transduction have also been reported (*20*). To utilize local resources for the development of novel foods that in addition to being nutritious possess health-protective properties, we undertook a systematic study on polyphenolic complexes in selected native Australian fruits. Here we report for the first time on the antioxidant properties of these fruits evaluated in 2,2-diphenyl-1-picrylhydrazyl (DPPH) and ferric reducing antioxidant power (FRAP) assays and the levels of total phenolics (Folin−Ciocalteu method) and ascorbic acid (HPLC-DAD). We found that the antioxidant activity of fruit extracts is strongly correlated with the level of total phenolic compounds. Various groups of phenolic compounds such as flavonoids (e.g., anthocyanins, flavan-3-ols) and phenolic acids contribute to this activity. The antioxidant activity of the complex will be governed by the amounts and variety of aglycons, the number of glycosides, acylation and its pattern, and the type of interactions among molecules. Therefore, we undertook a study toward complete characterization of polyphenolic complexes. In this paper we present original data on the identification and quantification of anthocyanins (HPLC/ESI-MS−MS; ESI = electrospray ionization).

MATERIALS AND METHODS

Plant Material. Fully ripened fruits of muntries (*K. pomifera* F. Muell.) were purchased from Tanamera Bush Foods, South Australia, Illawarra plum (*P. elatus* R. Br. ex Endl.), Burdekin plum (*P. timorense* DC. Leenh), Cedar Bay cherry (*E. carissoides* F. Muell.), Davidson's plum (*D. pruriens* F. Muell. var. *pruriens*), and Molucca raspberry (*R. moluccanus* var. *austropacificus* van Royen) were purchased from the Australian Native Foods "Playing with Fire", NSW, and Tasmanian pepper (*T. lanceolata* R. Br.) was purchased from grower Russell Langfield in Tasmania. 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 they were analyzed.

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.

Sample Preparation. The samples were prepared according to Kammerer et al. (*21*) 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 methanol (80%)/0.1% HCl (v/v) with 2 h of stirring under a nitrogen atmosphere to prevent oxidation. The extracts were centrifuged (10 min, 5000 rpm), and the material was reextracted two times with 3 mL of the organic solvent. Aliquots (9 mL) of the combined supernatants were stored frozen (−20 °C) until they were analyzed. For anthocyanin identification (LC/ESI-MS−MS), approximately 1 mg of lyophilized tissues 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, and the supernatants were combined and filtered through a 0.20 μm nylon filter.

Total Phenolics. The total phenolic content was determined using the Folin−Ciocalteu assay (*22*). Diluted fruit extracts were directly assayed at 750 nm with gallic acid serving as the standard. Results were expressed as micromoles of total phenolics (gallic acid equivalents, GAEs) per gram of fresh weight (μmol of GAEs/g of FW).

Antioxidant Activity (DPPH and FRAP Assays). Radical scavenging activity (RSA) of the fruit extracts was determined using the DPPH assay according to Brand-Williams et al. (*23*) with modifications (*24*). The principle of this assay is based on the reduction of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH•), which has an absorption maximum at 550 nm. All reactions were conducted in 96-well microplates with a total volume of 300 μL in each well. The sample solution (75 μL) containing the evaluated extracts at concentrations of 0, 5, 10, 25, 50, and 75 μL in 0.1 M 2-morpholinoethanesulfonic acid (MES) buffer (pH 6.0) containing 50% ethanol was added to 150 μL of the same buffer. Furthermore, 75 μL of 0.4 mM DPPH• solution in 50% ethanol was added to the mixture and the resulting mixture shaken vigorously for 2 min at room temperature. The decrease of DPPH• absorbance was measured immediately at 550 nm. The RSA of the samples was calculated by using the following equation: RSA (%) = [(absorbance of the control − absorbance of the samples)/absorbance of the control] × 100. The RSAs of the fruit extracts were expressed in terms of IC_{50} concentration in micromoles of Trolox required for a 50% decrease in the absorbance of DPPH radicals. Results were expressed as micromoles of Trolox equivalents (TEs) per gram of fresh weight (μmol of TEs/g of FW).

Table 1. Contents of Total Phenolics and Ascorbic Acid and Antioxidant Activity (DPPH and FRAP) of Seven Native Australian Fruits and Blueberry (Cv. Biloxi)

fruit	total phenolic content, μmol of GAEs/g of FW	RSA,[c] μmol of TEs/g of FW	TRC,[d] μmol of Fe^{2+}/g of FW	ascorbic acid content, μmol/g of FW
muntries[a]	67.12 ± 4.62	15.42 ± 0.72	267.55 ± 32.07	0.91 ± 0.08
Tasmanian pepper	82.51 ± 5.52	11.81 ± 0.77	186.71 ± 18.14	ND[b]
Molucca raspberry	21.91 ± 0.80	5.29 ± 0.26	66.58 ± 2.59	ND
Davidson's plum	16.75 ± 1.03	3.20 ± 0.24	49.29 ± 1.00	ND
Illawarra plum	68.21 ± 2.30	8.85 ± 0.54	214.79 ± 7.75	ND
Cedar Bay cherry	64.95 ± 4.07	9.57 ± 1.02	233.30 ± 14.53	ND
Burdekin plum[a]	100.50 ± 7.40	27.12 ± 1.47	283.38 ± 17.93	1.29 ± 0.08
blueberry[a]	26.00 ± 0.64	10.35 ± 0.80	52.74 ± 2.66	0.076 ± 0.002

[a] Total phenolic, DPPH, and FRAP values of these fruits are corrected for ascorbic acid. [b] Not detectable. [c] RSA = radical scavenging activity, DPPH• assay. [d] TRC = total reducing capacity, FRAP assay.

The FRAP assay is a simple and frequently used method to assess the total reducing capacity of samples (*25*) and was adopted with minor modifications (*26*). A 30 μL portion of water and 10 μL of antioxidant solution (fruit extracts) were mixed with 200 μL of FRAP reagent consisting of ferric chloride and 2,4,6-tripyridyl-*s*-triazine (TPTZ). The absorbance was measured after 8 min at 595 nm. The reducing capacity was calculated using the absorbance difference between the sample and the blank and a further parallel Fe(II) standard solution. Results were expressed as micromoles of Fe^{2+} per gram of fresh weight (μmol of Fe^{2+}/g of FW).

It should be noted that the values obtained in the DPPH, FRAP, and total phenolic assays of blueberry, muntries, and Burdekin plum were corrected for ascorbic acid.

Ascorbic Acid. The centrifuged (5 min, 10.000 rpm) and diluted (1:10 with 5% metaphosphoric acid) samples 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 sulfuric acid to pH 2.2 following the method of Vazquez-Oderiz et al. (*27*). 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 an L-ascorbic acid calibration curve and calculated as micromoles per gram of fresh weight (μmol/g of FW). The limit of detection (S/N = 3) was calculated to be 0.187 nmol/mL, and the recovery was about 90%. In the third extract, only traces of ascorbic acid could be detected.

Identification of Anthocyanins (System I, HPLC/ESI-MS−MS). Identification of anthocyanins was conducted using an LC/MS system consisting of a Waters 2695 gradient HPLC separation module, an autoinjector, a 996 diode array ultraviolet/visible (UV/vis) absorbance detector (Waters Corp., Milford, MA), and a triple-quadrupole ion-tunnel mass spectrometer (Quattro Ultima, Micromass Ltd., Manchester, U.K.) equipped with a Z-spray ESI source. Calibration of the mass spectrometer was performed using sodium iodide and cesium iodide. HPLC analysis was performed on a 150 × 3.0 mm i.d., 3 μm Atlantis dC_{18} column (Waters Corp.) at 35 °C. The solvent system consisted of a gradient mobile phase from 5% solvent B to 20% solvent B in 25 min and then to 5% solvent 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 the 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 the precursor ion scan were further fragmented using the product ion to obtain the potential aglycon and sugar composition in the structure.

Quantification of Anthocyanins and Ascorbic Acid (System II, HPLC-DAD). Quantification of anthocyanins in fruit extracts was carried out following the method of Terahara et al. (*28*). The HPLC system consisted of two LC-10AD pumps, an SPD-M10A diode array detector, a CTO-10AS column oven, a DGU-12A degasser, a SIL-10AD autoinjector, and an SCL-10A system controller (Shimadzu Co., Kyoto, Japan) equipped with a 250 × 4.6 mm i.d., 5 μm Luna C18(2) column (Phenomenex). Analytical HPLC was run at 35 °C and monitored at 520 nm. The following solvents in water with a flow rate of 1 mL/min were used: A, 1.5% phosphoric acid; B, 1.5% phosphoric acid, 20% acetic acid, and 25% acetonitrile. The elution profile was a linear gradient elution for solvent B of 25% to 43% over 30 min in solvent A, followed by 10 min to 85% solvent B and then to 25% solvent 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 (CEs) per gram of fresh weight (μmol of CEs/g of FW). The limit of detection (S/N = 3) for cyanidin 3-glucoside was calculated to be 6.0×10^{-3} nmol/mL, and the recovery was about 85%. In the third extract, only traces of anthocyanins could be detected.

Equipment. Antioxidant Activity (DPPH and FRAP) and Total Phenolics. Measurements were done in microplates using 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).

RESULTS AND DISCUSSION

Antioxidant Activity and Total Phenolic and Ascorbic Acid Contents. Antioxidant activity as evaluated in the DPPH and FRAP assays and the levels of total phenolics and ascorbic acid in fruits are shown in **Table 1**. The total phenolic content ranged from a low of 16.75 μmol of GAEs/g of FW (Davidson's plum) to a high of 100.5 μmol of GAEs/g of FW (Burdekin plum). With respect to the total phenolics, Davidson's plum closely followed by Molucca raspberries (21.91 μmol of GAEs/g of FW) are comparable to blueberries. However, the level of total phenolics in Burdekin plums was 3.9-fold and in muntries, Tasmanian pepper berries, Illawara plums, and Cedar Bay cherries 2.5−3.2-fold greater than that in blueberries. Radical scavenging activity values ranged from a low of 3.2 μmol of TEs/g of FW for Davidson's plum to a high of 27.12 μmol of TEs/g of FW for the Burdekin plum. The total reducing capacity values ranged from a low of 49.29 μmol of Fe^{2+}/g of FW (Davidson's plum) to a high of 283.38 μmol of Fe^{2+}/g of FW (Burdekin plum). Most of the evaluated samples possess higher antioxidant potential than blueberries. On the basis of the fresh weight of the fruit, the Burdekin plum had the highest RSA, TRC, and total phenolic values. These were 2.6-fold, 5.4-fold, and 3.9-fold higher than in the blueberry control, respectively.

Table 2. Relationship between the Levels of Phenolic Compounds and Antioxidant Activity (DPPH and FRAP Assays) for Seven Native Australian Fruits Examined in This Study

	r^a
total phenolics (μmol of GAEs/g of FW) vs FRAP (μmol of Fe^{2+}/g of FW)	0.904
total phenolics (μmol of GAEs/g of FW) vs DPPH (μmol of TEs/g of FW)	0.846

[a] r = coefficient of correlation (calculated with Microsoft Excel, version 9.0).

Ascorbic acid was found in negligible amounts (range 0.076 μmol/g of FW (blueberries) to 1.29 μmol/g of FW (Burdekin plum)).

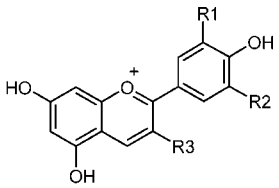
Relationship between Antioxidant Activity and Amounts of Phenolic Compounds. High positive correlations were detected between the FRAP and DPPH assays and total phenolics (**Table 2**). Previous studies reported a linear relationship between the total phenolic content and the antioxidant activity in berry crops and herbs (*29*, *30*). 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. To elucidate the contribution of individual groups of phenolic compounds to the antioxidant activity, we undertook a systematic study of phenolic compounds present in the fruits.

Anthocyanins in Selected Native Australian Fruits. All fruits evaluated in this study accumulate anthocyanins. The pigments are present in the flesh (Tasmanian pepper berry, Illawarra plum, Burdekin plum, Davidson's plum, and Molucca raspberry) or in the skin (Cedar Bay cherry and muntries). Anthocyanins, along with chlorogenic acids, were identified as the main sources of antioxidant activity of four different types of berries (*28*), and it can be anticipated they contribute to the antioxidant activity of Australian native fruits. Anthocyanins in fruit extracts, separated and identified using HPLC/ESI-MS−MS, are presented in **Table 3** and **Figures 1** and **2**. A total of nine different anthocyanidin glycosides were detected across the seven cultivars. The composition of the anthocyanins showed that the predominant sugars (glucose and sambubiose) were combined with cyanidin, delphinidin, pelargonidin, peonidin, and petunidin at the C-3 position (**Figure 2**). Besides 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 (**Figure 2** and **Table 3**). Cyanidin 3-glucoside was the main anthocyanidin glucoside in muntries and Illawarra plum and the only anthocyanidin glucoside in Burdekin plum and Cedar Bay cherry. Among the four detected anthocyanin compounds 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 the total anthocyanin content), peonidin 3-sambubioside (14.8% of the total anthocyanin content), and petunidin 3-sambubioside (3.6% of the total anthocyanin content). The remaining two species, Tasmanian pepper berry and Molucca raspberry, both contained cyanidin 3-rutinoside as the primary anthocyanin (73% and 81.2% of the total anthocyanin content, respectively) with either a low amount of cyanidin 3-glucoside (27%, Tasmanian pepper berry) or small amounts of cyanidin 3-glucoside and pelargonidin 3-rutinoside (14.3% and 4.5%, respectively, Molucca raspberry). The

Table 3. Identification (HPLC/ESI-MS–MS) and Quantification (HPLC-DAD) of Anthocyanins in Seven Native Australian Fruits and Blueberry (Cv. Biloxi)

fruit	compound	MS–MS fragment ions, m/z	μmol of CEs/g of FW[a]
muntries	delphinidin 3-glucoside	465, 303	0.219 ± 0.015
	cyanidin 3-glucoside	449, 287	0.621 ± 0.044
	total		0.84 ± 0.06
Tasmanian pepper	cyanidin 3-glucoside	449, 287	5.705 ± 0.485
	cyanidin 3-rutinoside	595, 449, 287	15.425 ± 1.311
	total		21.13 ± 1.81
Molucca raspberry	cyanidin 3-glucoside	449, 287	0.368 ± 0.002
	cyanidin 3-rutinoside	595, 449, 287	2.086 ± 0.017
	pelargonidin 3-rutinoside	579, 433, 271	0.116 ± 0.002
	total		2.57 ± 0.02
Davidson's plum	delphinidin 3-sambubioside	597, 303	0.273 ± 0.011
	cyanidin 3-sambubioside	581, 287	0.763 ± 0.016
	peonidin 3-sambubioside	595, 301	0.188 ± 0.008
	petunidin 3-sambubioside	611, 317	0.046 ± 0.006
	total		1.27 ± 0.04
Illawarra plum	cyanidin 3-glucoside	449, 287	19.296 ± 0.975
	pelargonidin 3-glucoside	433, 271	0.094 ± 0.004
	total		19.39 ± 1.00
Cedar Bay cherry	cyanidin 3-glucoside	449, 287	0.966 ± 0.026
Burdekin plum	cyanidin 3-glucoside	449, 287	6.072 ± 0.513
blueberry	delphinidin 3-galactoside	465, 303	2.057 ± 0.049
	delphinidin 3-glucoside	465, 303	1.046 ± 0.025
	cyanidin 3-galactoside	449, 287	0.363 ± 0.018
	delphinidin 3-arabinoside	435, 303	1.560 ± 0.008
	cyanidin 3-glucoside	449, 287	0.215 ± 0.003
	petunidin 3-galactoside	479, 317	1.060 ± 0.027
	cyanidin 3-arabinoside	419, 287	0.231 ± 0.003
	petunidin 3-glucoside	479, 317	0.843 ± 0.023
	peonidin 3-galactoside	463, 301	0.092 ± 0.001
	petunidin 3-arabinoside	449, 317	0.555 ± 0.017
	peonidin 3-glucoside	463, 301	1.438 ± 0.049
	malvidin 3-galactoside	493, 331	0.039 ± 0.001
	malvidin 3-glucoside	493, 331	1.250 ± 0.050
	malvidin 3-arabinoside	463, 331	0.755 ± 0.029
	total		11.51 ± 0.30

[a] Total anthocyanin level expressed in cyanidin 3-glucoside equivalents (CEs).



	R1	R2	R3
cyanidin 3-glucoside	– OH	– H	glucose
cyanidin 3-rutinoside	– OH	– H	rutinose
cyanidin 3-sambubioside	– OH	– H	sambubiose
delphinidin 3-glucoside	– OH	– OH	glucose
delphinidin 3-sambubioside	– OH	– OH	sambubiose
pelargonidin 3-glucoside	– H	– H	glucose
pelargonidin 3-rutinoside	– H	– H	rutinose
peonidin 3-sambubioside	– OCH_3	– H	sambubiose
petunidin 3-sambubioside	– OCH_3	– OH	sambubiose

Figure 1. Structures of anthocyanidin glycosides that occur in the native Australian fruits examined in this study.

Tasmanian pepper berry and the Illawarra plum exhibited by far the highest level of total anthocyanins (sum of all quantifiable monomeric anthocyanidin glycosides), whereas the muntries and Cedar Bay cherry exhibited the least amount (**Table 3**). Compared to blueberries (cv. Biloxi), the total anthocyanin contents of Tasmanian pepper berry and Illawarra plum were about 1.8- and 1.7-fold higher, whereas the total anthocyanin

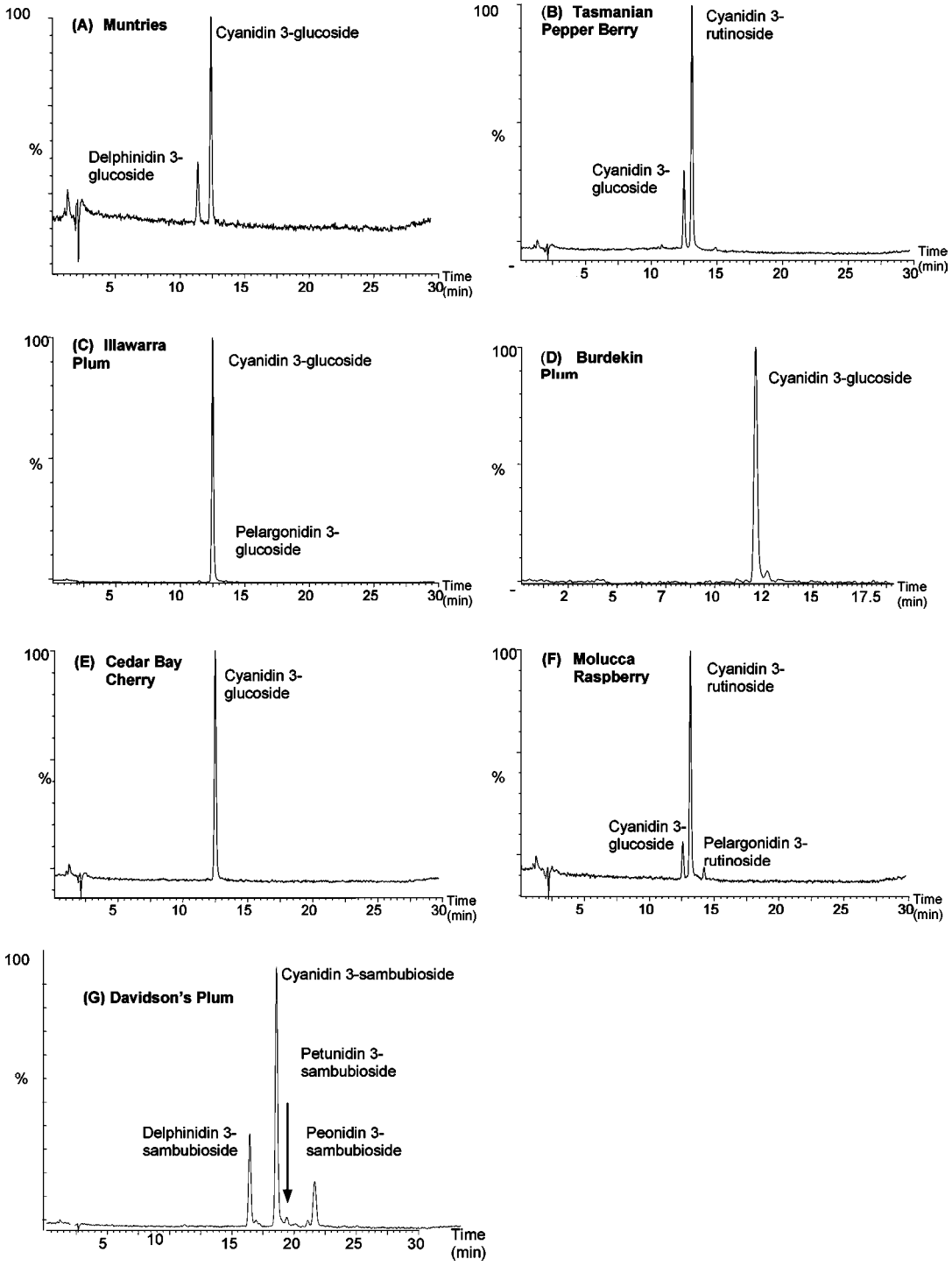


Figure 2. Reversed-phase HPLC chromatograms of anthocyanins detected at 520 nm from seven native Australian fruits.

contents of Burdekin plum, Molucca raspberry, Davidson's plum, Cedar Bay cherry, and muntries were only 52.8%, 22.3%, 11.0%, 8.4%, and 7.3% of that in the Biloxi blueberries, respectively. The levels of total anthocyanins in Tasmanian pepper berry (21.13 μmol/g of FW) and Illawarra plum (19.39 μmol/g of FW) were higher than in most well-known berries (including blueberries from different cultivars).

Taruscio and co-workers (*31*) 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 of CEs/g of FW. A content of 0.71−9.53 μmol of CEs/g of FW was found by Zheng and Wang (*29*) in cranberry (cv. Ben Lear), lingonberry (cv. Amberland), blueberry (cv. Serra), and chokeberry (wild). The total anthocyanin contents of strawberry (*Fragaria x ananassa* Duch. cv. Kent), raspberry (*Rubus idaeus* Michx. cv. Nova), highbush blueberry(*Vaccinium corymbosum* L. cv. Bluecrop), and lowbush blueberry (*Vaccinium angustifolium* Aiton) ranged between 0.155 (strawberry) and 4.35 (lowbush blueberry) μmol of malvidin 3-glucoside equivalents/g of FW as reported by Kalt et al. (*32*). These levels are about 99.3% and 77.6% lower than the values determined for Tasmanian pepper berry and Illawarra plum in the present study. In the study by Zheng et al. (*33*), the levels of total anthocyanins in highbush blueberries (*V. corymbosum* L. cv. Duke) were 80.8% and 79.1% lower than that of Tasmanian pepper berry and

Illawarra plum, respectively. To compare the anthocyanin concentrations in the present paper with the studies of Zheng and Wang (*29*), Taruscio et al. (*31*), and Zheng et al. (*33*), the original data (mg of CEs/g of FW and mg of CEs/100 g of FW, respectively) were converted to μmol of CEs/g of FW. Anthocyanin values reported by Wu et al. (*34*) for six black currant (*Ribes nigrum*) cultivars (range 3.22−5.86 mg/g of FW), four gooseberry (*Ribes grossularia*) cultivars (range 0.74 μg/g of FW to 0.10 mg/g of FW), and red currant (*Ribes rubrum*; 0.12 mg/g of FW) were significantly lower than those of Tasmanian pepper berry (9.48 mg/g of FW) and Illawarra plum (8.71 mg/g of FW), whereas chokeberry (*Aronia melanocarpa*; 14.8 mg/g of FW) and elderberry (*Sambucus nigra*; 13.74 mg/g of FW) had higher anthocyanin contents. It must be noted that in the study conducted by Wu et al. the quantification of individual anthocyanins was performed by utilizing the corresponding anthocyanidin 3-glucoside as a standard. In the crude extracts of native Australian fruits investigated in the present study neither anthocyanidin diglycosides nor acylated anthocyanin forms were detected.

Relationship between Antioxidant Activity and Amounts of Anthocyanins. The correlation coefficients between the levels of anthocyanins and FRAP and DPPH assays were low. The coefficient between anthocyanins (μmol of CEs/g of FW) vs FRAP (μmol of Fe^{2+}/g of FW) was 0.146, and that between anthocyanins (μmol of CEs/g of FW) vs DPPH (μmol of TEs/g of FW) was 0.051. This result clearly indicates that, contrary to other crops such as berries (*29*) and red-fleshed sweetpotato and purple corn (*35*), other phenolic compounds may play major roles as sources of antioxidant activities in the selected Australian native fruits. We are especially interested in Cedar Bay cherry, muntries, and Burdekin plum. The first two fruits accumulate anthocyanins at very low levels: 0.966 and 0.84 μmol of CEs/g of FW, respectively. These levels are approximately 12−14 times lower than that in blueberry. However, total phenolics and total reducing capacity of these fruits are 2.5-fold and 4.4−5-fold those of the control blueberry, respectively. The level of anthocyanins in Burdekin plum is about 50% of that in blueberry, and the antioxidant activity is 2.6-fold and 5.4-fold higher (DPPH and FRAP assays, respectively). To identify the source of exceptional antioxidant activities of these fruits, detailed identification of other phenolic compounds is in process.

In conclusion, this is the first study focusing on characterization of anthocyanin-rich polyphenolic complexes from selected native Australian fruits, identification of anthocyanins, and evaluation of antioxidant activities of fruit extracts. The antioxidant activity as gauged using DPPH and FRAP assays was strongly correlated with the total phenolic content. The selected Australian native fruits have been identified as a novel source of rich polyphenolic complexes with enhanced antioxidant activities. The results indicate putative application of these fruits for the development of health-promoting food products. Further studies on detailed identification of other phenolic compounds followed by studies of the fruit bioactivity *in vivo* (clinical trials) and *in vitro* (antimutagenic and anticancer activities) are in progress.

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