Hydrophilic phytochemical composition and anti-oxidant capacity of commercially grown native Australian fruits

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Abstract. Hydrophilic phytochemicals and antioxidant capacities of eight commercially grown native Australian fruits were determined. Kakadu plum contained a 6-fold higher and quandong a 1.9-fold higher level of total phenolic compounds (TP) than blueberry (*Vaccinum* sp., cv. Biloxi). Both fruits displayed superior oxygen radical scavenging capacity (ORAC assay) that was respectively 4.4-fold and 6.8-fold of that of blueberry. The total reducing capacity (TRC; Ferric Reducing Antioxidant Power, FRAP assay) of Kakadu plum and quandong exceeded the TRC of blueberry 13.1- and 2.3-times, respectively. Lemon aspen had lower TP and FRAP values than blueberry, however 2.4-fold higher antioxidant capacity in the ORAC assay. The primary sources of antioxidant capacities were cinnamic acids and flavonoids, including anthocyanins and flavonols, tentatively identified by the liquid chromatography mass spectrometry. The major organic acids detected were citric and malic acid. A high level of vitamin C was recorded for Kakadu plum and Australian citrus fruits.

Keywords: ethnic foods, phenolic compounds, organic acids, ORAC, *Terminalia ferdinandiana, Davidsonia pruriens, Santalum acuminatum, Syzygium luehmannii, Acronychia acidula, Citrus glauca, Citrus australasica.*

37 **1. Introduction**

38 Epidemiological studies have consistently shown that a high uptake of
39 fruits/vegetables is associated with reduced risk of developing chronic diseases. A
40 large group of phytonutrients responsible for this health benefit are phenolic
41 compounds. Their preventative effects against the development of degenerative
42 diseases such as cancer (Hertog, Bueno de Mesquita, Fehily, Sweetnam, Elwood &
43 Kromhout, 1996), cardiovascular diseases (Vita, 2005), neural degeneration (Youdim,
44 Spencer, Schroeter & Rice-Evans, 2002), diabetics and obesity (Tsuda, Horio, Uchida,
45 Aoki & Osawa, 2003) have been reported. Phenolic compounds are generally strong
46 antioxidants and primary understanding of their action was protection of cell
47 constituents against oxidative damage through scavenging free radicals and thereby
48 averting their deleterious effects on nucleic acids, proteins and lipids in cell (Rice-
49 Evans, 2001). Phenolics interact directly with receptors or enzymes involved in signal
50 transduction (Moskaug, Carlsen, Myhrstad & Blomhoff, 2005) which clearly indicates
51 that they play a specific function in our physiology. Understanding the role of dietary
52 phenolics will certainly open new prospects for their utilization in health-promoting
53 foods. Identifying novel sources of phenolic compounds and further understanding
54 their properties will be of interest of the food industry.
55 Utilising local edible plants for food is important from an economical point of view.
56 Exploring these plants as sources of physiologically active compounds offers
57 enormous opportunities for the development of novel foods. Over the last decades a
58 number of endemic edible Australian plants have entered commercial production
59 (Ahmed & Johnson, 2000). From the large variety of plants in the wild, growers have
60 selected the most productive forms, suitable for large plantations and

61 commercialisation. This study evaluated native Australian fruits of primary
62 importance to the Australian Native Food Industry.

63 Kakadu plum (*Terminalia ferdinandiana* Excell, *Conmbretaceae*) grows in the
64 Northern Territory and Western Australia (Wrigley, 1988). The fruit is yellow-green,
65 almond-sized and contains one large seed. It is fibrous and is used predominantly in
66 the form of a powder.

67 Davidson's plum (*Davidsonia pruriens* F. Muell., *Cunoniaceae*) originate from North
68 Queensland. The fruit resembles a plum, is 3-5 cm long, intensively pigmented, sour
69 and tangy. Due to large yields (Ahmed, et al, 2000) and wide application in both,
70 sweet and savoury products, it is rated among the best of native Australian fruits.

71 Quandong (*Santalum acuminatum*, A.D.C., *Santalaceae*) was an important staple for
72 the Australian Aboriginal population (Brand-Miller & Holt, 1998). It is widely
73 distributed throughout most southern regions of mainland Australia, including the arid
74 centre of the country, and in Western Australia. The tree is hemi-parasitic and can
75 attach to the roots of a wide range of species (Grant & Buttrose, 1978) which creates
76 an opportunity to extend the areas of quandong growth through utilization of roots of
77 the plants that are drought resistant. The fruit is bright red with a firm fleshy layer
78 surrounding an edible stone. The flesh is a good source of carbohydrate (17%) and
79 fibre (4%) (Brand-Miller *et al*., 1998) and is used for fruit-type flavour in sweet and
80 savoury products.

81 Riberry (*Syzygium luehmannii* (F.Muell.) L.A.S. Johnson, *Myrtaceae*) occurs in sub-
82 tropical rainforests on the east coast of Australia. The fruit, a pink to red berry with a
83 clove/cinnamon flavour, contains a single seed or is seedless. The berries are used in a
84 similar way as blueberries. The whole fruit can be blended for use in ice-cream and is
85 becoming popular with restaurants and food processors. The current production value

86 (3-5 tonnes from cultivation and smaller amount from wild harvest) is lower than the
87 demands but newly established orchards are expected to reduce this shortage (*Russel*
88 *Glover, Woolgoolga Rainforest Products; personal communication*).

89 Lemon aspen (*Acronychia acidula* F. Muell, *Rutaceae*) is a small to medium sized
90 native tree of highland rainforests from central to north Queensland. The fruit is small,
91 yellow, approximately 20-25 mm in diameter with small dark seeds encased in husks.
92 It has a spicy citrus aroma and a strong, acidic lemon flavour (Low, 1991) and is used
93 in sweet and savoury products. Current retail product categories include simmer
94 sauces, chutney, relishes and aspen flavoured mineral water.

95 Australian desert lime (*Citrus glauca* (Lindl.) Burkill; *Rutaceae*) grows in Queensland
96 and New South Wales, with some isolated occurrences in central South Australia. The
97 tree is 2 to 4 m high, bearing small (4 cm diameter) green to yellow coloured fruit
98 (Low, 1991), which is used for distinctive, recognisably citrus flavour in sweet and
99 savoury products. The production volume reaches 10 to 15 tonnes per annum. Desert
100 lime has been identified as one of the most resilient *Citrus* species, being
101 comparatively heat, drought, and cold tolerant.

102 Finger lime (*Citrus australasica* F.Muell; *Rutaceae*) originates from the rainforests in
103 southern Qld and northern NSW. The oblong fruit is about 6-7cm long and about 1
104 cm in diameter. Forms that bear fruits of different colours: green, yellow, pink, red
105 and purple have been selected from the wild and are grown commercially. The
106 cylindrical fruit has globular vesicles resembling caviar. These are becoming popular
107 as a gourmet food. The fruit is made into drink, marmalade, pickle and is even dried
108 and used as a flavoring spice.

109 Previous evaluation of the above listed fruits for the presence of water, protein, fat,
110 carbohydrates, selected vitamins and minerals has indicated a similar composition to

111 common western foods in the same category (Miller, James & Maggiore, 1993;
112 Ahmed et al., 2000). Our research has focused on their evaluation as a source of
113 health-promoting phytochemicals, including major hydrophilic and lipophilic
114 compounds as well as their antioxidant capacities, and comparing their composition
115 with other commonly consumed fruits. In the present paper we have characterised the
116 commercially produced Australian fruits with regard to their antioxidant capacity, the
117 level of total phenolics and identity of the major hydrophilic phytochemicals: phenolic
118 compounds identified by liquid chromatography mass spectrometry (LC/MS) and
119 organic acids identified by high performance liquid chromatography – diode array
120 detector (HPLC-DAD). It should be noted that the results obtained in this study
121 originate from a single lot of samples produced during one vegetative season using
122 plant sources selected by the Australian Native Food Industry Ltd. Variations in the
123 levels of phenolic compounds and antioxidant capacities arising from genetic
124 diversity and environmental factors were not evaluated in this study.

125

126 **2. Materials and Methods**

127

128 *2.1. Plant material.*

129 Samples were selected and provided by the Australian Native Food Industry Ltd.
130 (ANFIL). Australian desert lime was obtained from the Australian Desert Limes
131 company (Queensland, Australia). Kakadu plum, green finger limes, pink finger limes
132 and lemon aspen were obtained from the Australian Produce Company Pty Ltd
133 (Queensland, Australia). Davidson's plum was obtained from the Australian
134 Rainforest Products (NSW, Australia). Dry sample of quandong was supplied by the
135 Australian Native Food "Outback Pride" and frozen sample was purchased from the

136 Tanamera Bush Foods, South Australia, Australia. Riberry sample was supplied by
137 the Woolgoolga Rainforest Products, NSW. Blueberries (*Vaccinum spp.* cv. Biloxi)
138 from the Blueberry Farm of Australia, Corrindi (NSW, Australia), were used as a
139 control and were from a very late harvest after most harvesting for fresh market sales
140 was completed. The frozen samples were freeze-dried on arrival. In the case of plums,
141 the fruits were defrosted to allow stone removal, immediately frozen using liquid
142 nitrogen and freeze-dried. The freeze-dried samples were finely ground and placed in
143 air-tight containers. Subsequently they were stored at -20°C until analyzed.
144
145 *2.2. Reagents and standards.*
146 Unless otherwise stated, all chemicals were purchased from Sigma-Aldrich (Sydney,
147 Australia) and were of analytical or HPLC grade. Cyanidin 3-glucoside, cyanidin 3-
148 sambubioside, cyanidin 3-rutinoside, and cyanidin 3,5-diglucoside, were purchased
149 from Polyphenols Laboratories AS (Hanaveien, Norway). Deionized water was used
150 throughout.
151
152 *2.3. Extraction of hydrophilic compounds.*
153 An aliquot of the ground sample (250 mg) was extracted with 5 mL of 80% aqueous
154 methanol/1.0% HCl (v/v) under a nitrogen atmosphere to prevent oxidation. The
155 samples were sonicated for 10 minutes, centrifuged [10 min, 5000 rpm; centrifuge
156 Jouan C3i (Jouan S.A., France) rotor AC 100.10], and the supernatants collected. The
157 pellets were re-extracted two more times. Aliquots of the combined supernatants (15
158 mL) were filtered with 13 mm x 0.45 µm polytetrafluoroethylene (PTFE) membrane,
159 flushed with nitrogen and stored at 0.5°C until analyzed. The extraction was carried
160 out in triplicate for each sample. The analysis was conducted within 3 days.

161 *2.4. Total phenolic content (Folin-Ciocalteu assay).*

162 The total phenolic content (TP) was determined using the Folin-Ciocalteu (F-C) assay
163 (Singleton & Rossi, 1965). Diluted extracts were directly assayed at 600nm with
164 gallic acid as a standard. Measurements were done in microplates using a microplate
165 reader model Multiscan RC, version 4 (Labsystems, Finland) operated by the
166 DeltaSoft3 program (Elisa Analysis for the Macintosh with interference for the
167 Multiscan Microplate Readers, BioMetallics, Inc., 1995). The analysis was conducted
168 in triplicate. Ascorbic acid readily reacts with the F-C reagent and enhances the F-C
169 value. To avoid this effect, the level of ascorbic acid was measured before the
170 medium was alkalised with Na_2CO_3 (the assay relies on the transfer of electrons in
171 alkaline medium from phenolic compounds to phosphomolybdic /phosphotungstic
172 acid complexes to form blue complexes) and than subtracted from the F-C value
173 (Singleton *et al*., 1965). Results were expressed as micromoles of total phenolics
174 (gallic acid equivalents, GAE) per gram fresh weight (μMol GAE/gFW) or per gram
175 dry weight (μMol GAE/gDW). In the case of plums, the stone was removed and fresh
176 weight was corrected to reflect on the amount of compounds present in the edible part.

177 *2.5. FRAP (Ferric Reducing Antioxidant Power) assay.*

178 The assay was conducted according to Benzie & Strain (1996) with minor
179 modifications. Thirty μL of water and 10 μL of fruit extracts (diluted, as needed to
180 obtain a clear reading) were mixed with 200 μL FRAP reagent consisting of ferric
181 chloride and 2,4,6-tripyridyl-*s*-triazine (TPTZ). The absorbance was measured after 4
182 min at 600 nm. The reducing capacity was calculated using the absorbance difference
183 between sample and blank and a further parallel Fe(II) standard solution. Results were
184 expressed as micromoles of Fe^{2+} per gram fresh weight (μmol Fe^{2+}/g FW).
185 Measurements (in triplicate) were done in microplates as described for total phenolics.

186 *2.6. Oxygen Radical Absorbance Capacity for hydrophilic compounds (ORAC-H)*
187 *assay.*

188 The ORAC-H assay for oxygen radical scavenging capacity was conducted according
189 to Prior, Wu & Schaich (2005) and Ou, Hampsch-Woodill & Prior (2001). The
190 samples (in triplicate) were mixed with a fluorescein (15 nM) solution and a solution
191 of 2,2'-azobis-(2-amidinopropane) dihydrochloride (AAPH, 360 mM) both in
192 phosphate buffered saline (PBS, 75 mM, pH 7.0). Both AAPH and PBS buffer were
193 warmed to 37°C prior to use. The fluorescence was recorded until it reached zero
194 (excitation wavelength 495 nm, emission wavelength 515 nm) in a Varian Cary
195 Eclipse Fluorescence Spectrophotometer (Varian Australia Pty Ltd.) equipped with an
196 automatic thermostatic autocell holder at 37°C. A calibration curve was constructed
197 daily by plotting the calculated differences of area under the fluorescein decay curve
198 between the blank and the sample for a series of standards of Trolox solutions in the
199 range of 6.25 - 75 µg/L. The results were expressed as µmol Trolox equivalents per
200 100 gram fresh weight (µmol Trolox Eq./g FW).

201 *2.7. Analysis of phenolic compounds by high performance liquid chromatography-*
202 *diode array detector (HPLC-DAD) and liquid chromatography-photodiode array-*
203 *mass spectrometry (LC-PDA-MS/MS).*

204 *2.7.1. HPLC-DAD analysis.*

205 Quantification of phenolic compounds in extracts was carried out using a High
206 Performance Liquid Chromatography system that consisted of two LC-10AD pumps,
207 SPD-M10A diode array detector (DAD), CTO-10AS column oven, DGU-12A
208 degasser, SIL-10AD auto-injector and SCL-10A system controller (Shimadzu Co.,
209 Kyoto, Japan) equipped with a 250 x 4.6 mm i.d., 5 μ Luna C18(2) column

210 (Phenomenex, NSW, Australia). The following solvents in water with a flow rate of
211 1.0 mL/min were used: A, 0.5% Trifluoroacetic acid (TFA) in water and B, 95%
212 acetonitrile and 0.5% TFA in water. The elution profile was a linear gradient elution
213 for B of 10% over 10 minutes followed by an increase to 50% over 45 min, and then
214 to 80% over 15 minutes. The column was washed with 100% solvent B for 10
215 minutes. Analytical HPLC was run at 25°C and monitored at 280 (hydroxybenzoic
216 acids and flavanols), 326 (hydroxycinnamic acids, stilbenes), 370 (flavonols) and 520
217 nm (anthocyanins). Hydroxybenzoic acids and flavanols were quantified as gallic
218 acid equivalents (μMol GA E/gFW), cinnamic acids were quantified as chlorogenic
219 acid equivalents (μMol CHA E/gFW), flavonols and stilbenes were quantified as rutin
220 equivalents (μMol R E/gFW) and anthocyanin compounds were quantified as
221 cyanidin 3-glucoside equivalents (μMol C3G E/gFW).

222 *2.7.2. LC-PDA-MS/MS analysis.*

223 LC-PDA-MS/MS analysis was carried out on a Quantum triple stage quadrupole
224 (TSQ) mass spectrometer (ThermoFinnigan, NSW, Australia) equipped with a
225 quaternary solvent delivery system, a column oven, a photo-diode array (PDA)
226 detector and an autosampler. An aliquot (20 μl) from each extract was
227 chromatographed on a Luna C_{18}(2) analytical column (150 mm x 2.1 mm, 5 μm
228 particle size), (Phenomenex), which was heated to 30°C in an oven. The mobile
229 phase consisted of 0.5% formic acid in water (A) and 0.5% formic acid in acetonitrile
230 (B) at the rate of 220 μl/min. A linear gradient was used (0% B to 100% B over 40
231 min). Ions were generated using an electrospray source in the positive or negative
232 mode under conditions set following optimisation using solutions of cyanidin-3-
233 glucoside, chlorogenic acid and rutin. The PDA was monitoring signals at 520, 370,

234 320 and 280 nm. MS experiments in the full scan (precursor and product-specific)
235 and the selected reaction monitoring (SRM) mode were carried out.

236 *2.8. Extraction and analysis of vitamin C and organic acids.*

237 Organic acids were extracted from powdered samples and stabilised using 4.5% meta-
238 phosphoric acid according to Vazquez-Oderiz, Vazques-Blanco, Lopez-Hernandez,
239 Simal-Lozano & Romero-Rodriguez (1994). A freeze-dried powder (50 mg) of each
240 sample was mixed with 1500 μL of 4.5% m-H_3PO_4, vortexed and sonicated for 5
241 minutes to enhance the extraction process. Subsequently, the samples were
242 centrifuged (5 min, 12000rpm), the supernatants were collected and the extraction was
243 repeated twice. The supernatants were pooled (4.5 mL). The extracts were prepared
244 and analysed in triplicate. Representative samples (10 μL) were injected into HPLC
245 (equipment details as above). The compounds were separated under isocratic
246 conditions using water acidified with sulphuric acid to pH 2.2 following the method
247 of Vazquez-Oderiz *et al*. (1994). Detection was carried out at 215 and 245 nm at a
248 flow rate of 1.0 ml/min. Vitamin C was identified by comparing the retention time (at
249 215nm) and characteristic UV-VIS spectra with those of synthetic L-ascorbic acid
250 (Sigma, Sydney, Australia). The results were quantified using an L-ascorbic acid
251 calibration curve and calculated as milligrams vitamin C per gram fresh weight (mg/g
252 FW). Similarly, standards of citric acid, L-malic acid and oxalic acid (Sigma, Sydney,
253 Australia) were monitored at 245 nm and their chromatograms used to construct the
254 relevant calibration curves and to quantify the levels of organic acids.

255 **3. Results and Discussion**

256 *3.1. Folin-Ciocalteu assay and HPLC quantification of phenolic compounds.*

257 Among the commercially produced native Australian fruits evaluated in this study
258 Kakadu plum and quandong differed significantly from all other sources and the
259 blueberry control in respect to the level of TP. The level for Kakadu plum reached
260 159.6±1.2 μMol GAE/gFW (or 113.7±8.8 μMol GAE/gFW when the weight of a
261 stone was included; Table 1). The second high level of 50.4±0.4 μMol GAE/gFW
262 displayed quandong. The F-C assay, based on the reaction of phenolic compounds
263 with a colorimertic reagent, represents a fast screening method for measurement of
264 antioxidant capacity of food products and dietary supplements (Prior *et al*., 2005) and
265 is frequently used, which allows comparison of research data across a number of
266 studies. We have compared the TP values of native Australian fruits with that of a
267 blueberry (*Vaccinum sp*.), which is among fruits with the highest antioxidant capacity
268 due to the high TP content (Wang, Cao & Prior, 1996). The TP level of Kakadu plum
269 exceeded by 6-fold, and that of quandong exceeded by 1.9-fold the TP level of the
270 blueberry control (Table 1). These two Australian native fruits contained higher levels
271 of phenolic compounds per gram fresh weight than most common fruits, such as
272 cranberries (42.2 μMol GAE/gFW), blackberries (38.8 μMol GAE/gFW), apple (12.3
273 to 20.4 μMol GAE/gFW) and orange (19.8 μMol GAE/gFW) (USDA database on
274 Oxygen Radical Absorbance Capacity (ORAC) of selected foods, 2007; data
275 recalculated from mg GAE/g FW to μMol/g FW). The TP levels of all other
276 commercially grown Australian fruits evaluated in this study were lower than the TP
277 of blueberry.
278 At present quandong is sold in Australia predominantly as a dry fruit. A commercial
279 sample of dry fruit was compared with a sample of frozen quandong. The TP of dry
280 quandong was 193.2±17.0 μMol GAE/gDW, and was 10.4 % lower than the TP of the
281 frozen quandong (216.2±15.5 μMol GAE/gDW).

282 Australian desert lime contained 10.8±0.4 μMol GAE/gFW of TP and finger limes
283 contained from 6.8±0.4 to 9.2±0.5 μMol GAE/gFW (Table 1). Finger limes evaluated
284 in this study exhibited similar levels of TP to red (8.6 μMol GAE/g FW) and yellow
285 (10.9 μMol GAE/g FW) forms evaluated earlier (Netzel, Netzel, Tian, Schwartz &
286 Konczak, 2007). However commercially cultivated riberry analysed in the present
287 study had lower TP than riberry fruits collected from wild (13.08 μMol GAE/g FW)
288 (Netzel *at al.*, 2007).
289 The F-C assay is commonly used to estimate the level of phenolic compounds in food
290 products. However, the assay is not specific to phenolic compounds and other redox-
291 active molecules interfere in the measurement in an inhibitory, additive or enhancing
292 manner (Singleton *et al.*, 1965; Prior *et al.*, 2005). Among these molecules are
293 reducing sugars and vitamin C, pectins and organic acids, present in fruits. Moreover,
294 some phenolic compounds (e.g. salicylic acid, present in citrus) are less responsive in
295 this assay than others (Singleton *et al.*, 1965). Therefore the levels of phenolic
296 compounds in the fruits were evaluated by reversed-phase high performance liquid
297 chromatography.
298 In overall, the level of phenolic compounds in fruits, as evaluated by HPLC, varied
299 from the data obtained in the F-C assay. For the blueberry control the total level of
300 phenolic compounds as evaluated by HPLC was lower than the F-C values (Table 1).
301 Zheng and Wang (2003) have analysed blueberry (*cv*. Sierra) and found that the TP
302 level, as evaluated in the F-C assay, was 4.12 mg/gFW. Subsequently, they have
303 quantified the level of individual compounds in the blueberry extract by HPLC and
304 found that the sample contained 0.65mg/gFW of chlorogenic acid and 1.56 mg/gFW
305 of anthocyanins. These values were approximately 50% lower than values obtained in
306 the F-C assay, which is in agreement with our results.

307 Considerable variation was found in phenolic compounds in various fruits. Kakadu
308 plum extract contained exclusively compounds detected at 280nm, (possibly
309 hydroxybenzoic acids, flavanols, flavanones) at a level of 69.8±2.1 μMol GAE/gFW
310 (or 98.0±3.0 μMol GAE/gFW when the weight of stone was omitted) (Table 1). These
311 values represent approximately 2/3 of the TP values (F-C assay) discussed above.
312 Singleton *et al*. (1965) reported that sugars, beside ascorbic acid, aromatic amines and
313 unanticipated phenols, are responsible for the additive effect in the F-C assay.
314 Kahkonen, Hopia & Heinonen (2001) reported that removing sugars from berry
315 extract with a help of SPE treatment (Bond Elut C_{18} SPE) resulted in reduction of TP
316 (F-C assay). Similarly, in the present study, sugars in Kakadu plum extract could
317 contribute to the high TP value. Additionally, Kakadu plum contains exceptionally
318 high level of vitamin C (see *3.3. Vitamin C and organic acids*). Although the TP
319 values were corrected for ascorbic acid, the additive effect of Vitamin C due to the
320 exceptionally high level in the fruit may not be completely eliminated. Moreover, the
321 HPLC chromatogram of Kakadu plum extract is very complex. It is highly possible,
322 that beside phenolics, sugars and vitamin C, a number of other unknown compounds
323 are present and they interfere in the F-C assay. Similarly to Kakadu plum, the level of
324 phenolic compounds in quandong detected by HPLC was lower than that obtained in
325 the F-C assay (Table 1). Quandong, which exhibited second high TP value, contained
326 phenolic compounds of a different nature than Kakadu plum. These were mainly
327 hydroxycinnamic acids (30.1±0.9 μMol CHAE/gFW) and anthocyanins (0.37±0.01
328 μMol C3GE/gFW). The HPLC quantification of phenolic compounds in lemon aspen
329 indicates higher level of phenolics than the F-C assay. Similar, although less
330 pronounced effect, was visible for other fruits (riberry, finger lime and desert lime).
331 Presence of compounds interfering in the reagent-based assays and inhibiting the

332 reaction is highly possible, however further studies are needed to clarify it.
333 Davidson's plum, riberry and Australian citrus fruits extracts contained predominantly
334 compounds detected at 280nm, which suggests the presence of hydroxybenzoic acids,
335 flavonols, flavanones or proanthocyanidins. Anthocyanins were identified in
336 Davidson's plum, pink finger lime, riberry and quandong (Table 1).

337 *3.2. Antioxidant capacity: FRAP and ORAC assays*.

338 Kakadu plum extract exhibited superior total reducing capacity (TRC, FRAP assay),
339 which was 13-fold that of blueberry (Table 1). This effect could be due to the
340 presence of compounds, which in a similar manner contributed towards enhanced F-C
341 values as both assays are based the same single electron transfer mechanism. Among
342 these compounds could be sugars and vitamin C, however presence of other redox-
343 active compounds is also expected. The oxygen radical scavenging capacity (ORAC-
344 H assay) of Kakadu plum was 4.1-fold that of the blueberry control and lower than
345 that of quandong. According to Zheng & Wang (2003) sugars exhibit no antioxidant
346 activity in ORAC assay. This may partly explain the relatively lower ORAC values of
347 Kakadu plum. This study represents the first attempt to understand the phytochemistry
348 of Kakadu plum and further research may lead towards identification of other group
349 of compounds, which could contribute to the antioxidant capacity of this intriguing
350 fruit.

351 Quandong displayed approximately 2.4-times higher TRC than blueberry and
352 exhibited the highest ORAC value (6.5-fold that of the blueberry control) (Table 1).
353 Antioxidant activity of a phenolic mixture depends on the nature of phenolic
354 compounds and anthocyanins and hydroxycinnamic acids possess significantly higher
355 antioxidant potency in ORAC assay than hydroxybenzoic acids (Zheng *et al*., 2003).
356 The HPLC data (Table 1) indicates that quandong extract is rich in hydroxycinnamic

357 acids, flavonols and anthocyanins, which could be primary responsible for the high
358 ORAC values.
359 Lemon aspen performed superior to blueberry in the ORAC assay, however exhibited
360 low TRC. It also exhibited lower TP than blueberry in the F-C assay. Presence in this
361 fruit of compounds which could interfere in both assays is possible.
362 Total phenolic content and TRC of Davidson's plum evaluated in this study is similar
363 to that of Davidson's plum reported previously (Netzel *et al*., 2007). Among the
364 compounds contributing to the antioxidant capacity of this fruit are anthocyanins and,
365 possibly, hydroxybenzoic acids, flavonols, flavanones or proanthocyanidins which
366 can be detected by the HPLC at 280nm (Table 1).
367 Australian citrus fruits evaluated in this study exhibited lower antioxidant capacity
368 than blueberry in both antioxidant testing assays. Previously evaluated red finger lime
369 exhibited 3-fold lower antioxidant activity than blueberry in the Trolox Equivalent
370 Antioxidant Capacity (TEAC) assay, which similarly to FRAP is based on a single
371 electron transfer mechanism (Netzel *et al*., 2007). The TRC of the same fruit was
372 24.6±0.3 µmol Fe^{+2}/g FW (unpublished results), which is very similar to the TRC of
373 pink finger lime obtained in this study. The antioxidant capacities of green finger lime
374 and Australian desert lime were within the same range. Commercially produced
375 riberry sample evaluated in this study exhibited lower antioxidant potential (TRC and
376 ORAC values) than blueberry, and lower TRC than a riberry sample collected from
377 wild (TRC = 44.8±1.0, unpublished results).
378 *3.3. Identification of major phenolic compounds.*
379 The native Australian fruits of primary importance to the Australian Native Food
380 Industry are Kakadu plum, quandong, riberry and Davidson's plum. These fruits were
381 selected for further studies, including identification of the major phenolic compounds.

382 The compounds were separated and tentatively identified by using a reversed-phase
383 HPLC-DAD and LC-PDA-MS/MS (Table 2). The major groups of phenolic
384 compounds detected were: phenolic acids (benzoic and cinnamic) and flavonoids
385 (flavonols, flavanones and anthocyanins).

386 Quandong was a fruit that exhibited a very high antioxidant capacity (Table 1). The
387 LC/MS analysis revealed the presence of cyanidin 3-glucoside as the major
388 anthocyanin, minor amount of pelargonidin 3-glucoside and trace levels of cyanidin
389 3-rutinoside (Table 2). The total level of anthocyanin in quandong was 0.37 μmol
390 C3G E/g FW (Table 1) (or 1.57 μmol C3G E/g DW). In the commercial dry
391 quandong sample, the level of anthocyanin was reduced to 0.12 μmol C3G E/g DW
392 suggesting significant degradation of anthocyanin during the drying process. Other
393 components identified in the fresh extract of quandong included quercetin and
394 keampferol rutinosides as well as chlorogenic acid (Table 2). A series of notable
395 peaks at 280 nm, possibly due to benzoic acids, were also observed in the
396 chromatographic trace.

397 Anthocyanins were the major phenolic compounds detected in Davidson's plum. The
398 main anthocyanins were 3-sambubiosides of delphinidin, cyanidin, petunidin and
399 peonidin. These results confirmed our earlier findings (Netzel *et al.*, 2007).
400 Additionally, two new anthocyanins were detected: 3-sambubiosides of pelargonidin
401 and malvidin. Delphinidin 3-sambubioside contributed 47.5% of the anthocyanin
402 mixture and was followed by petunidin (23.7%), peonidin (14.7%) and cyanidin
403 (4.4%). The levels of pelargonidin and malvidin 3-sambubiosides were below 3%.
404 Other components found in small amounts included myricetin, rutin and quercetin
405 hexoside (Table 2).

406

407 Anthocyanins were the major compounds of riberry extract. The mixture consisted of
408 cyanidin 3-galactoside (81.6 %), cyanidin 3,5-diglucoside (11.9 %) and cyanidin 3-
409 glucoside (6.5%) (Table 2). The sample also contained notable amounts of other
410 glycosides such as quercetin and kaempferol rutinosides, myricetin and quercetin
411 hexosides and quercetin rhamnoside (Figure 1).

412 The reverse-phase HPLC trace obtained form the Kakadu plum extract was very
413 complex. Positive ionisation ESI-LC/MS suggested that quercetin/hesperitin-based
414 glucosides and keampferol/luteolin-based glycosides were part of the extract but
415 could not be quantified due to the complexity of the mixture. Other interesting
416 components present were those producing an m/z 291 during a product ion scan with
417 a precursor m/z of 451. Catechin exhibits m/z 291 in positive ionization mode so these
418 components could potentially be catechin-based components.

419 *3.4. Vitamin C and organic acids.*

420 A high level of vitamin C was detected in Kakadu plum (12.4±0.1 mg/gFW, Table 3).
421 This result is in agreement with previously reported levels of vitamin C in Kakadu
422 plum sample collected from the wild (Netzel *et al.*, 2007). The level of vitamin C in
423 Kakadu plum is slightly higher than that reported for acerola (*Malpighia emarginata*
424 DC.), a wild plant from Central America known as one of the richest and most
425 important food sources of vitamin C. A ripen acerola fruit contains from 9.44 to 10.40
426 mg vitamin C per gram fresh weight (Mezadri, Villano, Fernandez-Pachon, Garcia-
427 Parrilla & Troncoso, 2008), which is 76.1 to 83.9% of the vitamin C level in Kakadu
428 plum. The level of vitamin C in Kakadu plum is approximately 19-fold that in
429 strawberry (0.650mg/gFW; Agar, Streif & Bangerth, 1997) and in kiwifruit (0.649
430 mg/gFW; Agar, Massantini, Hess-Pierce & Kader, 1999).

431 Vitamin C was also present in commercial samples of Australian citrus fruits. Among
432 them, Australian desert lime contained 1.88±0.05 mg/gFW, which is approximately 2-
433 fold the level of vitamin C in Californian orange (0.83 mg/gFW, Vanderslice, Higgs,
434 Hayes & Block, 1990) and 5-fold the level of vitamin C in fresh mandarins (0.38
435 mg/gFW; Mitchell, McLauchlan, Isaacs & Williams, 1992). Among the finger limes,
436 the pink variety contained more vitamin C (3-fold the level in mandarins) than the
437 green variety (32% less than mandarins).

438 Citric acid is commonly present in fruits and it is the main organic acid of kiwi fruit
439 (9.85 mg/gFW), banana (3.59 mg/gFW) and strawberry (3.12 mg/gFW) (Perez, Olias,
440 Espada, Olias & Sanz, 1997). Citric acid was present in all fruits representing the
441 *Rutaceae* family evaluated in this study in the following order: pink finger lime >
442 green finger lime > lemon aspen > Australian desert lime. It dominated in finger limes
443 and lemon aspen (with the level ranging from 58.8±1.7 (pink finger lime) to 32.9±1.6
444 mg/gFW (lemon aspen) but not in the Australian desert lime which contained
445 predominantly malic acid (25.2±0.5 mg/gFW). Similarly to the Australian desert lime,
446 malic acid dominated in sweet lime (Clements, 1963). Malic acid has been identified
447 also in Davidson's plum, riberry and fresh quandong (Table 3). Malic acid is the main
448 organic acid of apple (4.12 mg/gFW), it was detected in kiwi fruit (1.9 mg/gFW),
449 banana (2.89mg/gFW), peach (2.82mg/gFW) and strawberry (1.11mg/gFW) (Perez et
450 al., 1997). Oxalic acid was identified in the fruits at very low levels. In case of citrus
451 fruits, peels could be the main source of this acid (Clements, 1963).

452 **4. Conclusions**

453 Two commercially grown native Australian fruits, quandong and Kakadu plum,
454 exhibited enhanced antioxidant capacity in comparison to blueberry, as evaluated in
455 two reagent-based assays: FRAP and ORAC-H. The main sources of antioxidant

456 capacity of quandong were cinnamic acids and anthocyanins. Phenolic compounds
457 (benzoic acids/flavanols/flavanones) and exceptionally high level of vitamin C
458 contributed towards the antioxidant capacity of Kakadu plum. Australian citrus fruits:
459 finger limes and Australian desert lime were found to be inferior to blueberry with
460 regards to antioxidant capacity however they are a good source of vitamin C. Citric
461 acid was the main organic acid detected in finger limes and lemon aspen, while malic
462 acid dominated in Australian desert lime, Davidson's plum, riberry and quandong.

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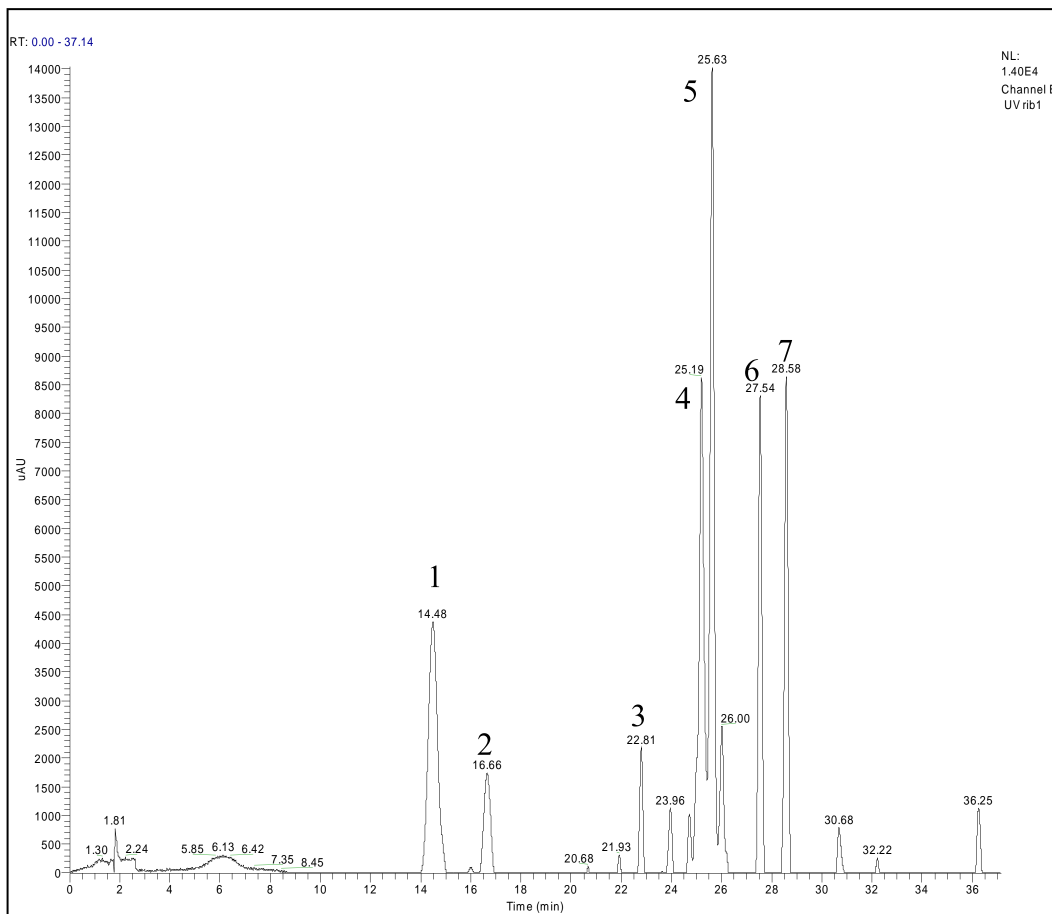

584 Figure 1. HPLC profile from the riberry extract at 370 nm; 1) Cyanidin 3-galactoside,
585 2) cyanidin 3-glucoside, 3) myricetin hexoside, 4) rutin, 5) quercetin hexoside 6)
586 kaempferol/luteolin rutinoside, 7) quercetin rhamnoside.
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Table 1. Total phenolic content and antioxidant capacity of selected commercially grown native Australian fruits. The data are means ±SD of 3 determinations.

Fruit	DW/FW ratio	Total phenolics (FC) (µmol GA E/g FW)	HPLC quantification* of phenolic compounds				FRAP (µmol Fe^{+2}/g FW)	ORAC-H (µmol TEq/g FW)
			280nm (µmol GA E./gFW)	326nm (µmol CHA E/gFW)	370nm (µmol R E/gFW)	520nm (µmol C3G E/g FW)		
Australian desert lime**	0.1957	10.8 ± 0.4	10.75 ± 1.6	1.32 ± 0.1	-	-	34.8 ± 2.3	44.9 ± 5.1
Kakadu plum**	0.1219 0.1712♦	113.7 ± 8.8 159.6 ±1.2♦	69.8 ± 2.10 98.0 ± 3.00♦	-	-	-	690.5 ± 48.4♦	315.4 ± 33.7♦
Lemon aspen	0.1549	9.5 ± 0.3	17.6 ± 0.70	-	-	-	14.0 ± 2.4	131.5 ± 11.4
Davidson's plum	0.0714 0.0804♦	14.1 ±1.0 15.9 ± 1.3♦	13.9 ± 0.20 15.4 ± 0.30♦	-	T	0.76 ± 0.01 0.85 ± 0.02♦	53.9 ± 4.0♦	83.1 ± 10.9♦
Finger lime (green)**	0.2090	6.8 ± 0.4	8.7 ±0.6	1.4±0.03	-	-	12.6 ± 0.5	45.9 ± 6.6
Finger lime (pink)**	0.1880	9.2 ± 0.5	10.6 ±2.7	0.45±0.01	-	0.06 ± 0.01	23.2 ± 0.8	65.1 ± 12.8
Riberry	0.0881	7.5 ± 0.7	11.9± 0.41	-	0.17 ± 0.01	0.69 ± 0.05	33.2 ± 1.9	49.9 ± 6.4
Quandong	0.2331	50.4 ± 0.4	-	30.1±0.91	1.15 ± 0.01	0.37 ± 0.01	123.0 ± 0.6	501.0 ± 64.1
Blueberry	0.1332	26.5 ±0.6	-	3.9 ± 0.05	-	11.5 ± 0.30	52.4± 2.78	76.9 ± 13.3

* HPLC quantification of phenolic compounds was calculated as: µmol Gallic acid E/gFW for compounds detected at 280nm, µmol Chlorogenic acid E/g FW for compounds detected at 326nm, µmol Rutin E/gFW for compounds detected at 370nm and µmol Cyanidin 3-glucoside E/gFW for anthocyanins (520nm);
** - Results of total phenolic compounds (F-C) corrected for Vitamin C; ♦ - stone weight excluded; FRAP: Ferric Reducing Antioxidant Power; ORAC-H: Oxygen Radical Absorbance Capacity - hydrophilic compounds; µmol TEq/gFW: micromole Trolox equivalent/g fresh weight.

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603 Table 2. Phenolic compounds identified in selected native Australian fruits.
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Compound	MS/MS		Quandong	Riberry	Davidson's Plum
	$[M+1]^+/[M-1]^-$	Fragments (*m/z*) (+/-)			
Cyanidin 3-galactoside	449/-	287/-	ND	0.25 ± 0.02*	ND
Cyanidin 3-glucoside	449/-	287/-	1.34 ± 0.01	0.02 ± 0.001	ND
Cyanidin 3-rutinoside	595/-	449, 287/-	T	ND	ND
Cyanidin 3,5-diglucoside	611/-	449, 287/-	ND	0.04 ± 0.006	ND
Pelargonidin 3-glucoside	433/-	271/-	0.27 ± 0.01	ND	ND
Delphinidin 3-sambubioside	597/-	303/-	ND	ND	0.16 ± 0.04
Cyanidin 3-sambubioside	581/-	287/-	ND	ND	0.02 ± 0.001
Pelargonidin 3-sambubioside	565/-	271/-	ND	ND	T
Peonidin 3-sambubioside	596/-	301/-	ND	ND	0.05 ± 0.01
Petunidin 3-sambubioside	611/-	317/-	ND	ND	0.08 ± 0.01
Malvidin 3-sambubioside	626/-	331/-	ND	ND	T
Chlorogenic acid	-/353	-/191	T	ND	ND
Myricetin	-/317	-/151	ND	ND	T
Myricetin hexoside	481/479	153/151	ND	T	ND
Quercetin rutinoside (Rutin)	610/609	303/301	0.53 ± 0.01	0.06 ± 0.01	T
Quercetin/hesperitin glucoside	465/-	303/-	ND	ND	ND
Quercetin hexoside	465/-	303/-	ND	0.05 ± 0.001	T
Quercetin rhamnoside	450/-	303/-	ND	0.03 ± 0.001	ND
Kaempferol	287/-		0.61 ± 0.01	0.03 ± 0.01	ND
Kaempferol/luteolin glucoside	449/447	287/285	ND	ND	ND
Kaempferol/luteolin rutinoside	595/-	287/-	T	T	ND

605 * Data are means ±SE of 3 independent determinations and are presented as mg/gFW; ND: not detected; T: trace (below 3%)
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611 Table 3. Ascorbic acid and organic acids in native Australian fruits.

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Fruit	Ascorbic acid	Citric acid	Malic acid	Oxalic acid
Australian desert lime	1.88 ± 0.05*	4.61 ± 0.19	25.24 ± 0.48	1.04 ± 0.10
Kakadu plum	8.84 ± 0.08 12.42 ± 0.12♦	ND	ND	0.74 ± 0.05 1.03 ± 0.08♦
Lemon aspen	ND	32.91 ± 1.60	2.33 ± 0.1	0.19 ± 0.01
Davidson's plum	ND	ND	30.9± 2.12 34.88 ± 2.39♦	0.09 ± 0.01 0.10 ± 0.01♦
Green finger lime	0.26 ± 0.01	46.81 ± 0.47	ND	0.08 ± 0.02
Pink finger lime	0.91 ± 0.02	58.82 ± 1.70	ND	0.20 ± 0.03
Riberry	ND	ND	17.2 ± 0.34	0.23 ± 0.01
Quandong	ND	ND	19.1 ± 1.33	0.40 ± 0.01

613 * Data are means ±SE of 3 independent determinations and are presented as mg/gFW; ♦ - weight of stone excluded; ND: not detected

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