Analytical Methods

Quantitative analysis of antiradical phenolic constituents from fourteen edible Myrtaceae fruits

Kurt A. Reynertson [a,1], Hui Yang [a], Bei Jiang [a], Margaret J. Basile [b], Edward J. Kennelly [a,*]

[a] Department of Biological Sciences, Lehman College and the Graduate Center, City University of New York,
250 Bedford Park Boulevard West, Bronx, NY 10468, United States
[b] Department of Neurology, University of Miami Miller School of Medicine, 1501 NW 9th Avenue, Miami, FL 33136, United States

Abstract

Many species of Myrtaceae are cultivated in home gardens throughout the tropics for their edible fruit, and have been used in traditional medicine to treat several inflammatory conditions. Fruit phenolics are important dietary antioxidant and anti-inflammatory constituents. We have investigated the antiradical activity, total phenolic content (TPC), and total anthocyanin content (TAC) of 14 underutilized Myrtaceae fruits, namely *Eugenia aggregata*, *E. brasiliensis*, *E. luschnathiana*, *E. reinwardtiana*, *Myrciaria cauliflora*, *M. dubia*, *M. vexator*, *Syzygium cumini*, *S. curranii*, *S. jambos*, *S. javanicum*, *S. malaccense*, *S. samarangense*, and *S. samarangense* var. *Taiwan pink*. An HPLC-PDA method was developed to quantify the amounts of cyanidin 3-glucoside (**1**), delphinidin 3-glucoside (**2**), ellagic acid (**3**), kaempferol (**4**), myricetin (**5**), quercetin (**6**), quercitrin (**7**), and rutin (**8**) present in MeOH extracts of the fruit. TPC ranged from 3.57 to 101 mg/g, TAC ranged from undetectable to 12.1 mg/g, and antiradical activity, measured as DPPH$^{\cdot}$ IC$_{50}$, ranged from very active (19.4 μg/ml) to inactive (389 μg/ml).
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1. Introduction

According to the Centers of Disease Control and Prevention, over half of all deaths in the United States are caused by diseases of the heart and malignant neoplasms (Minino, Heron, & Smith, 2006). Epidemiological evidence suggests that diets high in fruits and vegetables are linked to a reduced incidence of heart disease, cancer, and some neurodegenerative disorders (Arts & Hollman, 2005; Collins, 2005; Hu & Willett, 2002; Youdim & Joseph, 2001). Reactive oxygen species (ROS) are produced naturally in mammalian systems as a result of oxidative metabolism. However, ROS damage cell membranes and DNA, causing

cancerous mutations, and the oxidation of low-density lipoprotein is a major factor in the promotion of heart disease. ROS are also implicated in activating redox-responsive pro-inflammatory transcription factors, nuclear factor (NF)-κB and activator protein (AP)-1 (Rahman & Adcock, 2006). Inflammation is a major factor in the promotion of chronic inflammatory diseases, as well as the etiology of cancers and heart disease (Hu & Willett, 2002; Middleton, Kandaswami, & Theoharides, 2000). Oxidative damage is balanced by endogenous antioxidants, but additional protection, provided by nutritive and non-nutritive elements from food, is critical for disease chemoprevention.

Colourful fruits are a potentially rich source of many dietary phenolic antioxidants and are believed to play an important role in the prevention of many oxidative and inflammatory diseases (Arts & Hollman, 2005). The anthocyanin pigments are responsible for many of the bright fruit and flower colours, and act as strong antioxidants

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* Corresponding author. Tel.: +01 718 960 1105; fax: +01 718 960 8236.
E-mail address: edward.kennelly@lehman.cuny.edu (E.J. Kennelly).
[1] Current Address: Department of Pharmacology, Weill Cornell Medical College, 1300 York Avenue, New York, NY 10021, United States.

and anti-inflammatories, with antimutagenic and cancer chemopreventative activities (Kong, Chia, Goh, Chia, & Brouillard, 2003; Reynertson et al., 2006). Anthocyanins often account for much of the phenolic content of these fruits, but flavanols, procyanidins, phenolic acids, and ellagitannins may be the most predominant phenolics in some taxa. Polyphenolic compounds inhibit several oxidative and inflammatory enzymes (Middleton et al., 2000), and have shown antiallergenic, antiviral, antibacterial, antifungal, antitumor, and antihemorrhagic activities (Pietta, 2000). Flavonoids also inhibit the inflammatory transcription factors NF-κB and AP-1 (Rahman & Adcock, 2006).

The plant family Myrtaceae is pan-tropical in occurrence, with concentrations in South America, Southeast Asia, and Australia. The fleshy-fruited subfamily Myrtoideae includes many economically important food plants, agricultural crops, and ornamentals, including the Mediterranean genus *Myrtus* (myrtle), spices such as clove (*Syzygium aromaticum*), allspice (*Pimenta dioica*), and bay rum (*Pimenta racemosa*), and the fruits of *Psidium* (guavas), *Myrciaria*, *Eugenia*, *Syzygium*, *Plinia* and *Luma*.

Blueberries, cranberries, strawberries, grapes, cherries, and other colourful fruits have been the focus of much current research on dietary chemoprevention of cancers and heart disease (Kähkönen, Hopia, & Heinonen, 2001; Seeram, Lee, Scheuller, & Heber, 2006; Wang et al., 1999; Zheng & Wang, 2003). In this report, we have analyzed the phenolic content and antiradical activity of 14 edible fruits from 13 species of *Myrciaria*, *Eugenia* and *Syzygium*, namely *Eugenia aggregata* Kiaersk., *E. brasiliensis* Lam., *E. luschnathiana* Klotzsch ex O.Berg, *Myrciaria cauliflora* (Mart.) O.Berg, *M. dubia* (Kunth) McVaugh, *M. vexator* McVaugh, *Syzygium cumini* (L) Skeels, *S. curranii* (C.B.Rob.) Merr., *S. jambos* (L) Alston, *S. javanicum* Miq., *S. malaccense* (L)Merr. & L.M.Perry, *S. samarangense* (Bloom) Merr. & L.M.Perry and *S. samarangense* var. *Taiwan pink*.

These fruits are mostly red to purple drupes, 2–4 cm in diameter, although some species produce larger or less pigmented fruit. In the tropics, these species are often cultivated in home gardens, small-scale agricultural plots, or wild-harvested. They are primarily eaten fresh or used to make jams, desserts, wines, liquors, and vinegars, and can be found in local markets. In addition to their use as food, many of these fruits have been used in divergent traditional medical practices for a variety of illnesses and conditions. Most notably, the seeds of the jamun (*S. cumini*) are an important Ayurvedic medicine for diabetes. The rose apple (*S. jambos*) has been used in India as a tonic for the brain and for liver problems, as an astringent, and digestive (Kirtikar & Basu, 1988), and distilled to make rosewater (Morton, 1987). In Brazil, *E. brasiliensis* leaves have been used for gastrointestinal disorders and rheumatism, and the jaboticaba fruit (*M. cauliflora*) has been used as a treatment for hemoptysis, asthma, diarrhea, and chronic inflammation of the tonsils (Morton, 1987). Leaves

of *S. malaccense* have been used for a wide variety of inflammatory conditions in Western Samoa (Andersson Dunstan, Noreen, Serrano, Cox, Perera & Bohlin, 1997).

The majority of the phytochemical literature for these species has focussed on the aromatic terpenoid compounds found in the leaves (Wong & Lai, 1996), but phenolics have also been identified in some species. We previously reported flavonols, phenolic acids, anthocyanins, and depsides from *M. cauliflora* (Reynertson et al., 2006), as well as the occurrence of catechin and epicatechin in *E. aggregata* (Reynertson, Basile, & Kennelly, 2005). *Myrciaria dubia* was reported to contain cyanidin 3-glucoside and delphinidin 3-glucoside (Zanatta, Cuevas, Bobbio, Winterhalter, & Mercadante, 2005). Several flavonoids, ellagitannins, and phenolic acids have been identified from the fruits, seeds, and aerial parts of *S. cumini* (Bhatia & Bajaj, 1975; Bhatia, Bajaj, & Ghangas, 1971; Mahmoud, Marzouk, Moharram, El-Gindi, & Hassan, 2001), and flavonoids and ellagitannins have been found in the fruits of *S. samarangense* (Nair, Krishnan, Ravikrishna, & Madhusudanan, 1999; Nonaka, Aiko, Aritake, & Nishioka, 1992; Okuda, Yoshida, Hatano, Yazaki, & Ashida, 1982; Srivastava, Shaw, & Kulshreshtha, 1995). Ellagic acids and an anthocyanin were reported from *S. malaccense* (Andersson Dunstan et al., 1997). To the best of our knowledge, however, the phenolic constituents of *Eugenia brasiliensis*, *E. luschnathiana*, *E. reinwardtiana*, *Myrciaria vexator*, *Syzygium curranii*, *S. javanicum*, and *S. samarangense* var. *Taiwan pink* have not been reported in the literature, despite their widespread consumption in the tropics.

Here we report on the HPLC-PDA quantification of eight phenolic compounds in 14 fruits. Cyanidin 3-glucoside (**1**), delphinidin 3-glucoside (**2**), ellagic acid (**3**), kaempferol (**4**), myricetin (**5**), quercetin (**6**), quercitrin (**7**), and rutin (**8**) (Fig. 1) have been quantified to better
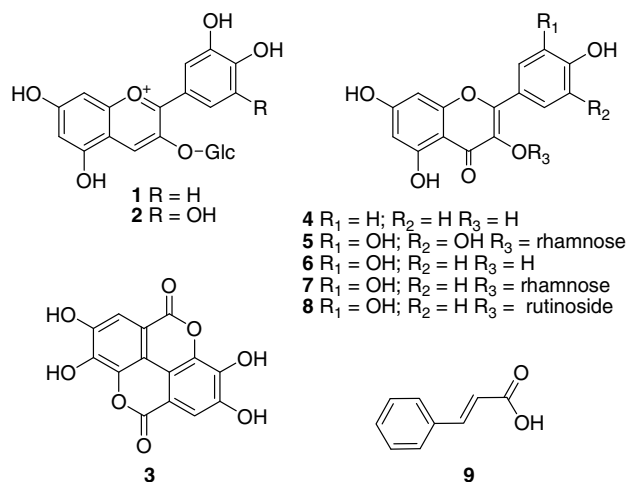

Fig. 1. Chemical structures of the phenolic compounds quantified or identified in the 14 Myrtaceae fruits. Compounds are numbered as follows: cyanidin 3-glucoside (**1**), delphinidin 3-glucoside (**2**), ellagic acid (**3**), kaempferol (**4**), myricetin (**5**), quercetin (**6**), quercitrin (**7**), rutin (**8**), and cinnamic acid (**9**).

understand the phenolic profile of these fruits. In addition, the total phenolic content (TPC) and total anthocyanin content (TAC) were determined, and each extract was evaluated for antiradical activity using the DPPH assay.

2. Materials and methods

2.1. General experimental

HPLC analyses were performed on a Waters (Milford, MA, USA) Alliance 2695 using a 250×4.6 mm Phenomenex (Torrence, CA, USA) C18 Aqua column and monitored with a Waters 996 PDA scanning device from 210 to 600 nm. A Perkin–Elmer (Wellesley, MA, USA) Lambda 2 UV/VIS spectrophotometer was used for total anthocyanin content (TAC) determinations. A Molecular Devices (Sunnyvale, CA, USA) Versamax tunable absorbance detector was used for the 1,1-diphenyl-2-picrylhydrazyl (DPPH) antiradical assay and total phenolic content (TPC) measurements. Extractions were performed at room temperature, using an ultrasonicator from VWR (West Chester, PA, USA; model 50 T, 45 W, 38.5–40.5 kHz). Reversed-phase C18 solid phase extraction (SPE) cartridges (500 mg, 4 ml) from Alltech (Deerfield, IL, USA) were used for sample preparation. Compounds 3–8, DPPH, and 2 N Folin–Ciocalteu reagent (FCR) were purchased from Sigma (St. Louis, MO, USA). Compounds 1 and 2 were previously isolated from *Myrciaria cauliflora* (Reynertson et al., 2006).

2.2. Plant material

Ripe fruits of Eugenia aggregata, *E. brasiliensis*, *E. luschnathiana*, *E. reinwardtiana*, *Myrciaria cauliflora*, *M. vexator*, *Syzygium cumini*, *S. curranii*, *S. jambos*, *S. javanicum*, *S. malaccense*, *S. samarangense*, and *S. samarangense* var. *Taiwan pink* were collected at the Fruit and Spice Park (Homestead, FL, USA), Rare Fruit and Vegetable Council of Broward County Research Garden (Southwest Ranches, FL, USA), The Kampong (Coconut Grove, FL, USA), and the University of Florida Tropical Resource and Education Center (Homestead, FL, USA). Fruits were frozen and shipped by overnight courier on dry ice to the laboratory, where they were kept in cold ($-20\,°C$) dark storage until processed. Voucher specimens were prepared, identified, and deposited at the Steere Herbarium of The New York Botanical Garden (Bronx, NY, USA). Dried, powdered camu-camu fruit (*M. dubia*) was supplied by Essential Living Foods (Los Angeles, CA, USA).

2.3. Extraction and sample preparation

Approximately 10 g of freeze-dried fruit were homogenized in a blender with 200 ml of MeOH–formic acid (9:1) and sonicated for 1 h. Extracts were centrifuged, filtered, and the marc extracted two more times. Extracts were combined and concentrated under reduced pressure at temperatures not exceeding 40 °C and brought up to 100 ml total. Aliquots (2–4 ml) were prepared for each experiment and stored at $-20\,°C$ until analyzed. Aliquots analyzed by HPLC and DPPH antiradical assay were dried, resuspended in 1–2 mL water, and subjected to SPE, eluting sequentially with water and MeOH. The MeOH fractions were collected, dried, and dissolved in DMSO for the DPPH assay or MeOH–formic acid (9:1) for HPLC analysis.

2.4. Quantitative HPLC-PDA analysis

Following SPE, two aliquots of each fruit extract were analyzed in triplicate ($n = 6$). HPLC-PDA analysis was performed using a gradient solvent system of 1% formic acid (A) and acetonitrile (B) as follows: 90–75% A over 30 min; 75–40% A from 30–45 min at a flow rate of 1 ml/min. Results were monitored from 210 to 600 nm. Sample concentrations ranged from 1.5 to 7 mg/ml. Peak area for each compound quantified in the test samples was integrated from the HPLC-PDA chromatogram using Waters Empower software and the concentration was calculated using the equation for each standard curve.

2.5. Standard curves, limits of detection and quantification, recovery

Stock solutions of compounds **1** and **3–8** were prepared in MeOH–formic acid (9:1), and combined to make a standard mixture. This stock mixture was diluted to make six standard solutions (ranging from 0.95 to 888 μg/ml) for HPLC-PDA quantification. Peak area for each standard was integrated from HPLC-PDA chromatograms at 520 nm for anthocyanins and 254 nm for all other compounds using Waters Empower software and plotted against concentration to create a linear curve. Delphinidin 3-glucoside (**2**) was quantified based on cyanidin 3-glucoside equivalents.

Minimum limits of detection (LOD), at a signal-to-noise (S/N) ratio of 3:1, and minimum levels of quantification (LOQ), at a S/N ratio of 10:1, were determined experimentally.

The percent recovery for the analytical method was calculated for three representative compounds, (**1**, **3**, and **8**). Each standard was accurately weighed (0.1 mg) and added to 1 g of freeze-dried *S. malaccense* fruit. The material was extracted, prepared, and analyzed, following the same method as used for HPLC-PDA quantification.

2.6. Total phenolic content

Analysis was performed by the Folin–Ciocalteu method (Kähkönen et al., 1999; Singleton & Rossi, 1965). Three aliquots were analyzed in triplicate ($n = 9$). Samples or gallic acid (100 μl) and 1 ml of FCR (diluted 10-fold) were

mixed in a vortexer and incubated for 5 min at room temperature (22 °C) prior to the addition of 1 ml of 10% Na_2CO_3 solution. This mixture was then allowed to stand for 90 min at room temperature, and the absorbance was determined at 765 nm. TPC was calculated as gallic acid equivalents (GAE) per g of dry weight.

2.7. Total anthocyanin content

Anthocyanin content was determined by the pH-differential method (Lee, Durst, & Wrolstad, 2005). Pigment concentration is calculated and expressed as cyanidin 3-glucoside equivalents per g of dry weight using the formula:

$$TAC(mg/g) = \frac{A \times MW \times DF}{\varepsilon \times 1}$$

where $A = (A_{520\,nm} - A_{700\,nm})$pH 1.0 $- (A_{520\,nm} - A_{700\,nm})$pH 4.5; MW (molecular weight) = 449 g/mol; DF = dilution factor; 1 = cuvette pathlength in cm; $\varepsilon = 26{,}900$ l/mol/cm, molar extinction coefficient for cyanidin 3-glucoside.

2.8. Antiradical activity

Samples for the DPPH· antiradical assay were assayed in triplicate, as previously described, using 400 μM DPPH (Yang et al., 2003). Gallic acid was used as a positive control ($IC_{50} = 30.0 \pm 2.9$ μM).

3. Results and discussion

3.1. General

Dark-coloured temperate fruits have recently generated considerable interest as a rich source of phenolic antioxidants. We have analyzed and quantified several antioxidative and anti-inflammatory flavonols, phenolic acids, and anthocyanins from 14 underutilized tropical Myrtaceae

Table 1
Results of antiradical DPPH assay (IC_{50} in μg/ml), TPC (mg GAE/g dry weight), and TAC (mg C3G/g dry weight)

	DPPH·	TAC	TPC
E. aggregata	84.6 ± 2.63	1.26 ± 0.45	25.3 ± 1.54
E. brasiliensis	42.7 ± 5.92	8.37 ± 0.23	24.8 ± 1.06
E. luschnathiana	38.0 ± 4.86	ND[a]	22.0 ± 0.21
E. reinwardtiana	110 ± 8.31	0.08 ± 0.03	9.25 ± 0.78
M. cauliflora	19.4 ± 0.28	2.78 ± 0.17	31.6 ± 0.39
M. dubia	57.2 ± 5.61	Tr[b]	101 ± 0.25
M. vexator	38.6 ± 2.40	6.84 ± 0.36	44.1 ± 1.21
S. cumini	389 ± 36.0	6.33 ± 0.10	9.95 ± 1.26
S. curranii	33.4 ± 2.52	12.1 ± 0.53	39.6 ± 0.77
S. jambos	92.0 ± 8.24	ND	8.69 ± 0.57
S. javanicum	81.4 ± 6.24	0.09 ± 4 × 10⁻³	3.57 ± 0.24
S. malaccense	269 ± 7.66	Tr	8.58 ± 0.12
S. samarangense	77.5 ± 4.19	0.07 ± 0.03	18.0 ± 0.70
S. samarangense var. Taiwan pink	157 ± 13.0	1.35 ± 0.10	23.8 ± 0.30

[a] ND: not detected.
[b] Tr: trace (below 0.01 mg/g).

Table 2
Minimum limits of detection and quantification of each standard compound

	LOQ (ng)	LOD (ng)
Cyanidin 3-glucoside (**1**)[a]	10.0	3.0
Ellagic acid (**3**)	8.3	2.5
Kaempferol (**4**)	11.0	4.0
Myricetin (**5**)	17.8	4.5
Quercetin (**6**)	16.4	4.5
Quercitrin (**7**)	b	b
Rutin (**8**)	25.0	8.0

[a] Cyanidin 3-glucoside was quantified at 520 nm, all others were quantified at 254 nm.
[b] The LOD and LOQ were not determined for quercitrin.

fruits. The anthocyanins are the most abundant compounds among those quantified (Tables 1 and 2) and are largely responsible for the bright colours of these fruits.

3.2. Total phenolic content, total anthocyanin content and antiradical activity

Results for TPC, TAC, and antiradical activity are displayed in Table 1. The TPC for most species falls within the range of 3.57 (*S. javanicum*) to 44.1 mg GAE/g (*M. vexator*). The TPC for *M. dubia* (camu-camu), however, is over double the TPC for *M. vexator* at 101 mg GAE/g. The camu-camu, however, is known to have high levels of ascorbic acid (Aragao, Ikegaki, Sato, Oliveira, & Park, 1996), which have been shown to interfere with the TPC measurement by the same oxidation/reduction reaction that detects phenolics (Prior, Wu, & Schaich, 2005; Singleton et al., 1965). TAC values range from undetected (*E. luschnathiana* and *S. jambos*) to 12.1 mg/g equivalents (*S. curranii*). Antiradical activity ranges from very active (*M. cauliflora*, 19.4 μg/ml) to inactive (389 μg/ml, *S. cumini*). *Myrciaria cauliflora* is the most active extract in the DPPH· assay, with only a slightly higher than average TPC (31.6 mg GAE/g) and TAC (2.78 mg/g). *Syzygium curranii* is the highest in anthocyanin content according to both the TAC method and HPLC method. In our analysis, TAC did not correlate strongly with antiradical activity ($\gamma^2 = 0.1147$), possibly because the anthocyanins make up only a portion of the overall phenolic profile. TPC was more closely correlated with antiradical activity, but did not reach significance ($\gamma^2 = 0.4542$). There was no correlation between the individual phenolics quantified and the antiradical activity, suggesting that the antiradical activity of these extracts is a complicated synergy that may also involve compounds not identified or quantified. The DPPH· antiradical activity of the standard compounds quantified from these fruits has been previously reported in the literature (Luo, Basile, & Kennelly, 2002; Ma, Yang, Basile, & Kennelly, 2004; Reynertson et al., 2006). The activities of these compounds are rather similar, and a comparison of their concentrations in these extracts does not point to any obvious trends.

3.3. Standard curves, limits of detection and quantification, recovery

All standard curves had a correlation coefficient (γ^2) value > 0.9998. The LOD and LOQ for each compound are given in Table 2. *Syzygium malaccense* was chosen as

a representative species for the recovery experiment because the experimental matrix being analyzed (lyophilized, deseeded fruit) was the same for each species. When using a relatively small amount of material (1 g), the constituents of *S. malaccense* were not sufficiently concentrated to interfere with the recovery analysis. Using the same
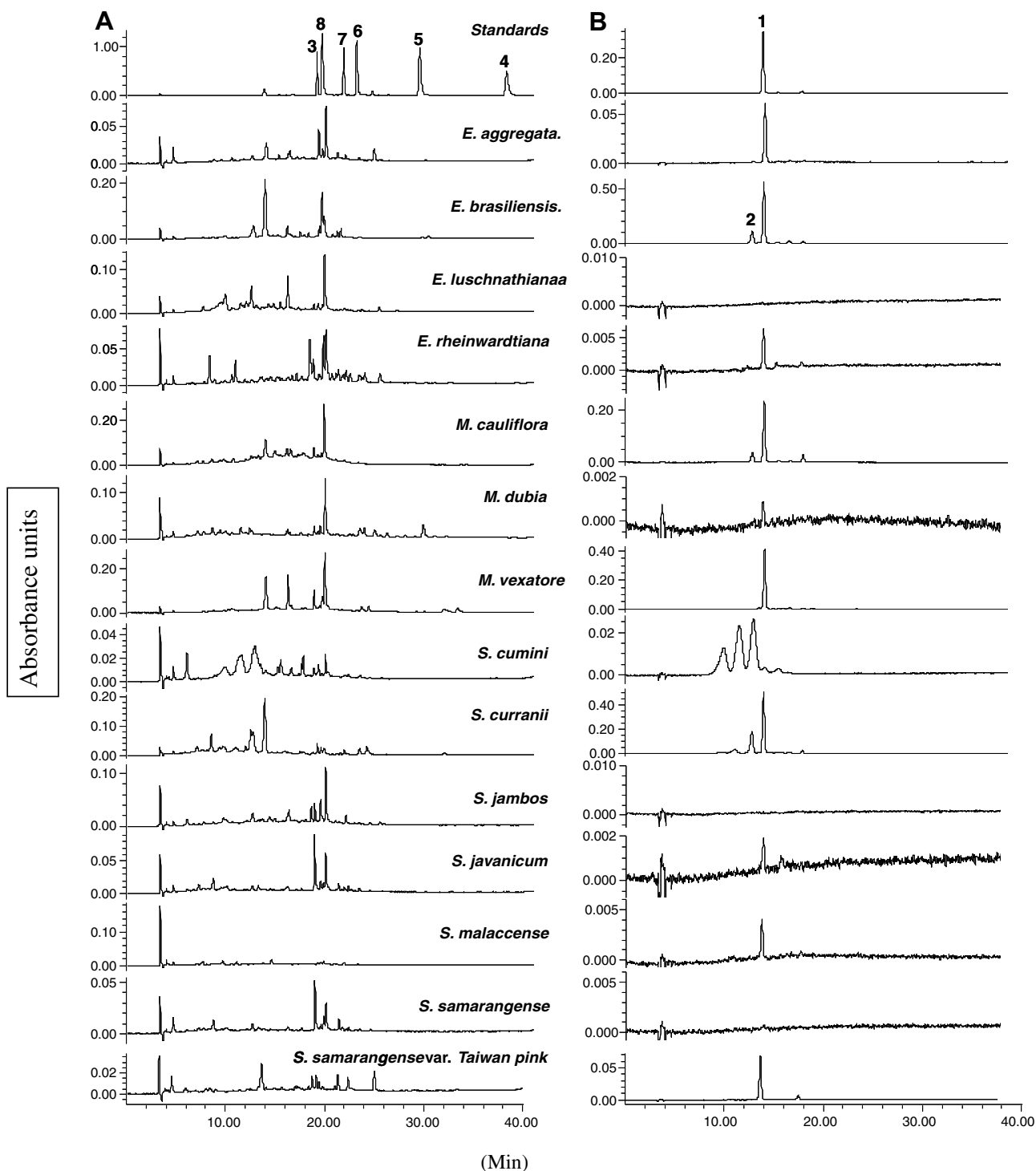

Fig. 2. HPLC-PDA chromatograms at (A) 254 nm and (B) 520 nm of the standard mixture and methanolic extracts of 14 edible Myrtaceae fruits. Compounds are numbered as follows: cyanidin 3-glucoside (**1**), delphinidin 3-glucoside (**2**), ellagic acid (**3**), kaempferol (**4**), myricetin (**5**), quercetin (**6**), quercitrin (**7**), and rutin (**8**).

analytical methods as for the HPLC-PDA quantification, it was determined that the average recovery of cyanidin 3-glucoside (**1**) was $113 \pm 12.6\%$; ellagic acid (**3**) was $80.1 \pm 9.5\%$; and rutin (**8**) was $96.1 \pm 9.6\%$.

3.4. Quantitative HPLC-PDA analysis

Chromatograms of the standard mixture and the 14 fruit extracts are presented in Fig. 2. In our HPLC analysis, **1** is the most abundant compound in *E. aggregata*, *E. brasiliensis*, *M. cauliflora*, *M. vexator*, *S. curranii*, *S. malaccense*, and *S. samarangense* var. *Taiwan pink* (Table 3). It was detected or quantified in all but the two species that do not produce fruit coloured dark-purple or red; the orange fruit of *E. luschnathiana* and yellow fruit of *S. jambos* have no detectable anthocyanins by HPLC-PDA or TAC measurement. *Eugenia brasiliensis*, *M. vexator* and *S. curranii* have far greater levels of **1** (10.2, 13.1, and 9.56 mg/g, respectively) than the next-highest species (*M. cauliflora*, 4.33 mg/g). The purple-skinned fruits *E. brasiliensis*, *E. aggregata*, *M. cauliflora*, *M. vexator*, *S. curranii* and *S. cumini* contain both **1** and delphinidin 3-glucoside (**2**) and are unsurprisingly the highest in TAC (Table 1). The only reddish fruit that contains both **1** and **2** is *M. dubia*; the remaining red to pink fruits *E. reinwardtiana*, *S. malaccense*, *S. samarangense* and *S. samarangense* var. *Taiwan pink*, contain only **1**. *Syzygium curranii* has far higher levels of delphinidin 3-glucoside than have any other fruit in the analysis (4.83 mg/g). *Syzygium cumini*, which is used by Ayurvedic practitioners in India as an antidiabetic medicine (Grover, Yadav, & Vats, 2002), has more **2** than **1**, based on the HPLC analysis, and the HPLC chromatogram indicates that the fruit may contain several delphinidin glycosides. Compound **2** is a potent antiradical compound (DPPH· $IC_{50} = 26.3 \, \mu M$), so it is remarkable

that *S. cumini* fruit pulp is essentially inactive in the DPPH· assay. However, while the fruits may contain many delphinidin glycosides, the TPC (9.95 mg/g) was below average for the 14 fruits tested, and comparable with other fruits with low antiradical activity ($IC_{50} > 100 \, \mu g/ml$). Seed extracts of *S. cumini*, the part most often used in Ayurvedic medicine, were previously shown to have high levels of total phenolics and good activity in the trolox equivalent antioxidant capacity (TEAC) and ferric reducing antioxidant power (FRAP) antioxidant assays (Luximon-Ramma, Bahorun, & Crozier, 2003).

Quercetin (**6**) and its glycosides quercitrin (**7**) and rutin (**8**) are prevalent in the 14 studied fruits. These flavonols are important dietary constituents, with many chemopreventive activities (Formica & Regelson, 1995). The concentration of **6** is <0.01 mg/g only in *S. malaccense*. *Syzygium curranii* and *M. dubia* had levels of **6** approximately six times higher (0.28 and 0.24 mg/g, respectively) than the other test fruits. Quercitrin (**7**) was also detected in every species, from <0.01 mg/g (*S. cumini*) to 0.18 mg/g (*S. samarangense* var. *Taiwan pink*). *Syzygium curranii* had levels of **7** (0.17 mg/g) similar to *S. samarangense* var. *Taiwan pink*. Rutin (**8**), detected in every fruit but *S. jambos*, was found at the highest levels in *E. aggregata* (0.48 mg/g).

Ellagic acid (**3**) and its derivatives are also abundant in this taxon (Lowry, 1968; Okuda et al., 1982). It is likely that ellagic acid derivatives are present in many of these extracts, and contribute considerably to the overall TPC and antiradical activity. In our analysis, **3** was identified and quantified. The three *Myrciaria* species have the highest levels of **3** (*M. cauliflora*, 0.52; *M. dubia*, 0.45; and *M. vexator*, 0.64 mg/g). It was found in every species except *E. aggregata*, *E. reinwardtiana*, and *S. samarangense* var. *Taiwan pink*. Generally, it was the most abundant compound by HPLC-PDA analysis in the red, orange,

Table 3
Results of HPLC-PDA quantification of common phenolics in 14 edible Myrtaceae fruits[a]

	Cyanidin 3-glc (**1**)	Delphinidin 3-glc (**2**)	Ellagic acid (**3**)	Myricetin (**5**)	Quercetin (**6**)	Quercitrin (**7**)	Rutin (**8**)
E. aggregata	1.42 ± 0.17[b]	$0.04 \pm 4.7 \times 10^{-3}$	ND[c]	$0.04 \pm 3.6 \times 10^{-3}$	$0.04 \pm 4.3 \times 10^{-3}$	$0.06 \pm 7.8 \times 10^{-3}$	0.48 ± 0.04
E. brasiliensis	10.2 ± 0.42	2.58 ± 0.13	0.26 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	0.06 ± 0.01	0.08 ± 0.01
E. luschnathiana	ND	ND	0.40 ± 0.01	Tr[d]	$0.04 \pm 2.98 \times 10^{-3}$	$0.07 \pm 2.31 \times 10^{-3}$	0.18 ± 0.01
E. reinwardtiana	$0.04 \pm 2.2 \times 10^{-3}$	ND	ND	$0.01 \pm 0.7 \times 10^{-3}$	$0.03 \pm 0.6 \times 10^{-3}$	$0.05 \pm 5.6 \times 10^{-3}$	$0.04 \pm 5.6 \times 10^{-3}$
M. cauliflora	4.33 ± 0.24	0.81 ± 0.06	0.52 ± 0.22	$0.02 \pm 2.6 \times 10^{-3}$	$0.04 \pm 7.6 \times 10^{-3}$	0.11 ± 0.03	0.21 ± 0.02
M. dubia	$0.02 \pm 3.2 \times 10^{-3}$	Tr	0.45 ± 0.04	ND	0.24 ± 0.02	$0.06 \pm 7.2 \times 10^{-3}$	$0.13 \pm 7.0 \times 10^{-3}$
M. vexator	13.1 ± 3.17	0.29 ± 0.06	0.64 ± 0.26	$0.03 \pm 4.4 \times 10^{-3}$	0.08 ± 0.02	0.05 ± 0.02	0.11 ± 0.02
S. cumini	0.14 ± 0.02	1.61 ± 0.50	$0.03 \pm 6.2 \times 10^{-3}$	ND	$0.01 \pm 8.0 \times 10^{-3}$	Tr	0.13 ± 0.02
S. curranii	9.56 ± 0.84	4.83 ± 0.41	0.09 ± 0.01	Tr	$0.28 \pm 9.0 \times 10^{-3}$	0.17 ± 0.02	0.32 ± 0.01
S. jambos	ND	ND	0.05 ± 0.02	ND	$0.01 \pm 0.3 \times 10^{-3}$	$0.03 \pm 4.0 \times 10^{-3}$	ND
S. javanicum	$0.01 \pm 1.4 \times 10^{-3}$	ND	0.05 ± 0.02	ND	$0.01 \pm 2.2 \times 10^{-3}$	$0.02 \pm 0.9 \times 10^{-3}$	Tr
S. malaccense	$0.02 \pm 1.5 \times 10^{-3}$	ND	$0.01 \pm 1.7 \times 10^{-3}$	ND	Tr	0.02 ± 0.01	$0.02 \pm 1.4 \times 10^{-3}$
S. samarangense	Tr	ND	0.09 ± 0.01	Tr	$0.02 \pm 2.2 \times 10^{-3}$	$0.02 \pm 6.0 \times 10^{-3}$	Tr
S. samarangense var. *Taiwan pink*	1.56 ± 0.10	ND	ND	ND	0.07 ± 0.02	$0.18 \pm 6.6 \times 10^{-3}$	0.16 ± 0.02

[a] Amount of compound per dry weight of plant material expressed as mg/g.

[b] Mean $\pm$ SD ($n = 6$).

[c] ND: not detected.

[d] Tr: amount was less than 0.01 mg/g.

and yellow-fruited species (*E. luschnathiana, S. javanicum, S. jambos, S. samarangense* and *M. dubia*).

Myricetin (**5**) was found in each of the *Eugenia* and *Myrciaria* species tested but *M. dubia* while, among the *Syzygium* species, it was detected only in *S. curranii* and *S. samarangense* at levels below 0.01 mg/g. Kaempferol (**4**) was detected in *E. brasiliensis, E. luschnathiana* and *S. curranii*, but at levels below 0.01 mg/g. *M. cauliflora, S. malaccense* and *S. jambos* were also found to contain *t*-cinnamic acid (**9**) by HPLC-PDA, but it was not quantified.

With cancers and heart disease being the leading causes of death in the United States, studies indicate that diets high in naturally occurring antioxidants and anti-inflammatories are important as a first-line strategy of chemoprevention (Arts & Hollman, 2005; Collins, 2005; Hu & Willett, 2002; Youdim & Joseph, 2001). We have shown that edible fruits in the Myrtaceae are a rich source of biologically active phenolic compounds, similar to other well-studied temperate berries and fruits (Kähkönen et al., 2001).

Anthocyanins, flavonoids, phenolic acids and tannins play primarily ecological roles, to attract pollinators and seed disbursers, and act as antifeedants and defence compounds against microbial infection (Middleton et al., 2000). Tropical Myrtaceae fruits are grown under conditions of high oxidative stress from intense sunlight and heat, and phenolic compounds inhibit lipid peroxidation and ultraviolet damage in plant tissues (Lee & Gould, 2002) in addition to protecting mammalian systems when eaten as fruit.

Acknowledgements

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