

Analytical, Nutritional and Clinical Methods

# Phytochemical flavonols, carotenoids and the antioxidant properties of a wide selection of Fijian fruit, vegetables and other readily available foods

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Received 10 April 2005; received in revised form 2 January 2006; accepted 20 January 2006

## Abstract

Frequent consumption of fruits and vegetables is associated with a lowered risk of cancer, heart disease, hypertension and stroke. This has been attributed to the presence of various forms of phytochemicals and antioxidants present in the foods, e.g. carotenoids and polyphenol compounds including flavonoids and anthocyanins. Seventy Fiji grown fruits and vegetables, and some other commonly consumed products, were analysed for their total antioxidant capacity (TAC), total polyphenol content (TPP), total anthocyanin content (TAT) as well as the major flavonol and carotenoid profiles. These data will be used to estimate the phytochemical and antioxidant intake of the Fijian population and will be a useful tool in future clinical trials.

Green leafy vegetables had the highest antioxidant capacity, followed by the fruits and root crops. A number of herbs also exhibited high antioxidant capacity. *Ipomoea batatas* (sweet potato) leaves have the highest TAC (650 mg/100 g) and are rich in TPP (270 mg/100 g), quercetin (90 mg/100 g) and  $\beta$ -carotene (13 mg/100 g). *Moringa oleifera* (drumstick) leaves also have a high TAC (260 mg/100 g) and are rich in TPP (260 mg/100 g), quercetin (100 mg/100 g), kaempferol (34 mg/100 g) and  $\beta$ -carotene (34 mg/100 g). *Curcuma longa* (turmeric ginger) has a high TAC (360 mg/100 g), TPP (320 mg/100 g) and is rich in fisetin (64 mg/100 g), quercetin (41 mg/100 g) and myricetin (17 mg/100 g). *Zingiber officinale* (white ginger) also has a high TAC (320 mg/100 g) and TPP (200 mg/100 g). *Zingiber zerumbet* (wild ginger), a widely used herb taken before meals is the richest source of kaempferol (240 mg/100 g).

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**Keywords:** Phytochemicals; Flavonols; Carotenoids; Antioxidant capacity; Food; Fiji

## 1. Introduction

Phytochemicals and antioxidant constituents in plant material have raised interest among scientists, food manufacturers, producers, and consumers for their roles in the maintenance of human health (Milner, 1999). Numerous epidemiological studies suggest that diets rich in phyto-

chemicals and antioxidants execute a protective role in health and disease. Frequent consumption of fruits and vegetables is associated with a lowered risk of cancer, heart disease, hypertension and stroke (Marco, Joseph, & John, 1997; Vinson, Su, Zubik, & Bose, 2001; Wolfe & Liu, 2003). Phytochemicals are bioactive substances of plants that have been associated in the protection of human health against chronic degenerative diseases. Antioxidants are compounds that help delay and inhibit lipid oxidation and when added to foods tend to minimize rancidity, retard

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the formation of toxic oxidation products, help maintain the nutritional quality and increase their shelf life (Fukumoto & Mazza, 2000).

The major groups of phytochemicals that may contribute to the total antioxidant capacity (TAC) of plant foods include polyphenols, carotenoids and the traditional antioxidant vitamins such as vitamin C and vitamin E. The vitamins are, however, not the only phytochemicals that can have a positive effect on the health of consumers. There are other phytochemicals present in plant foods that may have positive effects on the health of consumers and need further investigation. These phytochemicals may be present in small amounts but may be very important to the health of consumers.

Fruits and vegetables contain arrays of antioxidants with varying degrees of activities and are thus quite difficult to analyze. The TAC assay is a convenient way of assessing the total antioxidant level in foods and was used in this study to determine the amount of the collective antioxidant activity in a given food. There are various methods available in the assessment of total antioxidant capacity (Benzie & Szeto, 1999; Cao, Sofic, & Prior, 1996; Ghiselli, Serafini, Natella, & Scaccini, 2000; Gil, Tomas-Barberan, Hess-Pierce, Holcroft, & Kader, 2000; Prior et al., 1998; Pulido, Bravo, & Saura-Calixto, 2000; Wang, Cao, & Prior, 1996). In the light of the available resources, the ABTS (2,2'-azinobis (3-ethyl-benzothiazoline-6-sulphonate)) radical decolorization method was chosen as the most appropriate method to use. The total polyphenol (TPP) and total anthocyanin (TAT) content are also good indicators of antioxidant capacity and studies have reported a high correlation between antioxidant capacity and total polyphenols (Pellegrini et al., 2000; Simonetti, Piergiorgio, & Testolin, 1997). Several flavonols, e.g. quercetin, kaempferol, myricetin, and carotenoids (lutein, lycopene,  $\alpha$ - and  $\beta$ -carotene) are powerful antioxidants and were also selected as targets for this study.

Although most countries in the South Pacific are still regarded as developing nations, the prevalence of chronic degenerative disorders is similar or higher to the rate of the developed world (Coyne, 2000; Wahlqvist, 2001; Worsely, 2001). Given the importance of dietary habit and food components to health, the provision of phytochemical and antioxidant information of a range of foods readily available in the South Pacific Islands is vital to support the future work in assessing the protective status of people from chronic degenerative disorders. Any approach used needs to be effective and culturally appropriate to the community. Food-based approaches would be essential for sustainable solutions to combat the alarming prevalence of chronic cancer, coronary heart diseases and diabetes.

The objective of this study was to determine the TAC, TPP, TAT selected flavonols and carotenoids present in foods available in Fiji. These data will be used to estimate the phytochemical and antioxidant intake of the Fijian population and in future clinical trials. The determination

of individual anthocyanins was beyond the scope of this work. Anthocyanins contribute to the overall antioxidant activity of the food, however, recent work (Manach, Williamson, Morand, Scalbert, & Remesy, 2005) suggests that anthocyanin bioavailability is quite low, thus larger quantities of food need to be consumed for maximum benefit.

The “new” health benefits of these foods may prompt research into the assessment and determination of potential rich sources of antioxidant compounds in agricultural produce that could improve cultivar development, production practices, post-harvest storage and food processing.

## 2. Materials and methods

The experimental work was carried out at the University of the South Pacific and the Ministry of Agriculture located in Suva, Fiji and at the PIRVic Food Chemistry Laboratory located at DPI-Werribee, Vic., Australia.

### 2.1. Reagents

Quercetin, myricetin, morin, fisetin, kaempferol, lutein,  $\beta$ -carotene, lycopene, gallic acid, *tert*-butylhydroquinone (TBHQ), butylated hydroxy toluene (BHT), 2,2'-azinobis (3-ethyl-benzothiazoline-6-sulphonate) (ABTS) and trolox C; (( $\pm$ )-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), a water-soluble vitamin E analogue were obtained from Sigma Chemical Co. (Castle Hill, NSW, Australia).  $\beta$ -Carotene (30% fluid suspension in vegetable oil) was a gift from Roche, Australia. Hydrochloric acid, triethylamine, Folin–Ciocalteu reagent, formic acid, acetic acid, potassium chloride and sodium carbonate were obtained from BDH Chemicals (Kilsyth, Vic., Australia). Magnesium carbonate was obtained from Chem-supply (Gillman, SA, Australia). Ammonium acetate, sodium acetate and potassium persulphate were obtained from Ajax Chemicals (NSW, Australia). AR grade or HPLC grade dichloromethane, acetonitrile, acetone and methanol were obtained from Mallinckrodt Chemical (Australia). Isorhamnetin was a gift from Dr Rod Jones, DPI-Knoxfield, Melbourne, Victoria.

### 2.2. Preparation of standards and samples

#### 2.2.1. Standards

2.2.1.1. *Trolox C*; (( $\pm$ )-6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). A 1.25 mg/ml stock standard solution of trolox C was prepared by dissolving 125 mg of dry trolox C in 100 ml of ethanol. The solution was stored in the freezer. Working standards of between 0.06 mg/ml and 0.5 mg/1 ml were prepared by diluting the stock solution with ethanol.

2.2.1.2. *Gallic acid*. A 0.5 mg/ml stock standard solution of gallic acid was prepared by firstly dissolving 250 mg of dry gallic acid in 1 ml of 95% ethanol and then diluting to 500 ml with distilled water. The solution was stored in the

4 °C. Working standards of between 0.1 and 0.5 mg/ml were prepared daily by diluting the stock solution with distilled water.

**2.2.1.3. Flavonols.** Separate 500 µg/ml stock standard solutions of quercetin, myricetin, morin, fisetin, kaempferol and isorhamnetin were prepared by dissolving 10 mg of the dry standard in 20 ml of methanol. The solutions were stored in the freezer. Working standards of between 5 and 20 µg/ml were prepared by diluting the stock solution with methanol.

**2.2.1.4. Carotenoids.**

1. Separate 50–100 µg/ml stock standard solutions of lycopene and β-carotene were prepared by dissolving 1–2 mg of the standards in 20 ml of acetone. The solutions were stored in at –20 °C. Working standards of between 5 and 20 µg/ml were prepared daily by diluting the stock solution with acetone. The purity was determined from the molar absorptivity (β-carotene)  $E_{1\text{cm}}^{1\%} = 2590$  at  $\lambda$  max 450 nm (Ball, 1988) and lycopene  $E_{1\text{cm}}^{1\%} = 3450$  at  $\lambda$  max 472 nm (Merck Index, 1989).
2. 100 mg of the 30% suspension of β-carotene in vegetable oil was dissolved in 50 ml acetone. Working standards of between 5 and 20 µg/ml were prepared daily by diluting the stock solution with acetone. The purity was determined from the molar absorptivity  $E_{1\text{cm}}^{1\%} = 2590$  at  $\lambda$  max 450 nm (Ball, 1988).
3. An α-carotene standard material was not available for this study. The levels of α-carotene were estimated from the areas of the α-carotene peak (matched with a carrot extract) area related to that of the standard for β-carotene (CRC handbook, 56th edition, Page C-325).

### 2.3. Sampling and sample preparation

#### 2.3.1. Sampling

Sampling procedures were chosen so that data obtained represented foods consumed by people in Fiji. Representative samples of the same variety or cultivar were purchased from at least three different sites; the two major food markets in the urban centres of Suva and Nausori and two other smaller outlets in the vicinity of Suva and Nausori. Approximately 2 kg of each sample type was purchased from each site. For large items, e.g. *Colocasia esculenta* (taro) about 2–3 pieces were sampled while more pieces were sampled for smaller produce, e.g. *Syzygium malaccense* (malay apple). For leafy vegetables, e.g. *Ipomoea aquatica* (kangkong), 2–3 bundles, each bunch containing 10–12 leaves were purchased from each site. Representative samples from each site were prepared by compositing all halves of large items, 1–2 pieces of smaller produce and about 7–8 leaves were randomly picked, mixed and prepared as described in the sample preparation and storage section below. The different cultivars of foods were all pur-

chased on the same day. The Fiji Ministry of Agricultural Research Stations provided other foods that were not available in the markets. Fiji Agricultural officers performed the identification and confirmation of all the cultivars of foods.

#### 2.3.2. Sample preparation and storage

The edible portions of foods were immediately washed and processed according to their consumption state. Foods that are usually eaten when cooked were either steamed or boiled in a stainless steel cooking pot depending on the food type. All the roots and tree staples were boiled for approximately 20–25 min with minimum water just to cover the food. All other leafy vegetables and other foods were steamed for approximately 3–5 min. Smaller food portions were steamed for approximately 3 min and the big portions for approximately 5 min. After cooling, each food type was either diced or cut into smaller pieces and mixed thoroughly prior to sub-sampling into approximately three equal portions; one each for TAC, TPP and TAT analyses, and the other two for flavonol and carotenoid analyses. The sub-samples were packed in sealed plastic bags and stored at –20 °C until analysed or further processing into a freeze-dried form. Similarly, fruits and vegetables that are usually eaten in their raw state were washed, diced or cut into smaller pieces, mixed well and also divided into approximately three equal portions prior to packing and stored at –20 °C until analysed or for further processing into a freeze-dried form.

The third sub-sample of every food were freeze-dried as soon as possible after preparation, ground to a fine powder and stored in air tight containers at –20 °C until required for flavonol and carotenoid analyses or for transport to Australia. All samples were analysed as soon as possible after preparation.

#### 2.3.3. Sample extractions and assays

**2.3.3.1. TAC, TPP and TAT sample extraction.** Samples for TAC, TPP and TAT assays were extracted according to the method of Prior et al. (1998) and Sellappan and Akoh (2002a, 2002b) with some modifications. Briefly, approximately 10 g homogenised food was mixed with 45 ml of acetonitrile containing 4% acetic acid and blended for 5 min with a hand held Bamix mixer. The mixture was mechanically shaken for 30 min and then centrifuged at 5100g for 15 min at 10 °C. The supernatant (water-soluble fraction) was recovered and the pulp was further washed with 45 ml of acetonitrile containing 4% acetic acid and centrifuged. The resulting supernatants were combined and the volume adjusted to 100 ml with 95% ethanol. The solution was filtered before analysis.

**2.3.3.2. Total antioxidant capacity assay.** The TAC assay was determined with a Cintra 5 Spectrophotometer by the method of Pellegrini, Re, Yang, and Rice-Evans (1999), Rice-Evans and Miller (1994) and Sellappan and Akoh (2002a, 2002b). Briefly, ABTS<sup>+</sup> radical cation was

produced from the reaction of 7 mM 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and 2.45 mM potassium persulfate after incubation at room temperature in dark for 16 h. The ABTS<sup>•+</sup> solution was diluted with 95% ethanol to an absorbance of  $0.70 \pm 0.02$  at 734 nm. The trolox standards of different concentrations from 0.06 to 0.12 mg/ml were prepared to give 20–80% inhibition of the blank absorbance for linearity purpose. The ABTS<sup>•+</sup> solution (2000  $\mu$ L; absorbance of  $0.70 \pm 0.02$ ) was read at 734 nm before 200  $\mu$ L of the sample extract (drawn from the pooled extracts in the method extraction described above) was added, mixed for 10 s and absorbance was continuously taken at every 10 s intervals up to 1.0 min. Trolox equivalent antioxidant capacity of sample was calculated based on the inhibition exerted by standard Trolox solution at 1 min and expressed as milligrams of trolox equivalent antioxidant capacity (TEAC) per 100 g fresh weights.

**2.3.3.3. Total polyphenol assay.** The TPP content was determined by the Folin–Ciocalteu method (Sellappan & Akoh, 2002a, 2002b). Food extracts (0.5 ml) or gallic acid standard solutions were mixed with 2.5 ml of Folin–Ciocalteu's Reagent (FCR-1:10 dilution) and left to stand for 8 min at room temperature to allow for the FCR to react completely with the oxidizable substances or phenolates. 2.0 ml of Na<sub>2</sub>CO<sub>3</sub> (7.5% solution in water) was added to destroy the residual reagent. The absorbances were measured at 760 nm using a Cintra 5 UV–vis Spectrophotometer after incubating at room temperature for 2 h. Results were expressed as milligrams of gallic acid equivalents (GAE) per 100 g fresh weights.

**2.3.3.4. Total anthocyanin assay.** The total anthocyanin content of the food was estimated a Cintra 5 UV–vis Spectrophotometer by the pH-differential method (Sellappan & Akoh, 2002a, 2002b) using two buffer systems – potassium chloride buffer, pH 1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M).

Briefly, 0.4 ml of the extract was mixed with 3.6 ml of corresponding buffers and read against a blank at 510 and 700 nm. Absorbance (A) was calculated as:  $A = (A_{510} - A_{700})_{\text{pH } 1.0} - (A_{510} - A_{700})_{\text{pH } 4}$ . Monomeric anthocyanin pigment concentration in the extract was calculated as cyanidin-3-glucoside (mg/l) =  $A \times \text{MW} \times \text{DF} \times 1000 / (\text{MA} \times 1)$  (Sellappan & Akoh, 2002a, 2002b) where A: absorbance; MW: molecular weight (449.2); DF: dilution factor; MA: molar absorptivity (26,900). The total anthocyanin content was expressed as cyanidin-3-glucoside (mg/100 g).

**2.3.3.5. Flavonols sample extraction.** Flavonols were extracted according to methods of Crozier, Lean, McDonald, and Black (1997) Hertog, Hollman, and Venema (1992). Forty milliliters of 62.5% aqueous methanol containing 2 g/L of tertbutylhydroxyquinone (TBHQ) as an antioxidant was added to 0.5–1 g of freeze – dried sample.

Ten milliliters of 6 M HCl was added and the solution refluxed at 90 °C for 2 h. The mixture was cooled, made to 100 ml with methanol, mixed thoroughly and then sonicated for 5 min. An aliquot of the extract was then filtered through 0.5  $\mu$ m cellulose acetate filter disc prior to HPLC analysis.

**2.3.3.6. Carotenoids sample extraction.** Samples were homogenised with a laboratory grade food processor. To a 10 g wet or 1 g dry sample was added 1 g of heavy magnesium carbonate, 20 g sodium sulphate and 30 ml acetone and the sample extracted with a bamix blender for 5 min. The mixture was filtered through a Whatman No. 4 filter paper under vacuum. The residue, including the filter paper, was re-extracted with acetone (30 ml portions) until no residual colour was observed in the filter cake. The filtrates were combined and made to volume in either a 100 or 200 ml volumetric flask with acetone depending on the intensity of the colour in the sample. An aliquot of the extract was filtered through 0.5  $\mu$ m nylon filter disc before HPLC analysis. All extractions were conducted in a dark room.

**2.3.3.7. Moisture.** The moisture content of the samples were determined by drying 10 g of sample in an air oven at 105 °C until constant weight (16–18 h) (AOAC, 2000).

## 2.4. Apparatus

### 2.4.1. Fiji

**2.4.1.1. Spectrophotometry.** TAC, TPP and TAT analyses were performed with a Cintra 5, UV–vis Spectrophotometer, (GBC Scientific Equipment, Melbourne Australia) equipped with a temperature control thermostat model BCT-9090.

**2.4.1.2. HPLC.** The analyses were performed with a Varian ProStar UV/Vis detector Model 320, solvent binary delivery module model 210, solvent degasser (Varian, Australia) fitted with 717 Waters autosampler (Waters, Australia). Varian Star software version 5.51 was used to process the chromatographic data.

**2.4.1.3. Flavonols.** The flavonols were separated with a 250 mm  $\times$  4.6 mm 5  $\mu$ m C<sub>18</sub> OmniSpher column fitted with a 5  $\mu$ m C<sub>18</sub> ChromGuard column (Varian, Australia) using two mobile phases consisting of (A) 5% formic acid, 95% water and (B) 100% methanol and a flow rate of 1.0 ml/min. The elution profile was 0–8 min, 7% B in A (isocratic), 8–20 min, 7–15% B in A (linear gradient), 20–35 min, 15–65% B in A (linear gradient), 35–40 min, 65–80% B in A (linear gradient), 40–45 min 80–7% B in A (linear gradient). Flavonoids were detected at 370 nm and peak areas were used in the calculations. Myricetin, fisetin, morin, quercetin and kaempferol had retention times of 23.4, 23.9, 24.2, 25.3, 27.2 min, respectively.



**2.4.1.4. Carotenoids.** The carotenoids were separated with a 250 mm × 4.6 mm 5 µm C<sub>18</sub> Microsorb column (Waters, Rydalmere, Australia) fitted with a 5 µm C<sub>18</sub> ChromGuard column (Varian, Australia) using a mobile phase consisting of 95% methanol, 5% tetrahydrofuran with a flow rate of 2.0 ml/min. Carotenoids were detected at 450 nm and peak areas were also used in the calculations. Lycopene, α-carotene and β-carotene had retention times of 23.0, 25.8 and 27.0 min, respectively, with this system.

#### 2.4.2. Australia

**2.4.2.1. HPLC.** The analyses were performed with a Waters 600E HPLC pump (Waters, Rydalmere, Australia) equipped with an Agilent series 1100 autosampler and photodiode array detector (Agilent Technologies, North Ryde, Australia). Agilent Chemstation software was used to process the chromatographic data.

**2.4.2.2. Flavonols.** The flavonols were separated with a 250 mm × 4.6 mm 5 µm C<sub>18</sub> Hypersil column fitted with a 4 µm Novapak C<sub>18</sub> Guard column. Two mobile phases were used for elution – (A) 1% formic acid, 99% water and (B) 1% formic acid, 49% water and 50% methanol. The elution profile was 0–4 min, 10% B in A (isocratic), 4–21 min, 10–100% B in A (linear gradient), 21–41 min, 100% B (isocratic), 41–42 min, 100–10% B in A (linear gradient), 42–46 min, 10% B in A (isocratic). Flavonols were detected at 370 nm and peak areas were used in the calculations. The flavonols myricetin, quercetin, kaempferol and isorhamnetin had retention times of 23.8, 26.7, 31.2, 32.6 min, respectively. Retention times and spectral data collected with the photodiode array detector were used for peak confirmation.

**2.4.2.3. Carotenoids.** The carotenoids were separated with a 250 mm × 4.6 mm 5 µm C<sub>18</sub> Microsorb column fitted with a 4 µm C<sub>18</sub> Novapak guard column (Waters, Rydalmere, Australia). The mobile phase consisted of 75% acetonitrile, 20% 0.05 M ammonium acetate in methanol, 5% dichloromethane, 0.05% triethylamine and 0.1% BHT and the flow rate was 2.0 ml/min. The carotenoids were detected at 450 nm and peak areas were also used in the calculations. Lycopene, α-carotene and β-carotene had retention times of 9.5, 18.5 and 19.8 min, respectively. Retention time data and spectral information from the photodiode array detector were used for peak confirmation.

### 3. Results

#### 3.1. Antioxidant capacity, polyphenol, flavonol and carotenoid composition of Fijian foods

##### 3.1.1. Green leafy vegetables

The data displayed in Table 1 indicate that *Ipomoea batatas* (sweet potato) leaves contain the highest total antioxidant capacity followed by *Moringa oleifera* (drumstick) leaves, *Colocasia esculenta* (taro) leaves, *Albemoshus manihot* (edible hibiscus) and *Amaranthus viridis* (amaranth).

Considerable antioxidant capacity was also detected in *Ipomoea aquatica* (kangkong), *Athyrium esculentum* (edible fern), *Rorippa nasturtium aquaticum* (watercress) and *Brassica chinensis* (chinese cabbage). *Moringa oleifera* leaves contains the highest amount of total polyphenolics (gallic acid equivalents) followed by *Ipomoea batatas* leaves, *Colocasia esculenta* leaves, *Albemoshus manihot*, *Amaranthus viridis* and *Ipomoea aquatica*. *Brassica nigra* (mustard green), *Ipomoea aquatica* and *Brassica chinensis* also have considerable total polyphenolics. The richest source of quercetin and kaempferol in green leafy vegetable is *Moringa oleifera* followed by *Ipomoea batatas* leaves. The flavonol chromatographic profiles of *Ipomoea aquatica* and *Moringa oleifera* are depicted in Fig. 1. The minor changes in retention times observed between the standards and analytes in the samples could be due to either slight changes in the mobile phase between (gradient) runs, fluctuations in laboratory temperature, or small changes in stationary phase caused by other compounds adhering to the packing material.

Foods with the richest sources of β-carotene in descending order are *Moringa oleifera* leaves, *Amaranthus viridis*, *Ipomoea aquatica*, *Ipomoea batatas* leaves and *Colocasia esculenta* leaves. The carotenoid profiles of steamed *Moringa oleifera* is shown in the chromatogram depicted in Fig. 2. Chromatograms of steamed *Albemoshus manihot* is depicted in Fig. 3. Lutein was also detected in *Moringa oleifera* leaves, *Colocasia esculenta* leaves, *Ipomoea batatas* leaves and *Ipomoea aquatica* but not quantified.

##### 3.1.2. Other vegetables

Table 2 shows that among the other vegetables readily available in Fiji, *Solanum melongena* (red eggplants) contain the highest total antioxidant capacity followed by *Phaseolus vulgaris var* (red bean). Considerable antioxidant capacity was also detected in *Artocarpus heterophyllus* (jackfruit), *Phaseolus vulgaris var* (butter bean), *Phaseolus vulgaris var* (french bean), steamed *Daucus carota* (carrot) and *Momordica charantia* (bitter gourd). *Phaseolus vulgaris var* (red bean) contains the highest amount of total polyphenolics (gallic acid equivalents) followed by *Solanum melongena* and *Artocarpus heterophyllus*. *Phaseolus vulgaris* varieties of butter bean, french bean and steamed carrot also had considerable amounts of total polyphenolics. *Phaseolus vulgaris* (red bean) has the richest source of quercetin in these vegetables. Butter bean and french bean also contain moderate amounts of quercetin. Considerable amounts of myricetin and morin were also detected in red bean. Carrot, *Cucurbita maxima* (pumpkin) and *Lycopersicon esculentum* (tomato) contain high levels of β-carotene. Considerable amounts of α-carotene were also detected in carrots, tomatoes and pumpkin. Lutein was also present in pumpkin, red bean and tomatoes but not quantified.

##### 3.1.3. Fruits

*Carica papaya*, the Hawaiian variety and *Annona muricata* (soursop) contain the highest total antioxidant capacity.

Table 1  
Total antioxidant capacity, total polyphenols, total anthocyanin, flavonol, carotene and moisture content of green leafy vegetables grown in Fiji

Food item	Polyphenols (mg/100 g)			Flavonols (mg/100 g)						Carotenoids (mg/100 g)			% Moisture
	TAC (TEAC)	TPP (GAE)	TAT (C-3-G)	Myricetin	Fisetin	Morin	Quercetin	Kaempferol	Isorhamnetin	Lycopene	$\alpha$ -Carotene <sup>d</sup>	$\beta$ -Carotene	
<i>Ipomoea batata</i> var (orange) <sup>a</sup>	770	260	nd	26	7	6	78	3	na	nd	0.76	12	86
<i>Ipomoea batata</i> var (composite) <sup>a</sup>	650	270	nd	24	7	6	90	8	1	nd	2.1	13	87
<i>Ipomoea batata</i> var (Honiara) <sup>a</sup>	570	270	nd	20	7	5	78	6	na	nd	1.9	12	88
<i>Ipomoea batata</i> var (Tis3030) <sup>a</sup>	350	280	nd	16	2	6	46	5	na	nd	1.2	19	84
<i>Ipomoea batata</i> var (Papua) <sup>a</sup>	350	240	nd	18	5	5	43	8	na	nd	0.45	14	87
<i>Moringa oleifera</i> <sup>a</sup>	260	260	nd	nd	nd	1	100	34	2	nd	4.5	34	82
<i>Moringa oleifera</i> <sup>b</sup>	200	290	nd	nd	nd	nd	90	34	3	nd	10	28	83
<i>Albelmoshus manihot</i> <sup>a</sup>	100	81	nd	nd	nd	<1	8	2	na	nd	0.45	16	90
<i>Colocasia esculenta</i> var <sup>a</sup>	130	120	nd	1	nd	nd	1	nd	1	nd	0.56	19	86
<i>Amaranthus viridis</i> <sup>a</sup>	95	79	nd	5	nd	<1	8	nd	nd	nd	3.3	32	89
<i>Ipomoea aquatica</i> <sup>a</sup>	35	65	0.1	nd	<1	nd	24	4	nd	nd	0.22	20	87
<i>Athyrium esculentum</i> <sup>a</sup>	35	39	nd	<1	<1	<1	6	3	na	nd	0.36	3	93
<i>Rorippa nasturtium aquaticum</i> <sup>a</sup>	33	54	nd	nd	1	4	7	3	<1	nd	1.6	9.3	91
<i>Brassica chinensis</i> <sup>a</sup>	31	54	nd	<1	nd	nd	1	8	2	nd	1.2	7.4	92
<i>Brassica oleracea</i> var. <i>bullata</i> <sup>a</sup>	16	27	nd	nd	nd	nd	<1	nd	nd	nd	nd	nd	94
<i>Brassica nigra</i> <sup>a</sup>	14	58	nd	<1	<1	<1	<1	12	<1	nd	nd	nd	93
<i>Lactuca sativa</i> <sup>c</sup>	0	16	nd	1	1	nd	2	<1	na	nd	nd	1.9	95

nd – not detected, na – not analysed, t – trace, (values are average of  $n = 2$ ).

<sup>a</sup> Steamed.

<sup>b</sup> Boiled.

<sup>c</sup> Raw.

<sup>d</sup> Estimated.

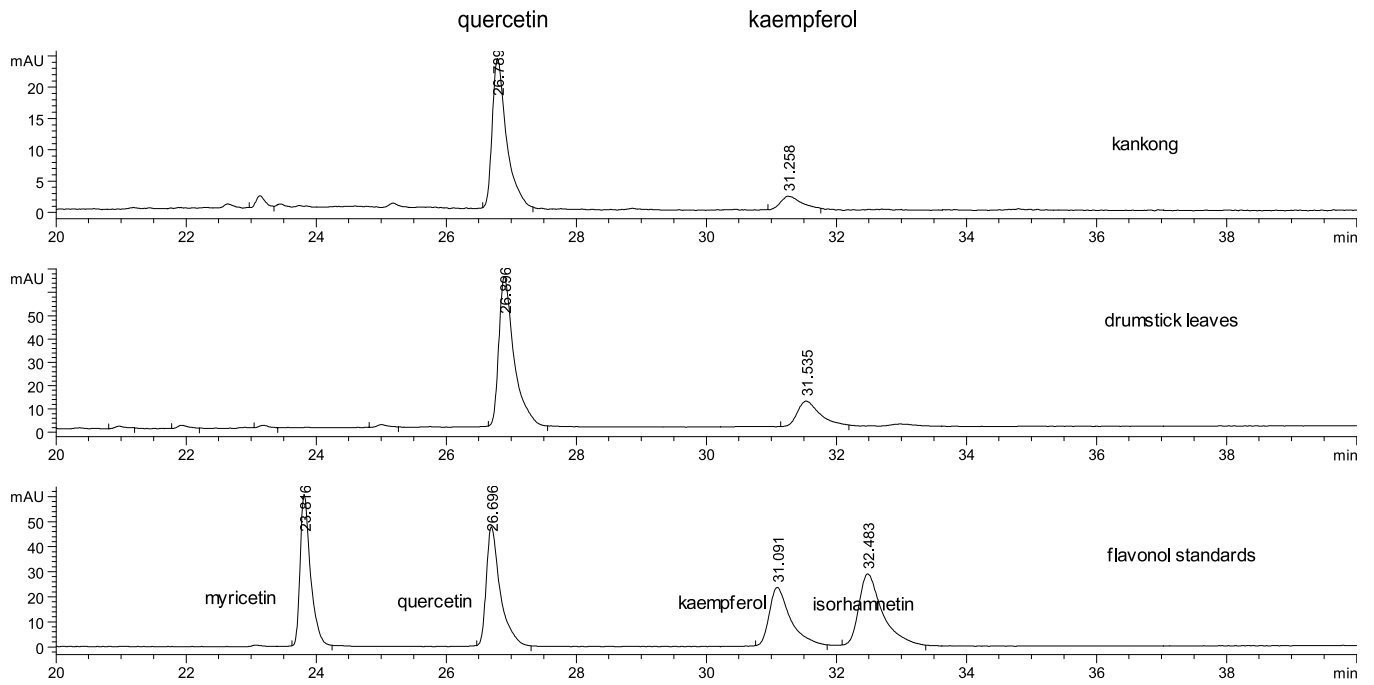


Fig. 1. HPLC chromatograms of steamed kangkong and steamed drumstick leaves compared to the standards using the conditions described in the flavonol method.

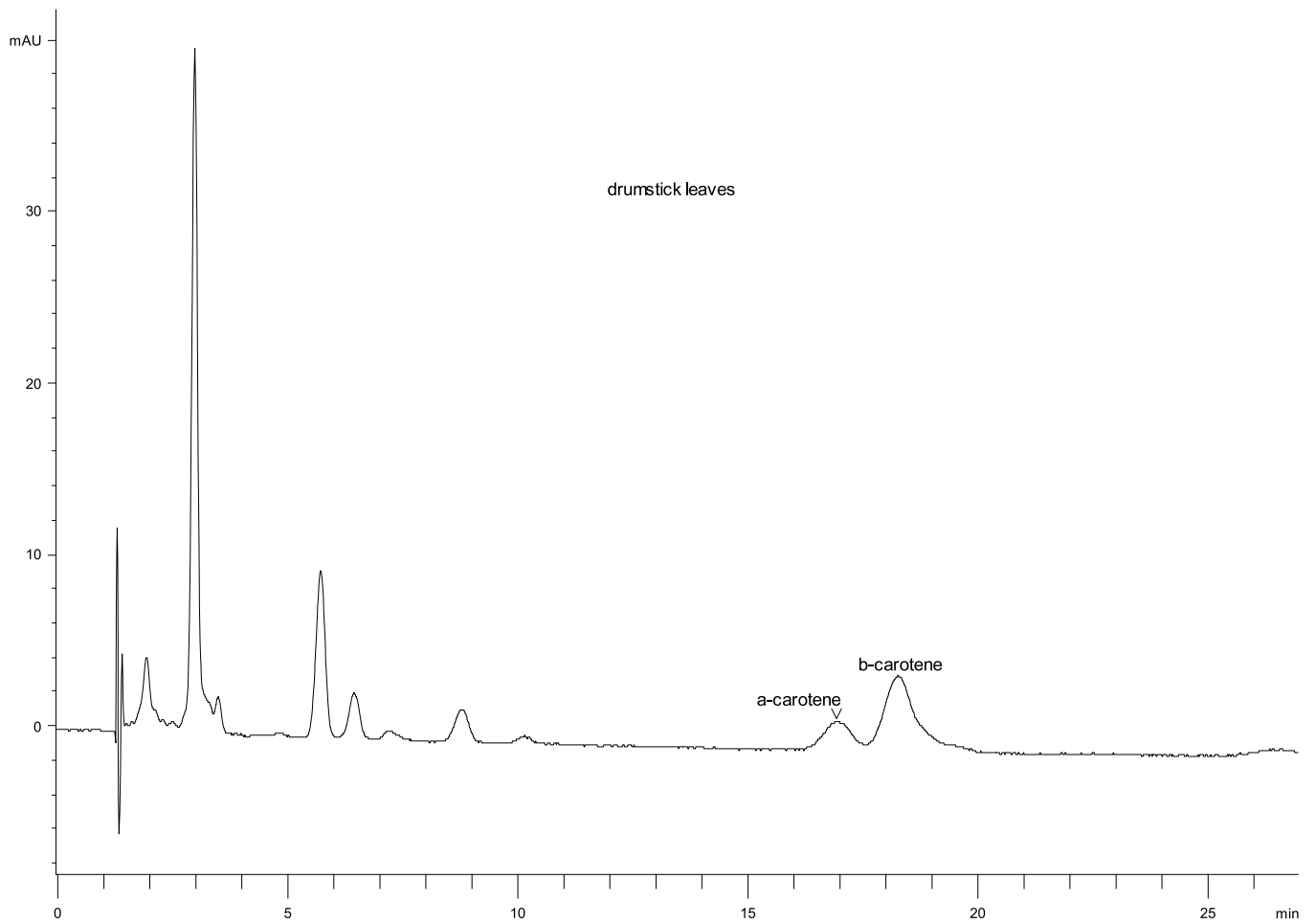


Fig. 2. HPLC chromatogram of steamed drumstick leaves using the conditions described in the carotenoid method.

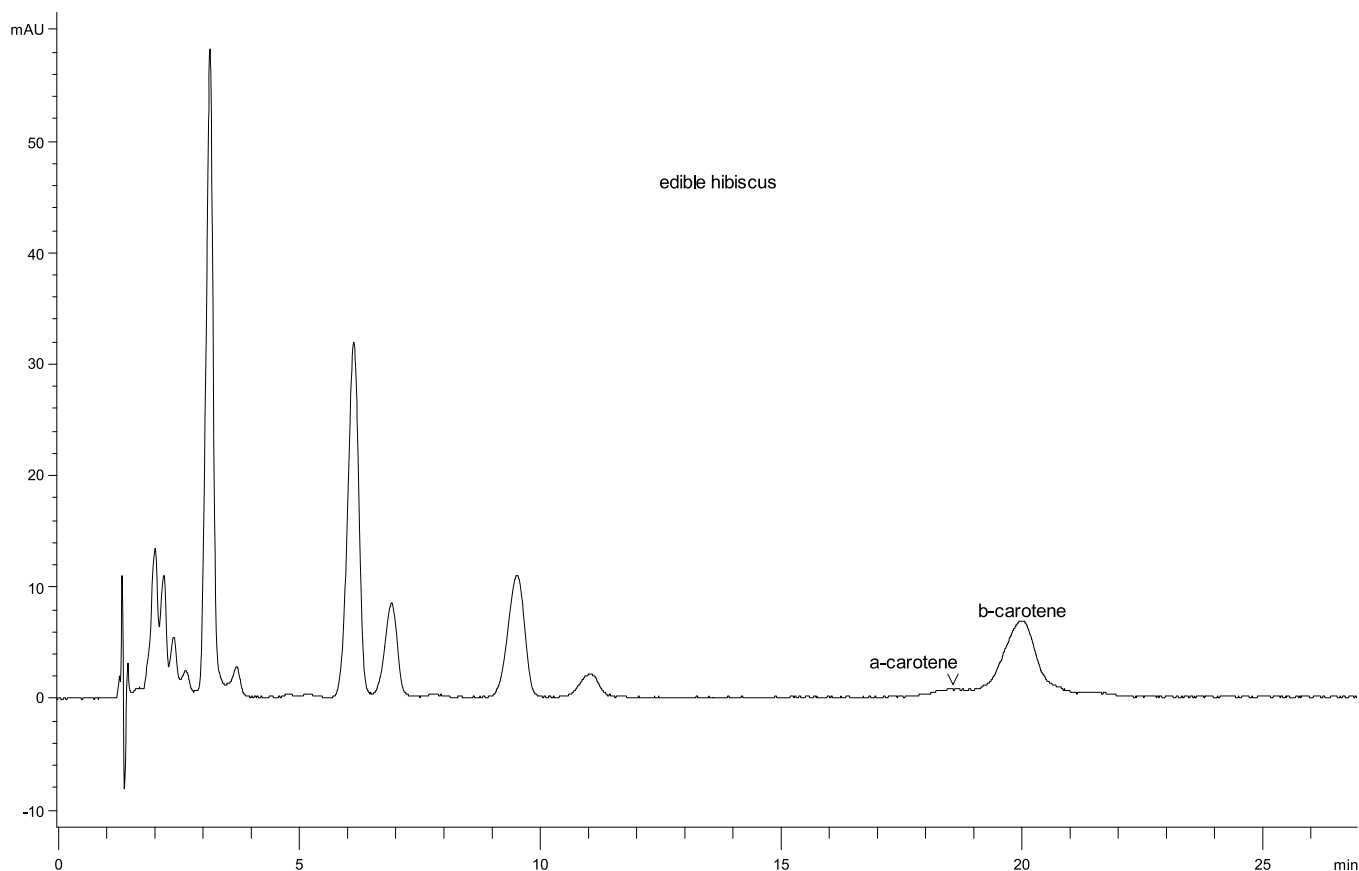


Fig. 3. HPLC chromatogram of steamed edible hibiscus using the conditions described in the carotenoid method.

ity among the fruits commonly grown in Fiji (Table 3). Considerable antioxidant capacity was also present in *Citrus sinensis*; the batiri variety, *Mangifera indica* (mango), local cherry, *Citrus reticulata* (mandarin) and *Ananas comosus* (pineapple). Batiri oranges and local cherry have considerable amounts of total polyphenolics (gallic acid equivalents). Generally, fruits are low in flavonol and carotenoid content compared to the leaves and other food groups. However, considerable amounts of flavonols were detected in Hawaiian papaya, local cherry, ripe banana and *Citrullus vulgaris* (watermelon). Moderate amounts of carotenoids were also detected in Hawaiian papaya, watermelon, ripe banana and *Syzygium malaccense* (malay apple).

#### 3.1.4. Root/tree staples

Orange *Ipomoea batatas* (sweet potato) contains much higher levels of total antioxidant capacity compared to the other traditional staples consumed in Fiji (Table 4). Interestingly, higher levels of antioxidant capacity were detected in coloured staples compared to their white counterparts, e.g. red *Dioscorea alata* (yam), red *Dioscorea esculenta* (sweet yam) and greenish *Colocasia esculenta* (taro). Staple roots/trees contain low levels of flavonols compared to the levels detected in the leaves. Only orange sweet potato is rich in  $\beta$ -carotene compared to the rest of the other root/tree staples.

#### 3.1.5. Miscellaneous foods and beverages

*Zingiber officinale* (ginger) contains the highest antioxidant capacity and total polyphenols however, is only used in small quantities in cooking (Table 5) and therefore may not provide high levels of antioxidants in the diet. *Gracilaria* sp (seaweeds) and *Cocos nucifera* (coconut) or products are low in antioxidant capacity. Coconut and or products appear to be low in both flavonols or carotenoids and this seems to be reflected in the low antioxidant capacity. *Curcuma longa* (yellow turmeric ginger) is a rich source of fisetin, quercetin and myricetin compared to the white *Zingiber officinale*. *Allium fistulosum* (spring onion) is a good source of kaempferol. The herbal *Zingiber zerumbet* (wild ginger), (*layalaya*), which is usually consumed before meals, contains the highest levels of kaempferol in the samples analysed.

#### 3.1.6. Quality control

All samples were analysed in duplicate and the mean of the two determinations reported (Greenfield & Southgate, 2003). Fully validated analytical methods were used to generate the data, e.g. carotenoids (Aalbersberg, 2002; Teng-Chuen Yu, Trenerry, Plozza, & Arcot, 2003), flavonols (Hertog et al., 1992). Recovery data were generated by spiking samples with pure reference standards prior to extraction. Good recoveries were obtained for  $\beta$ -carotene (96–99% in sweet potato, lettuce and tomato) and flavonols



Table 2  
Total antioxidant capacity, total polyphenols, total anthocyanin, flavonol, carotene and moisture content of other vegetables grown in Fiji

Food item	Polyphenols (mg/100 g)			Flavonols (mg/100 g)						Carotenoids (mg/100 g)			% Moisture
	TAC (TEAC)	TPP (GAE)	TAT (C-3-G)	Myricetin	Fisetin	Morin	Quercetin	Kaempferol	Isorhamnetin	Lycopene	$\alpha$ -Carotene <sup>c</sup>	$\beta$ -Carotene	
<i>Solanum melongena</i> (red) <sup>a</sup>	110	76	0.4	2	nd	nd	nd	nd	na	nd	nd	nd	93
<i>Phaseolus vulgaris</i> var (red) <sup>a</sup>	70	120	nd	3	<1	3	62	1	na	nd	0.28	0.8	89
<i>Phaseolus vulgaris</i> var (yellow) <sup>a</sup>	31	57	nd	<1	nd	nd	2	<1	na	na	nd	nd	90
<i>Phaseolus vulgaris</i> var (French) <sup>a</sup>	27	54	0.1	1	<i>t</i>	<1	4	<1	na	nd	0.72	0.78	92
<i>Momordica charantia</i> <sup>a</sup>	21	19	0.3	nd	<1	nd	nd	nd	<1	nd	nd	nd	92
<i>Lycopersicon esculentum</i> <sup>b</sup>	13	36	nd	<i>t</i>	<1	<1	1	<i>t</i>	nd	1.9	2.5	1.3	94
<i>Cucumis sativus</i> <sup>b</sup>	2	8	nd	nd	nd	nd	<1	<1	nd	nd	nd	nd	93
<i>Daucus carota</i> <sup>a</sup>	25	52	nd	nd	<1	<1	nd	nd	<i>t</i>	nd	6.2	21	91
<i>Daucus carota</i> <sup>b</sup>	2	16	nd	<1	<i>t</i>	<i>t</i>	<1	nd	<i>t</i>	nd	4.4	14	90
<i>Cucurbita maxima</i> <sup>a</sup>	5	23	nd	<i>t</i>	nd	<1	nd	nd	nd	nd	1.4	3.7	88
<i>Artocarpus heterophyllus</i> <sup>a</sup>	40	67	nd	<1	<1	1	nd	nd	nd	nd	nd	nd	80

\*\*Boiled, nd – not detected, na – not analysed, *t* – trace, (values are average of *n* = 2).

<sup>a</sup> Steamed.

<sup>b</sup> Raw.

<sup>c</sup> Estimated.

Table 3  
Total antioxidant capacity, total polyphenols, total anthocyanin, flavonol, carotene and moisture content of fruits grown in Fiji

Food item	Polyphenols (mg/100 g)			Flavonols (mg/100 g)						Carotenoids (mg/100 g)			% Moisture
	TAC (TEAC)	TPP (GAE)	TAT (C-3-G)	Myricetin	Fisetin	Morin	Quercetin	Kaempferol	Isorhamnetin	Lycopene	$\alpha$ -Carotene <sup>b</sup>	$\beta$ -Carotene	
<i>Carica papaya</i> (Hawaiian) <sup>a</sup>	100	26	0.06	3	<1	2	2	2	<1	1.7	nd	0.5	85
<i>Annona muricata</i> <sup>a</sup>	72	42	nd	<1	<i>t</i>	<i>t</i>	nd	nd	<1	nd	nd	nd	85
<i>Citrus sinensis</i> (Batiri) <sup>a</sup>	38	98	nd	na	na	na	na	na	na	na	na	na	92
Cherry local <sup>a</sup>	37	67	0.94	3	5	6	6	1	na	nd	nd	nd	85
<i>Mangifera indica</i> (Yalewa) <sup>a</sup>	34	48	nd	1	<1	1	<1	1	na	nd	nd	nd	87
<i>Cirus reticulata</i> <sup>a</sup>	26	28	<i>t</i>	1	nd	nd	<1	<1	na	nd	nd	0.03	89
<i>Ananas comosus</i> <sup>a</sup>	25	15	<i>t</i>	<1	<1	1	2	<1	na	nd	nd	0.17	79
<i>Cocos nucifera</i> (immature flesh) <sup>a</sup>	11	27	nd	<i>t</i>	nd	nd	<i>t</i>	nd	nd	na	na	na	82
<i>Syzygium malaccense</i> <sup>a</sup>	11	32	nd	<1	nd	<i>t</i>	<1	<i>t</i>	na	nd	0.14	0.18	93
<i>Musa nana</i> (ripe) <sup>a</sup>	9	9	nd	<1	2	10	<1	<1	nd	nd	0.82	0.72	77
<i>Citrullus vulgaris</i> <sup>a</sup>	1	14	nd	<1	<1	3	1	1	na	4	0.76	0.58	84
<i>Musa</i> sp. (ripe) <sup>a</sup>	<1	11	nd	nd	nd	nd	1	1	na	nd	<0.02	<0.02	68

\*Steamed, \*\*boiled, nd – not detected, na – not analysed, *t* – trace, (values are average of *n* = 2).

<sup>a</sup> Raw.

<sup>b</sup> Estimated.

Table 4  
Total antioxidant capacity, total polyphenols, total anthocyanin, flavonol, carotene and moisture content of staples grown in Fiji

Food item	Polyphenols (mg/100 g)			Flavonols (mg/100 g)						Carotenoids (mg/100 g)			% Moisture
	TAC (TEAC)	TPP (GAE)	TAT (C-3-G)	Myricetin	Fisetin	Morin	Quercetin	Kaempferol	Isorhamnetin	Lycopene	$\alpha$ -Carotene <sup>a</sup>	$\beta$ -Carotene	
<i>Ipomoea batatas</i> var (orange) <sup>b</sup>	64	43	nd	<1	<1	nd	nd	<1	nd	nd	nd	15	81
<i>Ipomoea batatas</i> var ( <i>Vulatolu</i> ) <sup>b</sup>	19	20	nd	nd	nd	<1	nd	nd	<1	nd	nd	0.06	72
<i>Ipomoea batatas</i> var ( <i>Papua</i> ) <sup>b</sup>	13	16	nd	nd	nd	nd	nd	nd	na	nd	nd	0.56	75
<i>Ipomoea batatas</i> var ( <i>Honiara</i> ) <sup>b</sup>	10	14	nd	nd	nd	nd	<1	<1	nd	nd	nd	nd	75
<i>Dioscorea alata</i> var ( <i>Veiva</i> ) (red) <sup>b</sup>	33	26	0.47	<1	nd	nd	<1	nd	nd	nd	nd	nd	72
<i>Dioscorea alata</i> var ( <i>Vurai</i> ) (white) <sup>b</sup>	3	8	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	67
<i>Dioscorea esculenta</i> var (red) <sup>b</sup>	27	38	0.09	<i>t</i>	nd	nd	nd	<1	nd	nd	nd	nd	68
<i>Dioscorea esculenta</i> var (white) <sup>b</sup>	10	17	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	67
<i>Dioscorea nummularia</i> <sup>b</sup>	26	47	nd	nd	4	nd	2	<i>t</i>	na	nd	nd	nd	62
<i>Colocasia esculenta</i> var ( <i>Tausala Samoa</i> ) <sup>b</sup>	26	39	nd	nd	nd	nd	nd	nd	nd	nd	nd	<0.02	68
<i>Colocasia esculenta</i> var ( <i>Wararasa</i> ) (greenish) <sup>b</sup>	25	20	nd	nd	nd	nd	1	2	nd	nd	nd	nd	66
<i>Colocasia esculenta</i> var common (white) <sup>b</sup>	13	12	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.05	66
<i>Xanthosoma sagittifolium</i> (red) <sup>b</sup>	10	12	0.62	nd	nd	nd	nd	nd	na	nd	nd	0.02	64
<i>Manihot esculenta</i> ( <i>Monroe</i> ), (yellow) <sup>b</sup>	28	14	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.52	59
<i>Manihot esculenta</i> var ( <i>Beqa</i> ), (yellow) <sup>b</sup>	17	13	nd	nd	nd	nd	nd	nd	na	nd	nd	0.43	59
<i>Manihot esculenta</i> var common (white) <sup>b</sup>	23	11	nd	nd	nd	nd	nd	nd	nd	nd	nd	<0.02	62
<i>Artocarpus alttilis</i> var ( <i>local</i> ) <sup>b</sup>	35	33	nd	<1	nd	nd	nd	nd	nd	nd	nd	nd	71
<i>Musa sp</i> (matured) <sup>b</sup>	18	16	nd	<i>t</i>	nd	nd	nd	nd	nd	nd	0.79	1.4	68

\*Steamed, # raw, nd – not detected, na – not analysed, *t* – trace, (values are average of  $n = 2$ ).

<sup>a</sup> Estimated.

<sup>b</sup> Boiled.

Table 5  
Total antioxidant capacity, total polyphenols, total anthocyanin, flavonol, carotene and moisture content of other miscellaneous foods and beverages grown in Fiji

Food item	Polyphenols (mg/100 g)			Flavonols (mg/100 g)						Carotenoids (mg/100 g)			% Moisture
	TAC (TEAC)	TPP (GAE)	TAT (C-3-G)	Myricetin	Fisetin	Morin	Quercetin	Kaempferol	Isorhamnetin	Lycopene	$\alpha$ -Carotene <sup>d</sup>	$\beta$ -Carotene	
Coconut juice	1	6	nd	na	na	na	na	na	na	na	na	na	100
<i>Sea weed</i>													
<i>Caulerpa racemosa</i> (Nama) <sup>c</sup>	14	22	nd	<1	nd	nd	nd	<1	nd	nd	2.6	0.81	90
<i>Gracilaria</i> sp (Lumi) <sup>a</sup>	0	8	nd	nd	<i>t</i>	nd	nd	nd	nd	nd	<0.02	0.03	93
<i>Seasoning</i>													
<i>Curcuma longa</i> (Turmeric ginger), (yellow) <sup>a</sup>	360	320	0.05	17	64	2	41	<1	na	nd	nd	nd	88
<i>Zingiber officinale</i> <sup>a</sup>	320	200	nd	<1	<1	nd	1	<i>t</i>	na	nd	nd	nd	81
<i>Allium fistulosum</i> <sup>a</sup>	11	41	nd	<i>t</i>	nd	<i>t</i>	<1	13	na	nd	nd	28	94
<i>Coconut products</i>													
Germinating coconut <sup>c</sup>	10	45	nd	<1	<i>t</i>	nd	<1	<1	nd	na	na	na	78
Fermented coconut <sup>c</sup>	6	47	nd	nd	nd	nd	nd	<1	<1	na	na	na	54
Coconut mature flesh <sup>c</sup>	6	19	nd	<i>t</i>	<i>t</i>	nd	nd	<i>t</i>	nd	na	na	na	80
Coconut cream (thick) <sup>b</sup>	5	15	nd	na	na	na	na	na	na	na	na	na	73
Coconut cream (thick) <sup>c</sup>	3	15	nd	na	na	na	na	na	na	na	na	na	64
<i>Herb</i>													
<i>Zingiber zerumbet</i> (layalaya) <sup>c</sup>	18	130	nd	<1	<1	nd	<1	240	nd	na	na	na	86

nd – not detected, na – not analysed, *t* – trace, (values are average of  $n = 2$ ).

<sup>a</sup> Steamed.

<sup>b</sup> Boiled.

<sup>c</sup> Raw.

<sup>d</sup> Estimated.

Table 6  
Comparison of levels of  $\beta$ -carotene, quercetin and kaempferol determined in Fiji and Australia

Food	Analyte	Fiji (mg/100 g)	Australia (mg/100 g)
<i>Brassica chinensis</i>	$\beta$ -Carotene	86	88
<i>Lycopersicon esculentum</i>	Lycopene	1.8	2.0
<i>Cirus reticulata</i>	$\beta$ -Carotene	0.03	0.03
<i>Ipomoea batatas</i> var (orange)	$\beta$ -Carotene	19	15
<i>Ipomoea batatas</i> var (Papua)	$\beta$ -Carotene	0.48	0.57
<i>Manihot esculenta</i> (Monroe), (yellow)	$\beta$ -Carotene	0.54	0.66
<i>Ipomoea batatas</i> var, composite leaves	Quercetin	85	90
<i>Amaranthus viridis</i>	Quercetin	9.2	8.3
<i>Ipomoea aquatica</i>	Quercetin	24	21
<i>Brassica nigra</i>	Quercetin	0.94	0.83
<i>Lycopersicon esculentum</i>	Quercetin	1.6	1.4
<i>Allium ascalonicum</i>	Quercetin	45	45
Germinating coconut	Kaempferol	0.24	0.25

(Values are average of  $n = 2$ ).

(myricetin, 114%; kaempferol, 82%; quercetin, 94% and isorhamnetin, 80% in a sample of onion). A number of samples were analysed seven times to demonstrate method repeatability. For flavonol analysis, a secondary reference material (freeze-dried onion) was developed for this work and analysed on different days to measure method reproducibility. The % CV ranged from 1.6% to 3.9%. A certified reference material for carotene analysis was not available, however, previous work at DPI-Werribee has demonstrated the robustness of the method (Teng-Chuen Yu et al., 2003). A number of samples were also transported to Australia and reanalysed for their  $\beta$ -carotene and flavonol content using an HPLC equipped with a photo diode array detector. The spectral data generated by the detector was used to verify the identity of the compounds in the chromatograms. In general, there were good agreement in the levels of analytes determined on the same samples in Fiji and Australia. This indicates that the analytes are relatively stable if stored at  $-20^\circ\text{C}$  in sealed containers. The data are displayed in Table 6.

#### 4. Discussion

Phytochemicals in fruit and vegetables have been proven effective in the prevention of certain chronic diseases. Epidemiological studies have shown relationships between fruit and vegetable intake and chronic diseases such as coronary heart diseases, certain cancers and diabetes. Due to the prevalence of chronic degenerative diseases worldwide including the South Pacific and Australia, the availability of information on phytochemicals and antioxidant rich foods will help individuals make informed choices in the consumption of foods that could help protect them from such chronic diseases.

The experimental data showed large variations in total antioxidant capacity, total polyphenols, flavonol and

carotenoid content of the foods assayed in this study. Prior and Cao (2000) documented factors such as cultivars, maturity and other environmental factors such as sunlight exposure that may influence the antioxidant capacity of fruits and vegetables. This is evident in the different cultivars of *Ipomoea batatas* roots and leaves. The results clearly show a six fold difference in total antioxidant capacity between cultivars of *Ipomoea batatas* roots and a 250-fold different in  $\beta$ -carotene between two types of *Ipomoea batatas* roots. The leaves of the *Ipomoea batatas* are also much richer in phytochemicals and antioxidants compared to their root counterparts.

Other studies have also highlighted the effect of preparation, processing and cooking on the levels of antioxidant capacity, with one study showing that cooking reduces the antioxidant capacity by 15% (Prior & Cao, 2000). However, Prior and Cao (2000) and our data indicate that uncooked carrots had a lower TAC compared to its steamed counterpart. The difference may be due to the types and the quantity of phytochemicals and the food matrix. This study showed that steamed carrot has higher levels of  $\alpha$ - and  $\beta$ -carotene than raw carrot, which might be a contributor to an increase in the total antioxidant capacity. This is important as carrot is eaten raw in salads and cooked in soup, stir-fried and steamed dishes. The type of cooking can also affect the total antioxidant capacity, e.g. steamed *Moringa oleifera* leaves have a higher total antioxidant capacity than the boiled *Moringa oleifera* leaves. This may be due to the leaching of phytochemicals into the cooking liquid (Castenmiller, West, Linseen, Hof, & Voragen, 1999). It should be noted, however, that some phytochemicals may be more bio-available when the food is cooked (Rock, Emehiser, Ruffin, Flatt, & Schwartz, 1998).

The variability in the compositions and quantities of phytochemicals and antioxidants in different foods indicate the importance of eating a variety of food sources in particular, coloured foods, in every meal.

The results of this study have been used to formulate a phytochemical constituent table that will be a useful tool for health professionals and consumers in choosing antioxidant and phytochemical rich foods. Plant breeders can also use this information in developing programs designed to increase antioxidant components in foods for human consumption.

The results presented in this paper are important in view of the reports that show the strong inverse association of antioxidants and ageing diseases (Willett, 1994). Furthermore, processes leading to coronary heart diseases and cancers are developed and initiated many years before the diseases manifest themselves (Marco et al., 1997). Therefore, it is important that educational campaigns are initiated to encourage young children to understand the health benefits of foods that are rich sources of antioxidants and phytochemicals to avoid and prevent the development of such diseases in the later stages of their lives.



## 5. Conclusion

Seventy Fijian foods were analysed for their total antioxidant capacity (TAC), total polyphenol content (TPP), total anthocyanin content (TAT) as well as the major flavonol and carotenoid profiles. Green leafy vegetables had the highest antioxidant capacity, followed by the fruits and root crops. A number of herbs also exhibited high antioxidant capacity. *Moringa oleifera* leaves and *Ipomoea batatas* leaves have high levels of quercetin and  $\beta$ -carotene. *Curcuma longa* has a high TAC value and is also rich in fisetin, quercetin and myricetin. *Zingiber zerumbet* (*Layalaya*), a widely used herb taken before meals has high levels of kaempferol. The phytochemical constituent table presented as a result of this study will be used to estimate the antioxidant intake of the Fijian population and in future clinical trials. Furthermore scientists can also use the data as an effective tool for breeding programs designed to increase the antioxidant and other phytochemical components in foods.

## Acknowledgements

The corresponding author wishes to acknowledge the support of Professor William Aalbersberg and the Chemistry Department of the University of the South Pacific in providing facilities, solvents and reagents and Mr. Aliko Turagakula of the Agronomy Department of the Fiji Ministry of Agriculture for the provision of some food samples and the identification and confirmation of sample cultivars. Mrs. Miliakere Nawaikula and Tawake of the Chemistry Department of the Fiji Ministry of Agriculture are also thanked for the privilege in the use the HPLC and the provision of solvents. Appreciation is also gratefully extended to the staff of the Food Chemistry Section of PIRVic at DPI-Werribee, in particular Mr. Philip Zeglinski. One of us (JL) wishes to thank the Ministry of Fijian Affairs, Fiji for financial support.

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