

# Health Benefits of Australian Native Foods

An evaluation of health-enhancing compounds



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Rural Industries Research and Development Corporation

# Health Benefits of Australian Native Foods

#### An evaluation of health-enhancing compounds

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RIRDC Pub. No. 09/133





Wattleseed

# **Preface**

Australia's unique biodiversity offers a basis for diversification in Australian agriculture in the future. This enabling research has identified opportunities for an industry with a comparative advantage in the food market place. This exciting new information on the health benefits of Australian native foods will assist in the development of this emerging industry.

Oxidative stress is believed to contribute to the "ageing process" and to diseases such as Alzheimer's disease, autoimmune and cardiovascular disease, cancer, cataractogenesis, diabetes, and macular degeneration. Lower levels of oxidative stress may reduce the probability of occurrence of these diseases at later stages in life and result in a healthier old age. Antioxidants are the compounds of our diet that reduce oxidative stress in the body. The major source of antioxidants preventing our bodies from oxidative stress is food. Therefore, the presence of antioxidants in the diet has the most conclusive role in the prevention/delaying of oxidative stress mediated diseases.

This research was developed to provide the native food industry with reliable information on the levels of health beneficial constituents, and the antioxidant capacities of commercially significant native fruits, herbs and spices. This report represents the first systematic evaluation of antioxidant capacity, and the identification of its sources; the presence of potentially bioactive phytochemicals (phenolic compounds and carotenoids) and selected vitamins. The research also included an evaluation of minerals, focusing especially on those that protect human DNA against mutations that can lead to the development of a range of chronic diseases.

Native species evaluated in this study exhibited superior antioxidant capacity as compared to the Blueberry standard, renowned worldwide as the 'health-promoting fruit.' In comparison to commonly consumed fruits that comprise predominantly hydrophilic antioxidants, native foods contained antioxidant activity in both hydrophilic and lipophilic fractions. This suggests more comprehensive protection from oxidative stress, and possibly more pronounced health benefits.

All of the evaluated plant species were found to contain vitamin E and folate. Rich sources of lutein, a compound essential for eye health are also present, as were magnesium, zinc and calcium, all important for the synthesis and self-repair of human DNA. Additionally, sources of valuable selenium were identified.

This report was funded from RIRDC Core Funds provided by the Australian Government and also from industry through the Australian Native Food Industry Ltd (ANFIL) and the Coles Indigenous Food Fund. It is an addition to RIRDC's diverse range of over 1900 research publications and is part of the New Plant Products R&D program which aims to facilitate the development of new industries based on plants or plant products that have commercial potential for Australia.

#### Peter O'Brien

Managing Director

The Rural Industries Research and Development Corporation

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# **Executive summary**

#### What the report is about

This report represents the first systematic evaluation of thirteen native Australian fruits, herbs and spices that are of primary importance within the Australian Native Food Industry, for antioxidant capacity and the presence of compounds with reported health benefits. The following compounds were targeted: phenolic compounds, carotenoids, vitamin C, vitamin E, provitamin A, folate and selected minerals: Zn, Mg, Ca, Fe, Se, P, Na, K, Mn, Cu and Mo.

### Who is the report targeted at?

The report is targeted at:

- the Australian native food industry
- the general food industry, and
- health-conscious Australian consumers with an interest in diversifying their daily diet with native Australian fruits, herbs and spices.

#### **Background**

The emerging Australian native food industry has enormous potential for the development and delivery of authentic Australian foods for both Australian and international communities. The rich Australian flora, containing over 25,000 native species, provides opportunities for the selection



of traditionally consumed sources that, besides being new attractive foods, possess significant health-enhancing properties.

Some native crops, including fruits, herbs and spices, have already entered commercial production. Products containing native foods are available in chain supermarkets, in delicatessens and specialty shops. Originally sold as tourist souvenirs they have also entered and enriched the everyday diets of Australian consumers. However, little is known about the composition of phytochemicals present in native fruits, herbs and spices. Systematic identification of compounds in native foods that have the potential to bring health benefits is required. This project has been designed to generate compositional data on thirteen selected native crops that are of a primary importance to the industry, with emphasis on compounds for which health benefits have been reported.

#### Aims/objectives

The aim of this project was to generate valuable and scientifically robust information about the levels of healthenhancing phytochemicals in Australian native plant sources. This information could then be used as a tool to educate the supply chain and consumers about products developed from native plants. This information should strengthen the position of the Australian Native Food Industry in the domestic and international market.

#### **Methods** used

A set of 13 dried herbs and spices or fresh fruit samples were selected to include commercial products representative of:

- fruit type (Kakadu Plum, Davidson's Plum, Desert Lime, Riberry, Lemon Aspen and Quandong), and
- herb/spice type (Tasmannia Pepper Berry and Leaf, Bush Tomato, Anise Myrtle, Lemon Myrtle and Wattleseed).

The fruits, herbs and spices were subjected to the following analyses:

- anti-oxidant capacity assays, ORAC (Oxygen Radical Absorbance Capacity) and FRAP (Ferric Reducing Antioxidant Power)
- total phenolic content, quantified by the Folin-Ciocalteu assay
- identification of the sources of antioxidant activities within the water-soluble (hydrophilic) fraction (major phenolic compounds) and lipid-soluble (lipophilic) fraction (major carotenoids, tocopherols and chlorophyll),
- analysis for vitamin C, vitamin E and folate, and
- analysis for minerals: Zn, Mg, Ca, Fe, Se, P, Na, K, Mn, Cu and Mo.

#### Results/key findings

This research has yielded a range of significant findings, suitable for either immediate or future use (pending confirmatory research), in the health-based marketing of native foods products.

- With the exception of Wattleseed and Australian Desert Lime, all evaluated samples displayed superior antioxidant capacity to the Blueberry control (ORAC assay). Outstanding antioxidant capacities were exhibited by Kakadu Plum and Quandong which belong to the same 'fruit' category as the Blueberry control. The sources of superior antioxidant capacity among the herbs and spices were Tasmannia Pepper Leaf, Lemon Myrtle and Anise Myrtle.
- The majority of the Australian native fruits,

- herbs and spices tested contain phytochemicals that provide antioxidant activity in both the hydrophilic and lipophilic environment. This suggests more comprehensive protection from oxidative stress and possibly more pronounced health benefits in comparison to commonly consumed fruits that are comprised predominantly of hydrophilic antioxidants.
- All of the evaluated native plant species contained vitamin E components that are fat-soluble strong antioxidants. The highest levels were found in the herbs [Anise Myrtle (59.7 mg/100g DW), Lemon Myrtle (21.2 mg/100g DW) and Tasmannia Pepper Leaf (17.6 mg/100g DW)] and fruits [Kakadu Plum (6.1 mg/100g DW), Quandong (4.8 mg/100g DW) and Australian Desert Lime (4.9 mg/100g DW]. The levels of vitamin E in Kakadu Plum and Quandong were comparable with the avocado benchmark (5.37-10.16 mg/100g DW).
- Phenolic compounds were identified as the major source of antioxidant capacity. Rich composition of phenolic mixtures and the presence of compounds with catechol-based structure are the primary source of antioxidant capacity of native fruits and herbs. This suggests a possible range of physiological activities that need to be confirmed using in vitro cell-based assays and/or in vivo animal / human trials.
- A high level of lutein, a carotenoid compound that plays an important role in

- eye health, was identified in Anise Myrtle (20.9 mg/ 100gDW). Lower levels of lutein (1.15 to 6.56 mg/100g DW) were also detected in Lemon Myrtle, Tasmannia Pepper Leaf, Kakadu Plum, Australian Desert Lime and Davidson's Plum. These native foods all possessed higher levels of lutein than avocado (0.6-1.05 mg/100g DW), which is considered to be a primary source of this health giving carotenoid.
- All of the evaluated native plant species contained folate with Australian Desert Lime being the richest source (420 μg/100g DW, i.e. double the amount of the recommended daily intake in 100g DW and over 10 times greater than Blueberry). Tasmannia Pepper Leaf contained 75% of the recommended daily intake in 100g DW and Quandong, Kakadu Plum, Riberry and Lemon Aspen among the fruits contained 50% of the recommended daily intake in 100g DW (i.e almost 3 times higher than Blueberry).



Tasmannia Pepper Leaf contained 75% of the recommended daily intake in 100g DW

 Outstanding levels of vitamin C were identified in Kakadu Plum (7% DW, 7000mg/100g DW)) and in Australian Desert Lime (1% DW, 962mg/100g

- DW) as compared with the Blueberry control (0.06% DW, 64.6mg/100g DW).
- Relative to the Blueberry control, many of the native foods tested possessed higher levels of important minerals. All of the native herbs, spices and fruits evaluated in this study contained three microelements important for genome health: magnesium (Mg), zinc (Zn) and calcium (Ca). Tasmannia Pepper Leaf and Wattleseed were identified as good sources of all these microelements. Lemon Myrtle was exceptionally rich in Ca and Anise Myrtle rich in Mg. Among the fruits, Quandong was the richest source of Mg and Zn, and Australian Desert Lime was the richest source of Ca.
- Among the herbs and spices, Bush Tomato, Tasmannia Pepper Leaf and Wattleseed were identified as the richest sources of iron (Fe). Among the fruits, the highest levels of Fe were detected in Quandong and Lemon Aspen.
- Bush Tomato and Wattleseed were the only two sources that contained selenium (Se), which is deficient in many soils and therefore foods.
- A high potassium:sodium (K: Na) ratio has been detected in *Davidsonia pruriens*, followed by Australian Desert Lime, *Davidsonia jerseyana* and Kakadu Plum. Based on this quality, application of the above mentioned fruits for the development of foods to reduce hypertension can be considered.



### Implications for relevant stakeholders

- There is an opportunity to promote native fruits, herbs and spices as exceptionally rich sources of antioxidants that are of both hydrophilic and lipophilic origin. This unique quality of Australian native fruits, herbs and spices will complement antioxidant profiles obtained from commonly consumed fruits that are lacking or display very low antioxidant activity of lipophilic compounds.
- High levels of phenolic compounds and their rich composition in the native foods evaluated, suggest a range of physiological activities that need to be confirmed using *in vitro* cell-based assays and/or in vivo in animal / human trials. Additional research will be required for the further substantiation of antioxidant testing data.
- The compositional data on vitamins (vitamin C, E, provitamin A, folate), lutein and minerals can be utilised by the industry when educating the market about health benefits. Quality assurance is required to

- ensure the nutrient content claim across different batches of the same plant source.
- Bioavailability of phytochemicals and microelements present in the fruits, herbs and spices is an important prerequisite of their potential health benefit. It is important to understand that diet affects bioavailability, for example, vitamin C enhances bioavailability whilst oxalic acid reduces it. For this reason it is important for the public to also consider overall diet rather than considering the health benefits of native foods in isolation.

#### Recommendations

Information obtained within this project can be used to promote and market products that are developed from the native plants evaluated in this study. Compositional nutrient data and levels of antioxidants (both hydrophilic and lipophilic) can be utilised by the industry provided quality assurance across different batches of the same product is maintained. Additional research will be required for the further substantiation of possible health effects related to antioxidant testing data.

# Introduction

The emerging Australian native food industry needs to compete with other larger, well established food industry sectors such as conventional fruit production (apples, bananas, oranges, etc.), herb and spice production, as well as with imported products. Credible research data on the presence of health-enhancing compounds in these unique Australian native sources will help to promote consumption among consumers and create market demand for native food products. This would subsequently be followed by higher demand, which would help the Australian native food industry to develop and grow.

Consumers are increasingly aware of the relationship between specific food components (e.g. fibre, antioxidants, etc.) and health. General compositional data of about 500 different indigenous foods has been collated and published by Miller et al., (1993) [1]. This includes information on the content of water, protein, fat, carbohydrates, vitamins C, B1 and B2 and selected minerals (Na, K, Mg, Ca, Fe, Zn, Cu, Pb, Cd and P) for about 100 native fruits. However, apart from this compositional data, the role that native fruits and herbs might play in human health is poorly understood. The opportunity therefore exists for the Australian native



Consumers are increasingly aware of the relationship between specific food components (e.g. fibre, antioxidants, etc.) and health.

food industry to 'discover' and substantiate the health attributes of their products and use this information to promote purchase and consumption of products made of Australian native plants.

The capability for evaluation of bioactive properties of foods is available to the Australian industry through CSIRO and its collaborators, who have established research programs aimed at developing the use of foods for disease prevention, healthy living and healthy aging.

This project was developed to provide the Australian native

food industry with reliable information on the levels of health beneficial constituents and antioxidant capacity of selected native fruits, herbs and spices that are of commercial significance. The project aligns with the capabilities of CSIRO in the area of in vitro substantiation of health properties of foods. It targets probable physiological activity of native fruits, herbs and spices, with the intention of conducting a targeted research program that could yield high quality data fit for publication and to support public communication of health messages to consumers.

# Objectives and planned outcomes

#### **Objectives**

The purpose of the project was to obtain comprehensive data on the presence of, and undertake analysis of selected native Australian fruits, herbs and spices with respect to components that are known to exhibit health-enhancing properties, specifically targeting general health and DNA stability. These targets are addressed by the determination of:

- total anti-oxidant capacities
- quantification and identification of major phenolic compounds
- quantification and identification of major carotenoids
- quantification of major vitamins (C, E isomers and folate)
- quantification of minerals: Zn, Mg, Ca, Fe, Se, P, Na, K, Mn, Cu and Mo.

#### **Planned Outcomes**

- Generation of scientific data with potential for publication and use in industry fact sheets, bulletins, consumers marketing and other communications media
- Generation of data useful for the benchmarking of Australian native fruits and herbs against other products (Blueberry) which can be used for industry development and marketing.



 ${\it Collecting freeze-dried sample}.$ 

# Methodology

# Sample selection and preparation

The selection of thirteen samples was provided by the industry. Samples of Tasmannia pepper (Tasmannia lanceolata R. Br., leaves and berries) were supplied by the company Diemen Pepper (Tasmania). Anise Myrtle (Syzygium anisatum), Lemon Myrtle (Backhousia citriodora) and Queensland Davidson's Plum (Davidsonia pruriens) were obtained from Australian Rainforest Products (NSW). Bush Tomato (Solanum centrale), Wattleseed (Acacia sp.) and Quandong (Santalum acuminatum) were supplied by Outback Pride. Australian Desert Lime (Citrus glauca) was obtained from Australian Desert Limes (Queensland) and Kakadu Plum (Terminalia ferdinandiana), Lemon Aspen (Acronychia acidula) and NSW Davidson's Plum (Davidsonia *jerseyana*) was provided by the Australian Produce Company Pty Ltd (Queensland). The Riberry sample (Syzygium luehmannii) was supplied by Woolgoolga Rainforest Products, (NSW).

Tasmannia pepper, Anise Myrtle, Lemon Myrtle, Wattleseed, Bush Tomato and Quandong arrived in a dry form. All other fruits were delivered frozen. The frozen samples were freeze-dried on arrival. In the case of plums, the fruits were defrosted to allow stone removal, immediately refrozen



Purification of phenolic compounds isolated from Davidson's Plum.

using liquid nitrogen and freeze-dried. The freeze-dried and dry samples obtained from the industry were finely ground. Subsequently they were stored at -20°C until analyzed.

It was important to source fruit, herb and spice samples from industry participants that were provided in the commercially available form i.e. harvested from plantations or controlled wild harvest using selected or clonally propagated cultivars. This ensured that results obtained were both indicative of industry products currently in fruit, herb and spice supply and also so research can be replicated and compared in future studies.

# Chemicals and reagents

Unless otherwise stated, all chemicals and standards with the exception of anthocyanins were purchased from Sigma-Aldrich (Sydney, Australia) and were of analytical or HPLC grade. Cyanidin 3-glucoside, cyanidin 3-sambubioside, cyanidin 3-rutinoside, and cyanidin 3,5-diglucoside were purchased from Polyphenols Laboratories AS (Hanaveien, Norway). De-ionized water was used throughout.

# Extraction of hydrophilic compounds

Two hundred and fifty mg of the pulverized samples were placed in test tubes and extracted with 5 mL of 80% aqueous methanol/1.0% HCl (v/v) under nitrogen atmosphere to prevent oxidation. The samples were sonicated for 10 minutes, centrifuged (10 min, 5000 rpm), and the supernatant collected. The pellet was re-extracted two more times. Aliquots of the

combined supernatants (15 mL) were flashed with nitrogen and stored at 0.5°C until analyzed. The extraction was carried out in triplicate for each sample. The analysis was conducted within three days.

# Extraction of lipophilic compounds

Two hundred mg of the pulverized samples were placed in test tubes and extracted with 10 mL of cold acetone. The samples were shaken for 20 minutes, centrifuged (10 min, 2500 rpm), and the supernatants were collected. The pellet was re-extracted two more times. Freshly prepared aliquots of the combined supernatants (30 mL) were filtered with  $13 \text{ mm} \times 0.45 \mu m \text{ PTFE}$  and immediately analyzed. The extraction was carried out in triplicate for each sample.

#### **Antioxidant testing**

#### **Total phenolics**

The Total Phenolics (or Folin-Ciocalteu) assay is used as a measure of total phenolic compounds in natural products. The basic mechanism is an oxidation/reduction reaction based on a single electron transfer.

The total phenolic content was determined using the Folin-Ciocalteu assay [2]. Diluted extracts were directly assayed at 600 nm with gallic acid serving as a standard. The analysis was conducted in triplicate and the results were corrected for vitamin C. Results were expressed as milligrams of total phenolics (gallic acid equivalents, GAE) per gram dry weight (mg GAE/g DW). Measurements were done in microplates using a microplate

reader model Multiscan RC, version 4 (Labsystems, Finland) operated by the DeltaSoft3 program (Elisa Analysis for the Macintosh with interference for the Multiscan Microplate Readers, BioMetallics, Inc., 1995).

# ORAC (Oxygen Radical Absorbance Capacity) assay

The ORAC assay is based on a hydrogen atom transfer reaction mechanism and evaluates the ability of sample to scavenge reactive oxygen species, which is one of the most common reactive oxygen species generated in the human body. In this aspect, the ORAC assay represents the most relevant system to human biology [3]. ORAC-H reflects water-soluble (hydrophilic) antioxidant capacity and the ORAC-L reflects the lipidsoluble (lipophilic) antioxidant capacity. However, it needs to be mentioned that ORAC-L measures predominantly the activity of tocopherols, and does not measure the antioxidant activity of carotenoids, since chemically carotenoids are not the chain breaking antioxidants [4]. ORAC-T represents the sum of ORAC-H and ORAC-L.

## **ORAC-H** (hydrophilic compounds)

The assay was conducted according to Prior *et al.*, 2005 and Ou *et al.*, 2001; [3, 5]. The samples (in triplicates) were mixed with a fluorescein (15 nM) solution and a solution of 2,2'-azobis-(2-methylpropionamidine) dihydrochloride (AAPH, 360 mM) both in phosphate

buffered saline (PBS, 75 mM, pH 7.0). Both AAPH and PBS buffer were warmed to 37°C prior to use. The fluorescence was recorded until it reached zero (excitation wavelength 495 nm, emission wavelength 515 nm) in a Varian Cary Eclipse Fluorescence Spectrophotometer equipped with an automatic thermostatic autocell holder at 37 °C.

A calibration curve was constructed daily by plotting the calculated differences of area under the fluorescein decay curve between the blank and the sample for a series of standards of Trolox solutions in the range of 6.25 - 75 µg/L. The results were expressed as µmol Trolox equivalents per 100 gram dry weight (µmol Trolox Eq./100g DW).

### ORAC-L (lipophilic compounds)

All the reagents were prepared using 75 mMol phosphate buffer (pH 7.4) as described for the ORAC-H assay. According to Huang et al., 2002 [4] samples and Trolox standards were made in 7% (w/v) randomly methylated β-cyclodextrin (RMCD) solvent to ensure solubility of the lipophilic antioxidant in the reaction mixture. The 7% RMCD solvent was made in a 50% acetone-water mixture (v/ v) and was shaken for 1 hour at room temperature on an orbital shaker at 200rpm prior to use.

The sample solution was ready for analysis after further dilution with 7% RMCD. The measurements were conducted as described for the ORAC-H method.

### FRAP (Ferric Reducing Antioxidant Power) assay

The FRAP assay measures the total concentration of redox-active compounds. It is based on ferric to ferrous ion reduction through a single electron transfer mechanism at low pH and measures not only antioxidants that are able to scavenge free oxygen radicals but also other redox-active compounds.

It is expected this assay captures the whole network of antioxidants with different chemical properties which cooperate in an integrated manner in the plant cell and might be needed by humans for proper protection against oxidative damage. Recently, this assay was selected to evaluate the content of antioxidants in 1113 foods consumed in the United States [6].

The assay was conducted according to Benzie and Strain (1996) [7] with minor modifications. Thirty µL of water and 10 µL extracts were mixed with a 200 µL FRAP reagent consisting of ferric chloride and 2,4,6-tripyridyls-triazine [TPTZ]. The absorbance was measured after 4 min at 600 nm. The reducing capacity was calculated using the absorbance difference between sample and blank and a further parallel Fe(II) standard solution.

Results were expressed as micromoles of Fe<sup>2+</sup> per gram dry weight (µmol Fe<sup>2+</sup>/g DW). Measurements (in triplicates) were done in microplates using a microplate reader model Multiscan RC, version 4 (Labsystems, Finland) operated by the DeltaSoft3 program (Elisa Analysis for the

Macintosh with interference for the Multiscan Microplate Readers, BioMetallics, Inc., 1995).

#### Analysis of phenolic compounds by HPLC-DAD and LC- PDA-MS/MS

#### **HPLC** analysis

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

The following solvents in water with a flow rate of 1.0 mL/min were used: A, 0.5% Triflouroacetic acid (TFA) in water and B, 95% acetonitrile and 0.5% TFA in water. The elution profile was a linear gradient elution for B of 10% over 10 minutes followed by an increase to 50% over 45 min, and then to 80% over 15 minutes.

The column was washed with 100% solvent B for 10 minutes. Analytical HPLC was run at 25°C and monitored at 280 (hydroxybenzoic acids and flavanols), 326 (hydroxycinnamic acids, stilbenes), 370 (flavonols) and 520 nm (anthocyanins). Hydroxybenzoic acids and flavanols were quantified as gallic acid equivalents (GA Eq.), cinnamic acids were

quantified as chlorogenic acid equivalents (CHA Eq.), flavonols and stilbens were quantified as rutin equivalents (R Eq.) and anthocyanin compounds were quantified as cyanidin 3-glucoside equivalents (C3G Eq.). The results are presented per gram of dry weight (e.g. mg C3G Eq/g DW).

#### LC-PDA-MS/MS analysis

LC-PDA-MS/MS analysis was carried out on a Quantum triple stage quadrupole (TSQ) mass spectrometer (ThermoFinnigan, NSW, Australia) equipped with a quaternary solvent delivery system, a column oven, a photo-diode array detector and an autosampler. An aliquot (20 µl) from each extract was chromatographed on a Luna  $C_{18}(2)$  analytical column (150) mm x 2.1 mm, 5 µm particle size), (Phenomenex, NSW, Australia), which was heated to 30°C in an oven.

The mobile phase consisted of 0.5% formic acid in water (A) and 0.5% formic acid in acetonitrile (B) at the rate of 220 µL/min. A linear gradient was used (0% B to 100% B over 40 min). Ions were generated using an electrospray source in the positive or negative mode under conditions set following optimisation using solutions of cyanidin-3-glucoside, chlorogenic acid and rutin.

The PDA was monitoring signals at 520, 370, 320 and 280 nm. MS experiments in the full scan (precursor and product-specific) and the selected reaction monitoring (SRM) mode were carried out.

# Analysis of lipophilic compounds by HPLC-DAD and HPLC-FD

Quantification and identification of lipophilic compounds was carried out using a HPLC system that consisted of two LC-10AD pumps, a SPD-M10A diode array detector, a RF-10AXL fluorescence detector, a CTO-10AS column oven, a DGU-12A degasser, a SIL-10AD auto-injector and a SCL-10A system controller (Shimadzu Co., Kyoto, Japan).

The mobile phase consisted of methanol, methyl tert-butyl ether and water in proportions: 81:15:4 (Solvent A) and 6:90:4 (Solvent B). The compounds were detected with the help of a YMC Carotenoid (C30, 4.6x520mm,  $5\mu$ ) column. For the analysis of tocopherols (including vitamin E), the detection was carried out at 290 nm with a flow rate of 1.0 ml/min. The elution profile was 0 to 20 % B over 10 minutes, followed by an increase to 100% over five minutes and isocratic run of 100% B over the next 5 minutes.

Detection of tocopherols was carried out using a fluorescence detector RF-10AXL. The detection of all other carotenoids was carried out at 445 nm with a flow rate of 1.5 ml/min. The elution profile was 0 to 100% B over 15 minutes, followed by 10 minutes of an isocratic run of 100% B. Calibration curves were prepared for eight standards: α tocopherol,  $\delta$  tocopherol,  $\gamma$  tocopherol, β carotene, chlorophyll a, chlorophyll b, lycopene and lutein. The solutions of tocopherols and β-carotene standards were prepared in

acetone; lutein, chlorophyll a and b, were dissolved in solvent A (81% MeOH, 15% MTBE, 4% H2O), and lycopene was dissolved in chloroform.

Lipophilic compounds in native fruits were identified by comparing the retention time and characteristic UV-VIS spectra with all available standards. The results, based on three independent measurements, were quantified using calibration curves and calculated as milligrams per 100g dry weight (mg/100g DW). The limits of detection were 15.0 µg/ml for αtocopherol,  $\delta$ -tocopherol and lycopene, and 3.9 μg/ml for βcarotene, lutein, chlorophyll a, chlorophyll b and γ-tocopherol.

#### **Analysis of vitamins**

### Extraction and analysis of Vitamin C

Vitamin C was extracted from powdered samples and stabilised using 4.5% metaphosphoric acid according to Vazquez-Oderiz et al. (1994) [8]. Fifty mg of samples were mixed with 1500  $\mu$ L of 4.5% m-H<sub>3</sub>PO<sub>4</sub>, vortexed and sonicated for five minutesto enhance the extraction process. Subsequently, the samples were centrifuged (5 min 12000rpm) to remove solid particles. The supernatants were collected and the extraction was repeated two more times. The supernatants were pooled together (4.5 mL). The extracts were prepared and analysed in triplicate.

Representative samples (10  $\mu$ L, three replicates) were injected into a HPLC. The HPLC system consisted of two LC-10AD pumps, a SPD-M10A diode array detector, a CTO-

10AS column oven, a DGU-12A degasser, a SIL-10AD auto-injector and a SCL-10A system controller (Shimadzu Co., Kyoto, Japan) equipped with a Luna (3μ C18(2), 4.6 x 250 mm, Phenomenex, USA) column. Analytical HPLC was run at 25°C and monitored at 215 and 245 nm.

Vitamin C was eluted under isocratic conditions using water acidified with sulphuric acid to pH 2.2 following the method of Vazquez-Oderiz et al. [8]. Detection was carried out at 245 nm at a flow rate of 1.0 mL/min. Ascorbic acid was identified by comparing the retention time and characteristic UV-VIS spectra with those of synthetic L-ascorbic acid (Ascorbic Acid, Sigma, Cat No L-0278). The results were quantified using an L-ascorbic acid calibration curve and calculated as micromoles per mL (μmol/mL). The limit of detection was 10<sup>-6</sup>g/mL (1 ug/ mL).

## Extraction and analysis of Vitamin E compounds

See Extraction and analysis of lipophilic compounds.

### Extraction and analysis of folate

The levels of folate were determined using an enzyme immunoassay for the quantitative analysis of folic acid, Ridascreen®Fast Folic Acid (Art.No. R3203) from R-Biopharm GmbH (Darmstadt, Germany). The detection limit was 1µg/kg DW. The results are presented as µg total folate per 100g dry weight (µg/100g DW).

#### **Analysis of minerals**

Multi element analysis has been performed on dry powders of fruits, herbs and spices using an Inductively Coupled Plasma – Mass Spectrometer (ICP-MS) that combines a high-temperature ICP source with a mass spectrometer. The ICP source converts the atoms of the elements in the sample to ions. These ions are then separated and detected by the mass spectrometer.

For analysis of all the elements the samples were weighed (~250mg) into 75ml glass digestion tubes then digested in 5ml of concentrated HNO<sub>3</sub>. After digestion the samples were diluted to 25ml with ultrapure H<sub>2</sub>O then analysed by ICP-MS. The results are expressed in μg/gDW (ppm).



Anise Myrtle in flush



Lemon Aspen fruit on tree

# Results and discussion

Samples evaluated within this project fall into two categories:

- herb/spice, and
- fruit.

The "herb/spice" category comprised of:

- Tasmannia Pepper (Leaves and Berries)
- Anise Myrtle
- Lemon Myrtle
- Bush Tomato
- Wattleseed.

The "fruit" category included:

- Australian Desert Lime
- Kakadu Plum
- Lemon Aspen
- two species of Davidson's Plum—Davidsonia pruriens and Davidsonia jerseyana
- Quandong
- Riberry.

#### **Antioxidant capacity**

Antioxidants are the compounds of our diet that reduce oxidative stress in the body. Oxidative stress occurs as a result of accumulation and action of free radicals, generated as by-products of normal and essential metabolic reactions and through exposure to environmental factors (e.g. cigarette smoke, pollutants, exposure to ionizing radiation, infections). The free radicals (or reactive oxygen species, ROS) are molecules of oxygen (or nitric oxide) with an unpaired electron. This electron makes the



Ground Lemon Myrtle. Within the herb/spice category, Tasmannia Pepper Leaf, Anise Myrtle and Lemon Myrtle exhibited the highest oxygen radical absorbance capacity which exceeded 2.5 to 4.0 times the activity of Tasmannia Pepper Berry and Bush Tomato and 41 to 66 times the activity of Wattleseed

molecule electrically charged. The charged molecule interacts with the nearest lipids, proteins and DNA and damages them. This damage contributes to the general decline in the optimum body functions. It is commonly believed to contribute to the "ageing process". It may also lead to oxidative stress mediated diseases (Alzheimer's disease, autoimmune and cardiovascular disease, cancer, cataractogenesis, diabetes, macular degeneration, etc.).

Lower levels of oxidative stress may reduce the probability of occurrence of these diseases at later stages in life. Preventing or reducing the oxidative stress may result in a healthier old age. The major source of antioxidants preventing our bodies from oxidative stress is food.

Therefore, the presence of antioxidants in the diet has the most conclusive role in the prevention/delaying of oxidative stress mediated diseases.

No single assay has universal acceptance as a measure of antioxidant capacity in foods. A variety of compounds exhibit antioxidant activity either directly or contribute to it, so a variety of assays have been developed, potentially focussing on different targets and mechanisms. In this evaluation two internationally recommended assays have been used: the oxygen radical

absorbance capacity (ORAC) assay, that represents the most relevant system to human biology and the ferric reducing ability of plasma (FRAP) assay [3].

The results of antioxidant testing on native herbs, spices and fruits are presented in Table 1, Figure 1 and Figure 2.

Within the herb/spice category, Tasmannia Pepper Leaf, Anise Myrtle and Lemon Myrtle exhibited the highest oxygen radical absorbance capacity which exceeded 2.5 to 4.0 times the activity of Tasmannia Pepper Berry and Bush Tomato and 41 to 66 times the activity of Wattleseed (Table 1; ORAC-T).

With the exception of Lemon Myrtle, the main source of antioxidant capacity in these sources was the hydrophilic fraction: 86% and 95% for Tasmannia Pepper Leaf and Anise Myrtle, respectively (Table 1; Figure 1).

In the case of Lemon Myrtle, the hydrophilic fraction contributed 56.2% and lipophilic fraction 45.8% to the total oxygen radical absorbance capacity. The high values of ORAC-L are due to the presence in Lemon Myrtle of an essential oil (monoterpene citral) that displays a high antioxidant capacity [9].

The contribution of the lipophilic fraction to the total oxygen radical absorbance capacity in Tasmannia Pepper Leaf was 14.0%, and in Tasmannia Pepper Berry was 18.5%.

The same three samples (Tasmannia Pepper Leaf, Anise Myrtle and Lemon Myrtle) exhibited superior antioxidant

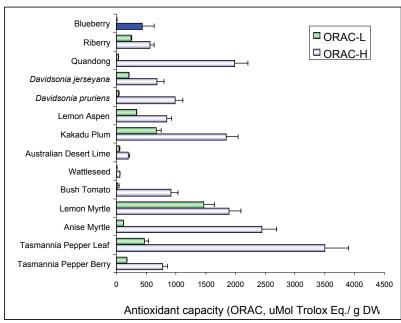


Figure 1. Antioxidant capacity of native Australian fruits, herbs and spices as evaluated in the ORAC assay

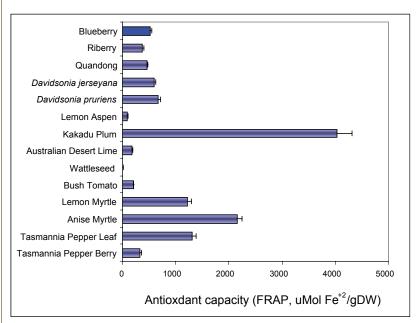


Figure 2. Antioxidant capacity of native Australian fruits, herbs and spices as evaluated in the FRAP assay

capacity to the Blueberry control in the FRAP assay in the following order: Anise Myrtle > Tasmannia Pepper Leaf > Lemon Myrtle. The levels of their antioxidant capacity exceeded 4-7 times the capacity of Tasmannia Pepper Berry and the Blueberry control, 10-6 times the antioxidant capacity of Bush Tomato and 120-69 times the antioxidant capacity of Wattleseed (Table 1; Figure 2).

Among the evaluated fruits, Kakadu Plum and Quandong exhibited superior oxygen radical absorbance capacity (ORAC-T) that was, respectively, 5.7 and 4.6 – fold of that of the Blueberry control (Table 1, Figure 1). The hydrophilic fractions contributed in 98.0% to the total oxygen radical absorbance capacity of Quandong, but only in 73.5% in the case of Kakadu Plum. The oxygen

Table I. Total phenolics and antioxidant capacity of selected native Australian herbs, spices and fruits

No	Sample	Dry matter content	Total phenolic content (mg GA Eq/g DW)	Total anthocyanins (mg C 3G Eq/g DW)	Total Reducing Capacity (FRAP) (µmol Fe <sup>+2</sup> /g DW)	ORAC-H (μmol TEq/gDW)	ORAC-L (μmol TEq/gDW)	ORAC-T (µmol TEq/gDW)
				Spic	е			
1	Tasmannia Pepper B.	100	16.86 ± 0.7	79.2 ± 4.1	332. 9 ± 19. 9	779.49 ± 82.60	176.87 ± 2.72	956.36
2	Tasmannia Pepper L.	100	102.06 ± 1.23	1.24 ± 0.02	1314.5 ± 67.9	3504.42 ± 392.5	572.70 ± 27.66	4077.12
3	Anise Myrtle	100	55.93 ± 4.67	ND	2158.0 ± 88.5	2446.06 ± 242.1	119.70 ± 0.15	2565.76
4	Lemon Myrtle	100	31.44 ± 5.9	ND	1225.3 ± 72.2	1889.82 ± 206.6	1470.05 ± 171.96	3359.87
5	Bush Tomato	100	12.40 ± 0.9	ND	206.2 ± 9.0	912.77 ± 117.69	18.56 ± 2.20	931.33
6	Wattleseed	100	0.76 ± 0.12	ND	17.8 ± 1.2	53.40 ± 7.93	8.14 ± 0.45	61.54
				Frui	t			
7	Australian Desert Lime	19.6	9.36 ± 0.35	ND	177.8 ± 11.7	197.17 ± 22.56	52.28 ± 0.73	249.45
8	Kakadu Plum	12.2	158.57 ± 12.29	ND	4032.5 ± 282.9	1841.97 ± 196.85	669.50 ± 81.15	2511.47
9	Lemon Aspen	15.5	10.49 ± 0.34	ND	90.2 ± 15.3	848.70 ± 73.70	343.95 ± 0	1192.65
10	Davidsonia pruriens	7.1	48.60 ± 2.48	47.80 ± 1.2	670.7 ± 49.3	982.41 ± 129.30	210.38 ± 2.06	1192.79
11	Davidsonia jerseyana	5.3	$50.25 \pm 6.34$	98.65 ± 6.5	599.8 ± 20.7	686.24 ± 109.83	214.04 ± 0.64	900.28
12	Quandong (dry)	90.1	32.87 ± 2.89	0.53 ± 0.1	454.9 ± 16.8	1987.99 ± 221.50	39.98 ± 1.00	2027.97
13	Riberry	8.8	23.62 ± 1.27	35.34 ± 2.5	376.9 ± 21.3	565.91 ± 72.39	251.31 ± 9.73	817.22
	Blueberry (control)	15.0	35.4	38.93 ± 0.99	397.1 ± 20.0	434.6*	2.4*	436.8*

[\*Source: Oxygen Radical Absorbance Capacity (ORAC) of selected foods – 2007. Retrieved on March 19, 2009 from http://www.ars.usda.gov. Values recalculated for DW based on DW=15%FW.]; ND – not determined

radical absorbance capacity of Queensland's Davidson's Plum (D. pruriens) and Lemon Aspen was 2.7-fold of that of the Blueberry control (ORAC-T; Table 1). These two crops were closely followed by the NSW Davidson's Plum (D. jerseyana) and Riberry, with approximately 2-times higher oxygen radical absorbance capacity than Blueberry. Australian Desert Lime displayed the lowest capacity at 57% of that of the Blueberry control. The contribution of compounds present in the hydrophilic fractions of these fruits was 82.4% for *D. pruriens*, 71.0% for Lemon Aspen, 76.2% for D. *jerseyana* and 69.2% for Riberry (Figure 1).

In the FRAP assay Kakadu Plum displayed an outstanding total reducing capacity of 4032.5 µmol Fe<sup>+2</sup>/gDW (Table 1; Figure 2) that was 10.5-fold of that of the Blueberry control. It was followed by Davidson's Plums (*D. pruriens* and *D. jerseyana*), Quandong, Blueberry and Riberry with

total reducing capacities within the range of 670.7 to 377.0 µmol Fe<sup>+2</sup>/gDW. Consistent with the results obtained in the ORAC assay, the lowest antioxidant capacity using the FRAP assay was with Australian Desert Lime.

Relatively high antioxidant activity has been detected in the lipophilic fractions of native fruits [e.g. Kakadu Plum (26.5%), Lemon Aspen (28.8%), Riberry (30.7%), Davidson's Plum (D. pruriens. 17.6% and D. jerseyana 23.8%), Australian Desert Lime (20.9%)] and herbs and spices [Tasmannia Pepper Leaf (14.0%), Tasmannia Pepper Berry (18.5%) and Lemon Myrtle (43.7%)] (Table 1; Figure 1). Lipophilic compounds such as vitamin E, carotenoids or chlorophyll are the possible source of this activity. In the case of the Blueberry control, the major source of antioxidant activity is within the hydrophilic fraction (99.5%). Similarly high activities within the hydrophilic

fractions were reported for apple (99.2%), cranberry (97.9%), grape (100%), plum (99.7%), orange (98.1%), oregano (100%), and potatoes (98.3%)[10].

Unlike hydrophilic antioxidants, which do not accumulate in the body and are excreted in urine, lipophilic antioxidants penetrate the lipoprotein cell membrane more easily and therefore reach a higher level of bioavailability [11]. The presence of antioxidant compounds that are active in both the hydrophilic and the lipophilic environment, within the same food source, may provide more comprehensive protection from oxidative stress. Subsequently this may result in higher levels of protection from ROS and possibly more pronounced health benefits. In this aspect, evaluated native food sources exceed the quality of the Blueberry control and other traditionally consumed fruits.

#### **Total phenolic content**

Phenolic compounds (phenolics) are water-soluble compounds and usually are the main phytochemicals present in hydrophilic extracts of plant materials. In our evaluation the major source of antioxidant activity is within the hydrophilic extract. Therefore the levels of phenolic compounds need to be evaluated and their effect on the antioxidant activity needs to be understood.

Within the 'herb/spice' category, Tasmannia Pepper Leaf contains the highest levels of phenolics that comprise about 10% of the dry leaf powder (102 mg/g DW; Table 1). Tasmannia Pepper Leaf is closely followed by Anise Myrtle (5.6% of dry weight) and Lemon Myrtle (3.1% of dry weight). The level of phenolic compounds in Blueberry is 3.54% of the dry weight [10, the values for Blueberry are recalculated from fresh weight using the average Blueberry dry weight of 15% of the fresh weight].

Kakadu Plum is the richest source of phenolic compounds among the fruits, with 4.7-times higher levels than that found in blueberries. The levels of phenolic compounds in Quandong are comparable to those found in blueberries.

However, the Quandong has displayed an outstanding antioxidant capacity, significantly higher than that of the Blueberry control. Antioxidant capacity is a function of both the level and the quality of phenolic compounds. This result suggests the presence of compounds that possess enhanced

antioxidant activity in Quandong. The levels of phenolic compounds in Davidson's Plums were 1.5-fold higher than that of Blueberry, while for other fruit samples, were significantly lower than in the Blueberry control (Table 1).

In 1996, antioxidant testing (ORAC assay) of more than 40 commercially available fruits and vegetables in the US market identified blueberries as the richest source of antioxidants [12]. Vitamin C and E were originally considered to be responsible for the high antioxidative potential of Blueberries. However, further research has confirmed that mainly the phenolic acids and anthocyanins present in blueberries in high concentrations, are responsible for the antioxidant properties [13]. Similarly, phenolic compounds present in berries, grape, rosemary and green tea are described as the active ingredients responsible for the health-preventative properties of these foods. [14].

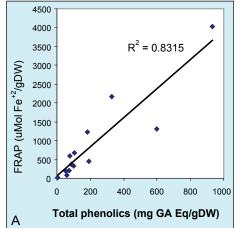
In order to understand the effect of phenolic compounds on the antioxidant capacity of the evaluated fruits, herbs and spices, correlations between the levels of phenolics and

antioxidant capacity data need to be investigated.

# Correlation between total phenolic content and antioxidant capacity

The correlation between the level of total phenolic contents and antioxidant capacities of the extracts evaluated in FRAP and ORAC-H assays is presented in Figure 3. A high positive correlation was achieved in both cases, indicating that phenolic compounds are primarily responsible for the antioxidant capacity of the hydrophilic extracts.

The presence of phenolic compounds in native Australian fruits, herbs and spices, at exceptionally high levels in some of the evaluated sources, promises potential healthenhancing effects from their consumption. However, antioxidant capacity of phenolic-rich extracts depends on the total level of phenolic compounds as well as on their molecular structure [15]. The knowledge of phenolics composition in extracts prepared from the native fruits, herbs and spices can be used to envisage their possible antioxidant/



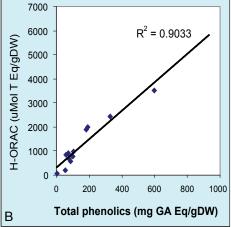


Figure 3. Correlation between total phenolic content and FRAP (A) and total phenolic content and ORAC - H(B)

enhanced health properties, which will further translate into potential health benefits, providing the benefits are confirmed in further studies and the same compounds are present in consumed foods and are bioavailable.

# Identification of major phenolic compounds

The composition of phenolic compounds in some evaluated fruits, herbs and spices was rich. Within each extract a number of compounds were visible at 280 nm (benzoic acids and flavanols, quantified as gallic acid equivalents), at 326nm (cinnamic acids, quantified as chlorogenic acid equivalents), at 370nm (flavonols and stilbens, quantified as rutin equivalents) and in coloured samples at 520 nm (anthocyanins, quantified as cyanidin 3-glucoside equivalents). Complete identification of compounds in such rich mixtures requires long-term research and therefore within the present study only the major compounds were targeted. The identified compounds are listed in Table 2.

#### Herb/Spice category

Tasmannia Pepper Leaf represents the richest source of phenolic compounds within this group. Tasmannia Pepper Berrry contains 6-fold less phenolic compounds than the leaf, yet the composition of both phenolic mixtures were very similar. Cyanidin 3-rutinoside followed by cyanidin 3-glucoside were the major anthocyanins present in the extract of berry at the total level of approximately 8%DW (79.2 mg C3G Eq./g DW, Table 2). Other minor components detected included rutin (0.2%DW), chlorogenic acid (0.15%DW), quercetin

Table 2. Major phenolic compounds identified in selected native Australian herbs and spices

No	Sample	Compound	Amount (mg/g DW)
		Spice	
1		Cyanidin 3-glucoside	23.95
		Cyanidin 3-rutinoside	55.26
	Tasmannia Pepper B.	Chlorogenic acid	1.46
	rasmannia r epper b.	Rutin	2.08
		Quercetin	0.86
		Kaempferol/Luteolin hexoside	T
		Caffeic acid	T
2		Cyanidin 3-glucoside	1.25
	Tanmannia Dannar I	Chlorogenic acid	30.02
	Tasmannia Pepper L.	Rutin	3.72
		Quercetin	17.85
3		Quercetin hexoside	
	Ania a Mandla	Quercetin pentoside	3.38
	Anise Myrtle	Myricetin	4.14
		Chlorogenic acid	
4		Hesperitin rhamnoside	3.82
	Laman Muntha	Hesperitin pentoside	P
	Lemon Myrtle	Myricetin	3.61
		Hesperitin hexoside	4.24
5		Quercetin rutinoside	Р
		Quercetin hexoside	P
		Chlorogenic acid	0.42
	Duch Tomoto	Caffeic acid	P
	Bush Tomato	Ferulic acid	0.82
		p-Coumaric acid	Р
		Hydroxybenzoic acid	0.33
		Kaempferol/Leutolin hexoside	Р
6		Rutin hexoside	Р
	Wattleseed	Quercetin hexoside	Р
		Kaempferol/Leutolin hexoside	Р

T- traces; P – possible (confirmation required)

hexoside (0.1%DW) and traces of kaempferol/luteolin hexoside as well as caffeic acids. Traces of anthocyanin (cyanidin 3-glucoside, 0.125%DW) were also present in the leaf extract. Chlorogenic acid was detected at very high levels of 3%DW, followed by quercetin (1.8%DW) and rutin (0.4% DW). Other minor compounds present in both extracts were not identified. Figure 4 presents the molecular structures of the detected phenolic compounds. The characteristic feature of these molecules (with exception of kaempferol) is the 'catechol' structure or presence of at least 2 HO- groups on a benzene ring. Phenolic compounds that possess this characteristic were

identified as the most powerful antioxidants and display a range of physiological functions [16, 17].

Chlorogenic acid is a highly bioactive molecule and a well absorbed molecule. It is an antioxidant and anti-mutagenic compound [17]. It slows the release of glucose into the bloodstream [18]. Plant-based extracts standardised to contain 16-20% chlorogenic acid (such as Blueberry leaf extract, [19]) are frequently sold as an antidiabetic food supplement. The same compound was found to protect collagen (the main skin protein) from damage [20]. Due to the high level of chlorogenic acid in the leaf, and the

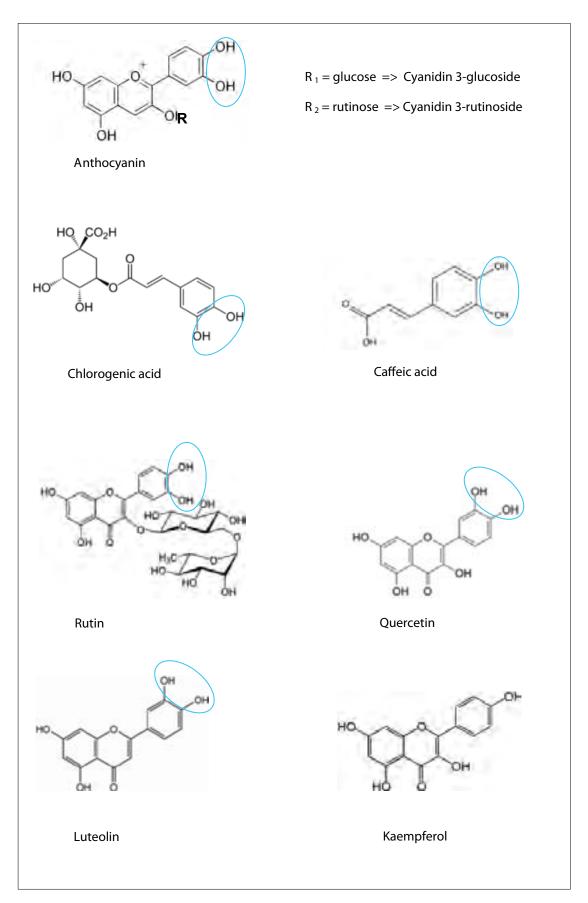


Figure 4. Molecular structures of phenolic compounds detected in Tasmannia Pepper Berry indicated catechol structure is necessary for antioxidant properties of a phenolic compound [15]

presence of rutin and quercetin in the same mixture, Tasmannia Pepper Leaf is an excellent candidate for the production of a supplement for the food and/or cosmeceutical industries.

Anise Myrtle extract contained several components with an m/z 303 aglycone (quercetin or hesperitin). These components were responsible for the major peaks at 370 nm. The group included a rhamnoside, a pentoside (i.e. glycoside with a 5-carbon sugar), a hexoside (glycoside with a 6-carbon sugar) and a rutinoside. Other detected components in minor amounts included myricetin and chlorogenic acid.

The major components in Lemon Myrtle extract were glycosides with a m/z 303 algycone. Given that in citrus the abundant aglycone is hesperitin it is more likely that this aglycone was present in the extract rather than quercetin (both exhibit an m/z of 303 in positive ionization). Major hesperitin glycosides found were hexosides, a ramnoside, and a pentoside. Traces of rutin/hesperidin and naringenin rutinoside were also found.

Bush Tomato contained quercetin rutinosides, a quercetin hexoside, a kaempferol/luteolin hexoside. Minor amounts of chlorogenic, caffeic, ferulic, coumaric and hydroxybenzoic acids were also detected in the extract.



**Bush Tomatoes** 

Similarly to Bush Tomato, phenolic compounds were present at minute quantities in the extract of Wattleseed. Major compounds detected were: rutin, quercetin and kaempferol/luteolin hexosides. Trace levels of chlorogenic acid were also found.

#### Fruit category

Kakadu Plum exhibited superior antioxidant capacity and the presence of phenolic compounds was identified as its source.

The HPLC chromatogram of Kakadu Plum extract revealed the presence of over 50 various compounds. However, the LC/MS scan showed the presence of over 100 compounds. Due to this exceptionally rich composition, many of the compounds co-eluted and this prevented their identification.



Dried Kadadu Plums

For complete identification of compounds, fractionation of the extract will be necessary. This approach was out of the scope of the current study. Nevertheless, quercetin/hesperitin glucosides and keampferol/luteolin glycosides were identified in the extract (Table 3). Other interesting components present were those producing an m/z 291 during a production scan with a precursor m/z of 451. Catechin exhibits 291 atomic mass unit (amu) in the positive ionization mode so these

components could potentially be catechin-based hexosecontaining glycosides.

Despite the problems with compound identification it is already clear that the exceptionally rich composition of the Kakadu Plum extract is the reason for the superior antioxidant capacity of this fruit. This could be due to an additive or a synergistic effect and/or presence of compounds with exceptional activities. The answer to this question should be a subject of future studies.

Quandong was the second fruit that displayed an outstanding antioxidant capacity.



Quandongs

The LC/MS analysis revealed the presence of cyanidin 3-glucoside as the major anthocyanin, minor amounts of pelargonidin 3-glucoside and trace levels of cyanidin 3- rutinoside (Table 3).

The total level of anthocyanin in dry Quandong was 0.53 mg C3G/g DW (Table 1) whilst in fresh (frozen) Quandong this level was 7.03 mg C3G/g DW (data not presented). The most probable reason of this drop would be degradation of valuable anthocyanins during the drying process.

Other components identified in the fresh extract included quercetin, keampferol rutinosides and chlorogenic acid. A series of notable peaks at 280 nm, possibly due to benzoic acids, were observed in the chromatographic trace but these components could not be identified within this study. Two major compounds detected at 326nm at levels 22.4 and 34.4 mg CHA Eq/gDW remain unidentified.

In Davidson's Plums, anthocyanins were the major phenolic compounds detected. The major forms found were sambubiosides of delphinidin, cyanidin and peonidin aglycones. Petunidin, pelargonidin and malvidin sambubiosides were present at lower levels.

The total amount of anthocyanins in *Davidsonia jerseyana* was 98.6 mg C3G/g DW and in *Davidsonia pruriens* was 47.8 mg C3G/g DW (Table 1).

The observed differences could be due to cultivar specificity and/or fruit maturity. Other components found in small amounts included myricetin, rutin and quercetin hexoside.

Anthocyanins are plant pigments responsible for the red, purple and blue colours of fruits and vegetables. They are an increasingly important group of natural food colorants.

Anthocyanins are potent antioxidants and a range of health benefits arising from their consumption have been reported, such as anti-diabetic effects [21] and reduction of obesity [22]. Cyanidin 3-sambubioside isolated from flowers of *Hibiscus sabdariffa* L. induced apoptosis (programmed cell death, known also as cell suicides) of cancer cells *in vitro* (cell culture studies) [23].

Table 3. Major phenolic compounds identified in selected native Australian fruits

No	Sample	Compound	Amount (mg/g DW)
		Fruit	
1	Australian Desert Lime*		
2	Kakadu Plum	Quercetin/Hesperitin glucoside Kaempferol/Luteolin glycoside	P P
3	Lemon Aspen	Kaempferol/Luteolin hexoside Quercetin hexoside Rutin Chlorogenic Acid Caffeic Acid Coumaric Acid Ferulic Acid	P P P P P
4	Davidsonia pruriens	Delphinidin sambubiose Cyanidin sambubiose Peonidin sambubiose Pelargonidin sambubiose Malvinidin sambubiose Myricetin Rutin Quercetin hexoside	22.70 2.08 7.07 T T P P
5	Davidsonia Jerseyana	Delphinidin sambubioside Cyanidin sambubioside Petunidin sambubioside Peonidin sambubioside	19.98 24.68 8.56 30.44
6	Quandong (dry)	Cyanidin-3-glucoside Pelargonidin-3-glucoside Quercetin rutinoside Kaempferol	0.523 0.01 2.22 1.65
7	Quandong (Fresh)	Cyanidin-3-glucoside Pelargonidin-3-glucoside Quercetin rutinoside Kaempferol	5.76 1.18 2.29 2.6
8	Riberry	Rutin Quercetin hexoside Cyanidin 3-galactoside Cyanidin 3 – glucoside Myricetin hexoside Kaempferol/luteolin rutinoside Quercetin rhamnoside Cyanidin 3,5 - diglucoside	0.65 0.64 28.8 2.3 P P P P

T- traces; P – possible (confirmation required)

Based on the high level of anthocyanins in the flesh, the potential application of Davidson's Plum as a source of a natural food colour with health-enhancing properties for a wide application in beverages and confectionery might be considered. Anthocyanins were also found in Riberry extract. The cyanidin-based galactoside

and glucoside were the major pigments and were present at the level of 35.3 mg C3G/g DW (Table 1). The simpler molecular structure of these anthocyanins in comparison with Davidson's Plum will be responsible for colour loss in Riberry products. The sample also contained notable amounts of other glycosides such as

<sup>\*</sup> This extract contained components that require further investigation in order to establish their identity. Major peaks in the extract exhibited m/z 682 (fragments: 454, 438), m/z 454 (fragments 182, 210, 226), m/z 334 (fragment 164).

quercetin and kaempferol rutinosides, myricetin and quercetin hexosides and quercetin rhamnoside (Figure 5).

Research over the last 20 years has generated evidence on possible health effects of phenolic compounds.

At first, in the 1990s, phenolic compounds of red wine (polymerised anthocyanins and resveratrol) were found responsible for the 'French paradox' phenomena – low incidence of cardiovascular diseases despite a high fat diet [24, 25, 26].

At the same time the hydrophilic extract from *Vaccinum myrtillus* (bilberry) containing 36% anthocyanins was found to cure



Lab preparation of Davidson's Plums

altered conditions of capillary fragility and permeability and normalized ophthalmological disorders resulting in an increase of vision ability in the dark [27]. Kamei *et al.* in 1995 suggested

possible cancer suppression by anthocyanins [28].

Blueberry supplemented diets fed to mice over eight weeks resulted in measurable

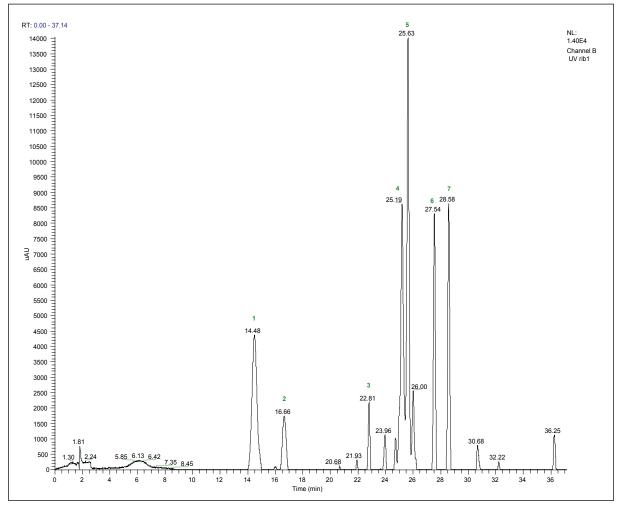


Figure 5. HPLC profile at 370 nm from the Riberry extract 1) cyanidin 3-galactoside, 2) cyanidin 3-glucoside, 3) myricetin hexoside, 4) rutin, 5) quercetin hexoside, 6) kaempferol/luteolin rutinoside, 7) quercetin rhamnoside

improvement in normal agerelated declines in behavioural parameters (balance, coordination and memory) [29] and nutritional intervention through a phenolic rich diet has been proposed as an avenue to prevent cognitive and behaviour deficits that occur in aging [29, 30].

A vast amount of research data generated to date, including epidemiological studies, confirmed the earlier reports and a number of health benefits arising from the consumption of phenolic compounds can be summarised as: suppression of neural degeneration [29, 30, 31], possible cancer chemoprevention [ 32, 33, 34], reduction of cardiovascular diseases [35], diabetes and obesity [21, 22]. The mechanisms of the health effects of phenolic compounds are still under investigation.

It needs to be remembered that these plant-produced compounds are xenobiotics (compounds that are present in the human body but not produced by humans) and if present in very large quantifies may exhibit toxic effects. However, until now, the toxicity levels of phenolic compounds have not been observed.

An intake of flavonoids, phenolic compounds that dominate in fruits and vegetables, and are linked to health effects, is estimated at a maximum level of a few hundreds milligrams per day (up to 650mg/day).

The average uptake is only 23 mg/day, with 70% of the contribution coming from quercetin, predominantly obtained from apples and onions [34].

Health-maintaining properties of some traditional diets, such as the Mediterranean or Japanese diet, that are well known for their rich composition of fruits and vegetables, are undisputed. Synergistic interactions between various compounds originated from fruits and vegetables or their additive effects are proposed as the most probable mechanism of disease prevention [34].

Research over the last decade has established that nutritional quality of diet improves with a greater diversity of food items or food groups [36, 37].

Subsequently, increased dietary diversity and especially presence of a range of flavonoids, a group of phenolic compounds, is linked to improved health [34, 38].

The Australian native fruits, herbs and spices contain an exceptionally rich composition of phenolic compounds present at relatively high levels. With the observed multiplicity of phytochemicals, native fruits, herbs and spices may contribute greatly to diversify and enhance the health-maintaining properties of the daily diet.

# Identification of major lipophilic compounds

Lipophilic extracts of 13 samples were evaluated for the presence of  $\alpha$ -,  $\gamma$ - and  $\delta$ -tocopherol,  $\beta$ -carotene, lycopene, lutein as well as chlorophyll a and chlorophyll b with a help of authentic standards. The compounds were identified through comparison of the retention time, UV-VIS spectra characteristics and co-chromatography. The following compounds were detected: tocopherols (vitamin E), lutein, chlorophyll and carotene.

#### Tocopherols (vitamin E)

Tocopherols were the major lipophilic phytochemicals detected in all the evaluated samples. Tocopherols identified in this study are the main components of vitamin E. Their levels are presented in Table 5 and the results are discussed under the chapter 'Vitamins'.

The Australian native fruits, herbs and spices contain an exceptionally rich composition of phenolic compounds present at relatively high levels. With the observed multiplicity of phytochemicals, native fruits, herbs and spices may contribute greatly to diversify and enhance the health-maintaining properties of the daily diet.

Table 4. Lipophilic compounds identified in selected native Australian herbs, spices and fruits (Lipophilic tocopherols identified in this study are the main components of vitamin E and their levels are presented in Table 5.)

No	Sample	Lutein (mg/100g DW)	Chlorophyll a (mg/100g DW)	Chlorophyll b (mg/100g DW)	β carotene (mg/100g DW)						
	Herb/Spice										
1	Tasmannia Pepper B.	ND	ND	ND	ND						
2	Tasmannia Pepper L.	1.56 ± 0.04	15.83 ± 0.02	$3.65 \pm 0.02$	2.30 ± 0.09						
3	Anise Myrtle	20.86 ± 1.66	412.56 ± 29.72	50.57 ± 4.26	ND						
4	Lemon Myrtle	6.56 ± 0.40	135.66 ± 0.09	25.06 ± 0.29	ND						
5	Bush Tomato	ND	ND	ND	ND						
6	Wattleseed	ND	ND ND ND		ND						
			Fruit								
7	Australian Desert Lime	1.51 ± 0.07	ND	6.89 ± 0.23	ND						
8	Kakadu Plum	1.52 ± 0.09	15.91 ± 0.5	3.58 ± 0.15	ND						
9	Lemon Aspen	ND	ND	ND	ND						
10	Davidsonia pruriens	1.15 ± 0.11	ND	1.96 ± 0.15	ND						
11	Davidsonia jerseyana	1.41 ± 0.30	ND	ND	ND						
12	Quandong (dry)	ND	ND	ND	ND						
13	Riberry	ND	ND	ND	ND						
Blueberry*		0.08	_	-	-						

(ND - not detected, T - traces)

#### **β-Carotene**

β-Carotene (Figure 6) is a red-orange lipophilic pigment and is the most common carotenoid among over 600 molecules identified to date. Plant carotenoids are the primary dietary source of pro-vitamin A and β-carotene is the most efficient pro-vitamin A. Carotenoids are efficient free-radical scavengers, however, studies of supplementation with large doses of β- carotene in heavy smokers have shown an increase in cancer risk [39].

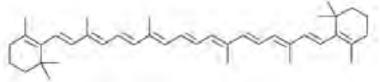


Figure 6. Molecular structure of  $\beta$ -carotene

 $\beta$ -carotene was identified only in the leaf of Tasmannia pepper, present at a level of 2.3 mg/100 gDW (Table 4).  $\beta$ -carotene is present in some vegetables with the richest sources being:

- avocado, spinach, kale, sweetpotato leaf, carrot and fruits:
- papaya and mango.

The level of  $\beta$ -carotene in the Australian avocado, "Hass," is from 0.02 to 0.07 mg/100 g DW [40; the values are recalculated from fresh weight data using an average dry weight of avocado of 26%].



Tasmannia Pepper Leaf

<sup>\*</sup>See Appendix Table A

#### Lutein

Lutein (Figure 7) plays an important role in eye health. It improves visual function and symptoms in atrophic age-related macular degeneration (ARMD) that is the leading cause of vision loss in aging Western societies [41]. Lutein protects the retina from damage by inhibiting inflammation [42]. Lutein is used as a **food** colour **additive** and nutrient supplement in a wide range of applications at concentrations ranging from 2 to 330 mg/kg. Lutein is purified from oleoresin extracted from the petals of marigold (*Tagetes erecta*) and is used as a food additive with the E number E161b [43].

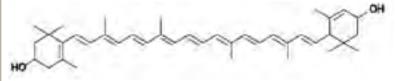


Figure 7. Molecular structure of lutein

Among the evaluated herbs and spices, Anise Myrtle contained the highest level of lutein: 20.9 mg/100g DW, and was followed by Lemon Myrtle (6.6 mg/100g DW) and Tasmannia Pepper Leaf (1.6 mg/100g DW) (Table 4). Lutein was not present in the other evaluated spice sources.

Kakadu Plum, Australian Desert Lime and Davidson's Plum were identified as containing lutein at the approximate level of 1.5 mg/100g DW (Table 4). These fruits contain more lutein than the Australian "Hass" avocado (from 0.615 to 1.05 mg/100g DW; the values are recalculated from fresh weight data using an average dry weight of avocado of 26%) [39]. Avocado is one of the primary sources of lutein. Other sources of lutein are corn, orange pepper, kiwi fruit, grapes, spinach, orange juice and zucchini [44]. The level of lutein in Blueberry is 0.5 mg/100g DW (Appendix Table A).



Lemon Myrtle

#### Chlorophyll a and b

Chlorophyll is the major pigment present in plants and occurs in several distinct forms. Among them chlorophylls a and b are the major types found in higher plants. Chlorophyll molecules display antioxidant capacity and are considered to be implicated in the reduction of oxidative stress in human body, that subsequently is linked with the prevention of chronic diseases [45].

Chlorophyll levels in evaluated samples are affected by sample maturity (e.g. Davidson's Plum) and drying techniques (leaf).

As expected, chlorophylls were identified in Anise Myrtle, Lemon Myrtle and Tasmannia Pepper Leaf as well as Kakadu Plum (Table 4). Chlorophyll *a* dominated over the chlorophyll *b* in the ratio of 8.2 (Anise Myrtle), 5.5 (Lemon Myrtle), 4.4 (Tasmannia Pepper Leaf and Kakadu Plum).

Degradation of chlorophyll (loss of green colour) and synthesis of pigments (anthocyanin - red and blue colour and /or carotenoids – yellow and orange colour) are the major compositional changes that occur during fruit ripening [46]. The presence of the chlorophyll *b* in *Davidsonia pruriens* may suggest that the fruit has been harvested and analysed before it reached full maturity. This could possibly affect the level of anthocyanin (Table 1).

Table 5. Vitamins identified in selected native Australian herbs, spices and fruits

		Vitamin C	Vitamin E o	omponents (m	g/100g DW)	Vitamin E	Folate	Vitamin A*		
No	Sample	(mg/100g DW)	α-tocopherol β-tocopherol		γ-tocopherol	(mg/100g DW)	(µg/100g DW)	(mg/100g DW)		
	Herb/Spice									
1	Tasmannia Pepper B.	ND	0.24 ± 0.09	$0.02 \pm 0.01$	$0.93 \pm 0.10$	1.198	87.0	ND		
2	Tasmannia Pepper L.	ND	17.41 ± 1.79	$0.19 \pm 0.06$	Т	17.835	160.0	$0.2 \pm 0.007$		
3	Anise Myrtle	$66.7 \pm 7.5$	49.40 ± 0.87	1.63 ± 0.01	8.66 ± 0.02	59.696	100.0	ND		
4	Lemon Myrtle	ND	20.21 ± 0.33	$0.36 \pm 0.02$	0.36 ± 0.01	21.231	71.0	ND		
5	Bush Tomato	ND	3.414 ± 0.73	$0.50 \pm 0.03$	0.66 ± 0.04	4.573	100.0	ND		
6	Wattleseed	ND	4.65 ± 0.26	$0.34 \pm 0.02$	0. ± 0.015	5.286	100.0	ND		
				Fruit						
7	Australian Desert Lime	962.3 ± 23.4	3.58 ± 0.91	$0.42 \pm 0.09$	ND	3.999	420.0	ND		
8	Kakadu Plum	7252.8 ± 68.6	5.97 ± 0.63	0.11 ± 0.06	ND	6.079	110.0	ND		
9	Lemon Aspen	ND	1.82 ± 0.31	$0.06 \pm 0.02$	ND	1.885	110.0	ND		
10	Davidsonia pruriens	ND	$0.52 \pm 0.04$	$0.38 \pm 0.02$	$0.26 \pm 0.03$	1.16	40.0	ND		
11	Davidsonia jerseyana	ND	$0.05 \pm 0.002$	$1.60 \pm 0.23$	1.25 ± 0.29	2.905	34.0	ND		
12	Quandong (dry)	ND	4.24 ± 0.28	$0.30 \pm 0.02$	0.24 ± 0.01	4.786	120.0	ND		
13	Riberry	ND	2.60 ± 0.51	$0.02 \pm 0.01$	ND	2.615	110.0	ND		

\*Vitamin A is presented in mg retinol activity equivalent recalculated from β-carotene (RAE, 1μg of retinol = 12 μg of "dietary" beta-carotene) [58], ND – not detected, T- traces.

#### **Identification of** vitamins

Vitamins, named originally the 'vital amines' by their discoverer, Kazimierz Funk in 1912, are essential regulators of physiological processes in humans and are essential for the growth and development of the human body. Vitamins cannot be stored in the body. It is essential that vitamins are available in our daily food. In organic foods they are present in very low amounts and therefore in our evaluation they are presented in milligrams per 100 g dry weight (mg/100 g DW). Oversupply of vitamins doesn't have a benefit - they are eliminated as a waste or can even be toxic (fat soluble vitamins).

Vitamins C and E are powerful antioxidants active in hydrophilic (vitamin C) and lipophilic (vitamin E) environments. Other vitamins, such as nicotinic acid (also called niacin or vitamin B3). B12, vitamin A and folate are essential in maintaining genome health. Among them, vitamins B3 and B12 are of animal origin, while vitamin A and components of folate are gathered from plant sources. β-carotene, a lipophilic compound, is the most efficient pro-vitamin A.

#### Vitamin C

Vitamin C (Figure 8) is a water soluble highly effective antioxidant that even in small amounts can protect indispensable molecules in the body, such as proteins, lipids (fats), carbohydrates, and nucleic acids (DNA and RNA) from damage by free radicals and reactive oxygen species (ROS). An efficient reduction of oxidative stress by vitamin C has been observed in cells grown in vitro [47] and in life subjects [48]. Vitamin C is required for the synthesis of collagen, an important structural component of blood vessels, tendons, ligaments, and bone and is implemented in the prevention of a number of diseases [49].

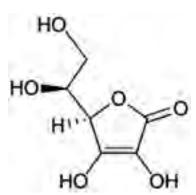


Figure 8. Molecular structure of Vitamin C

Among the evaluated herbs and spices, Anise Myrtle was the only source of vitamin C. The vitamin made up 0.07% of the dry weight (66.7 mg/100 g DW, Table 5). The richest source

of vitamin C was Kakadu Plum with an outstanding level of 7252.3 mg/100 g DW (7.2% of the dry weight). This result supports earlier published reports [1, 50]. Vitamin C was identified also in Australian Desert Lime at the level of 962.3 mg/100g DW (0.9 % of the dry weight) (Table 5). The level of vitamin C in Blueberry is 64.6 mg/ 100g DW (Appendix Table A).



#### Vitamin E

Although Vitamin E has been known as an essential nutrient for reproduction since 1922, due to the potent antioxidant properties, the impact of vitamin E in the prevention of chronic diseases is believed to be associated with oxidative stress. Recently Azzi (2007) has suggested involvement of vitamin E in signal transduction [51]. A number of other functions were identified for various forms of vitamin E, such as neutralisation of mutagens by  $\gamma$ -tocopherol and protection of neurons by tocotrienols [52, 53].

$$\begin{array}{lll} \alpha\text{-tocopherol, }R_1 = R_2 = R_3 = CH_3 & \gamma\text{-tocopherol, }R_1 = R_2 = CH_3 R_3 = H\\ \alpha\text{-tocotrienol, }R_1 = R_2 = CH_3 : R_2 = H\\ \beta\text{-tocopherol, }R_1 = R_3 = CH_3 : R_2 = H\\ \beta\text{-tocotrienol, }R_1 = R_3 = CH_3 : R_2 = H\\ \beta\text{-tocotrienol, }R_1 = R_3 = CH_3 : R_2 = H\\ \delta\text{-tocotrienol, }R_1 = R_2 = R_3 = H\\ \end{array}$$

Figure 9. Molecular structures of various forms of Vitamin E

Vitamin E is the term for a group of tocopherols and tocotrienols (Figure 9), of which  $\alpha$ -tocopherol has the highest biological vitamin E activity. The most common components of vitamin E are:  $\alpha$ -tocopherol,  $\beta$ -tocopherol and  $\gamma$ -tocopherol and the same have been evaluated in this study. The sum of  $\alpha$ -tocopherol,  $\beta$ -tocopherol and  $\gamma$ -tocopherol is presented as the level of vitamin E (Table 5).

The highest levels of vitamin E (the sum of  $\alpha$ -,  $\gamma$ - and  $\delta$ -tocopherol) were contained in the sample of Anise Myrtle (59.7 mg/100gDW), followed by Lemon Myrtle (21.2 mg/100gDW) and Tasmannia Pepper Leaf (17.6 mg/100gDW) (Table 5).  $\alpha$ -Tocopherol was the main compound that contributed to the total tocopherol mixtures in these three sources, respectively, 82.8%, 95.18% and 98.90%. In case of Anise Myrtle, the contribution of  $\delta$ - and  $\gamma$ -tocopherols to the tocopherol mixture was 2.7% and 14.5%.

The levels of vitamin E in Wattleseed, Bush Tomato and Tasmannia Pepper Berry were significantly lower: 5.3, 4.57 and 1.2 mg/100g DW (Table 5).  $\alpha$ -Tocopherol was the main compound in the tocopherol mixtures of Bush Tomato and Wattleseed with the contribution of 74.6% and 87.9%, respectively.  $\delta$ -Tocopherol was the main compound in Tasmannia Pepper Berry and made 78.0% of its tocopherol mixture.

Significantly lower levels of vitamin E were detected in the fruit samples. Kakadu Plum had the greatest amount (6.1 mg/100gDW) and was followed by Quandong (4.8 mg/100gDW), Australian Desert Lime (4.0 mg/100gDW), Davidsonia jerseyana (2.9 mg/100gDW), Riberry (2.6 mg/100gDW), Lemon Aspen (1.9 mg/100gDW) and Davidsonia pruriens (1.2 mg/100gDW) (Table 5). The composition of the tocopherol mixture in the fruit

samples differed. α-Tocopherol was the main compound in the tocopherol mixtures of Kakadu Plum (98.2%), Quandong (88.6%), Australian Desert Lime (89.6%), Riberry (99.3%) and Lemon Aspen (96.5%). Two other tocopherols, γ- and δ-tocopherol, dominated in both Davidson's Plum samples: 55% and 43.1% in *D. jerseyana*, 33.0% and 22.1% in *D. pruriens*, respecitively.

According to the USDA Database on food composition [54], high levels of vitamin E can be found in the following foods: avocado, nuts, red palm oil, seeds, spinach, vegetable oil, wheat germ, wholegrain foods, milk and asparagus. One of the richest sources of tocopherols is avocado. Australian avocado "Hass" contains from 4.60 to 8.27 mg/ 100g DW α-tocopherol, 0.45 to 0.89 mg/100g γ-tocopherol, and 0.32 to 1.00 mg/100g DW  $\delta$ -tocopherol. The level of vitamin E in Kakadu Plum and Quandong is comparable with that of avocado 'Hass' [40; the values are recalculated from fresh weight data using an average dry weight of avocado of 26%]. The level of vitamin E in Blueberry is about 3.7 mg/100g DW (Appendix Table A).



In the fruit samples the richest source of vitamins C and E was Kakadu Plum

#### **Folate**

Components of folate are water-soluble compounds (known also as vitamin B9) that play a key role in the methylation cycle and in DNA biosynthesis (Figure 10). They are required for the repair and synthesis of human DNA and for chromosome segregation. Breaks in the DNA strand and chromosome malsegregation are the main forms of genome damage. Adequate levels of folate, combined with the presence of Zn, Mg, Ca and vitamin B12 in the diet, can prevent genome damage that occurs due to oxidative stress, nutrient deficiency or calory excess [55]. Folate deficiency results in an increased risk of cardiovascular disease and dementia as well as neural tube defects [56, 57].

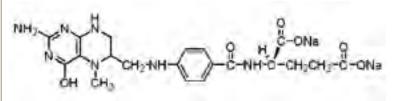


Figure 10. Molecular structure of folate

All samples of native species evaluated in this study contained folate. Among herbs and spices, the highest level of 160 ug/ 100gDW was present in Tasmannia Pepper Leaf. Anise Myrtle, Bush Tomato and Wattleseed contained 100ug/100gDW each and were followed by Tasmannia Pepper Berry (87  $\mu g/100gDW)$  and Lemon Myrtle (71  $\mu g/100gDW)$  (Table 5).

Among fruit samples, Australian Desert Lime contained the highest levels of folate with 420  $\mu g/100~g$  DW, followed by Quandong (120  $\mu g/100~g$  DW), Kakadu Plum, Riberry, Lemon Aspen (110  $\mu g/100~g$  DW) and Davidson's Plum (34-40  $\mu g/100g$ DW) (Table 5). The level of folate in Blueberry is 39.6  $\mu g/100g$ DW (Appendix Table A).



**Bush Tomato** 



Among fruit samples, Australian Desert Lime contained the highest levels of folate

#### Vitamin A

The most common form of vitamin A is retinol (Figure 11). Only precursors of vitamin A (provitamin A) are present in food and these are converted into retinol in the thin intestine.

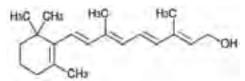


Figure 11. Molecular structure of retinol

The most common provitamin A is  $\beta\text{-carotene}.$  In this study the levels of  $\beta\text{-carotene}$  are recalculated into vitamin A [58]. Tasmannia Pepper Leaf was identified as a sole source of provitamin A with the level 0.2 mg retinol equivalents/100gDW (or 200µg retinol equivalents/100gDW) (Table 5). The level of vitamin A in Blueberry is 19.8 µg retinol equivalents/100gDW (Appendix Table A).



Tasmannia Pepper Leaf was identified as a sole source of provitamin A

## Identification of minerals

Trace elements, beside vitamins, form another group of micronutrients that are essential regulators of physiological processes in humans. Micronutrient deficiency is known to cause specific acute illnesses and may cause predisposition to a number of chronic diseases. Deficiency of micronutrients during pregnancy and childhood are linked to growth problems early in life and to increased incidence of chronic diseases later in life. Fresh fruits and vegetables are important contributors to the supply of minerals in the diet. The levels and composition of minerals in fruits and vegetables are affected by their presence or absence in soils and fertilizers.

This study aimed to establish a systematic inventory of the levels of selected micronutrients that are considered to be critical for the stability of the human genome and other essential physiological processes. Among the minerals essential to maintain DNA are: zinc (Zn), magnesium (Mg) and calcium (Ca). Zinc is required for DNA synthesis and repair; magnesium for DNA synthesis and chromosome segregation and calcium is needed for chromosome segregation [55]. Other minerals evaluated in this study are: iron (Fe),, manganese (Mn), copper (Cu), molibdenum (Mn), selenium (Se), potassium (K) and sodium (Na).

### Magnesium, zinc and calcium

Magnesium (Mg) is a component of the chlorophyll molecule, which is responsible for the green colour in fresh produce. According to the National Health and Medical Research Council, 1991, the recommended daily intake (RDI) of Mg for adults (expressed as mean daily intake) is 270 mg (women) and 320 mg (men) (Appendix Table B). In the US diet about 26% of magnesium comes from fresh fruits and vegetables [46].

Magnesium (Mg) has been identified in all evaluated samples, with Wattleseed, Anise Myrtle and Tasmannia Pepper Leaf containing above 200 mg/100g DW (Table 6, Figure 12A). Similar levels of Mg were identified in Quandong, Davidson's Plum (D. jerseyana) and Kakadu Plum. The content of Mg in all other sources was between 100 and 200 mg/100g DW, with the exception of Australian Desert Lime that contained 94.5 mg/100g DW. The level of Mg in Blueberry is 39.6 mg/100gDW (Appendix Table A).

The recommended daily intake of zinc (Zn) for adults is 12 mg (Appendix Table B). Tasmannia Pepper Leaf was identified as the richest source of zinc, with 6.5 mg/100gDW. It was followed by the Tasmannia Pepper Berry and Wattleseed with the levels of 3.5 and 3.1 mg/100gDW (Table 6, Figure 12B). Bush Tomato, Anise Myrtle and Lemon Myrtle all contain between 1 and 2 mg/100g DW.

Among the evaluated fruits, Quandong and Lemon Aspen contained the highest amounts of Zn, 4.2 and 3.9 mg/100gDW, respectively. The level of Zn in Riberry was approximately 3-fold lower (1.3 mg/100g DW). Australian Desert Lime and Davidson's Plum (*D. jerseyana*) contained 1.1 and 1.0 mg/100g DW respectively and in this

aspect were similar to Blueberry (1.05 mg/100g DW; Appendix Table A).

Calcium is required for chromosome segregation, however most calcium in the body (approximately 98%) is contained in the bones. Ca is essential for bone health and strength. In addition, about 1% of Ca is used for nerve impulses and muscle contractions (including heart, kidney, and other organs) that sustain life and provide movement. The recommended daily intake of Ca for an adult is 800 and 1000 mg for men and women, respectively (Appendix Table B).

Lemon Myrtle was identified as the richest source of calcium with the content of 1583 mg/100g DW (Table 6, Figure 12C). It was followed by the Tasmannia Pepper Leaf and Wattleseed with 495 and 434 mg/100g DW, respectively.



Quandong on tree



Lemon Aspen fruit

Among the evaluated fruits, Quandong and Lemon Aspen contained the highest amounts of zinc.

Table 6. Minerals identified in selected native Australian herbs, spices and fruits The results are presented in mg/100g DW with exception of Se and Mo that are presented as  $\mu g/100g$  DW.

No	Sample	Zn	Mg	Ca	Fe	Se	Р	Na	K	Mn	Cu	Мо	K : Na
Herb/Spice													
1	Tasmannia Pepper B.	3.500	142.2	147.8	5.22	ND	126.0	27.45	1106.8	33.8	0.847	2.3	40.3
2	Tasmannia Pepper L.	6.565	212.1	495.1	11.35	ND	106.3	47.35	837.95	ND	0.6219	3.3	17.7
3	Anise Myrtle	1.440	247.4	261.45	5.86	ND	100.6	51.75	773.3	9.595	0.367	2.6	14.9
4	Lemon Myrtle	1.055	188.4	1583.15	5.77	ND	114.1	19.20	1258.7	1.28	0.474	5.5	65.5
5	Bush Tomato	1.850	160.3	117.05	26.50	6.65	256.5	4.66	2251	1.315	0.732	18.4	483.57
6	Wattleseed	3.105	255.1	434.4	10.90	31.7	227.5	43.90	1147.6	2.955	0.836	25.1	26.1
					F	ruit							
7	Australian Desert Lime	1.060	94.5	384.2	4.74	ND	127.8	2.24	1287.8	0.8775	0.641	7.7	574.9
8	Kakadu Plum	0.574	203.8	282.45	3.99	ND	52.45	10.45	1905.5	3.5	0.303	18.5	182.3
9	Lemon Aspen	3.925	147.6	133.35	13.25	ND	129.0	45.05	1512.9	10.025	0.834	12.9	33.6
10	Davidsonia pruriens	0.426	138.1	217.35	1.24	ND	94.45	1.77	1465.5	19.55	0.638	11.0	828.0
11	Davidsonia jerseyana	0.965	208.7	193.55	2.39	ND	105.5	9.90	1857.4	30.15	0.919	13.7	187.7
12	Quandong (dry)	4.240	217.9	133.3	16.55	ND	96.9	306.05	3456.2	0.288	0.100	55.6	11.3
13	Riberry	1.315	189.0	307.7	4.32	ND	118.8	47.10	1715.7	22.75	1.135	10.7	36.4

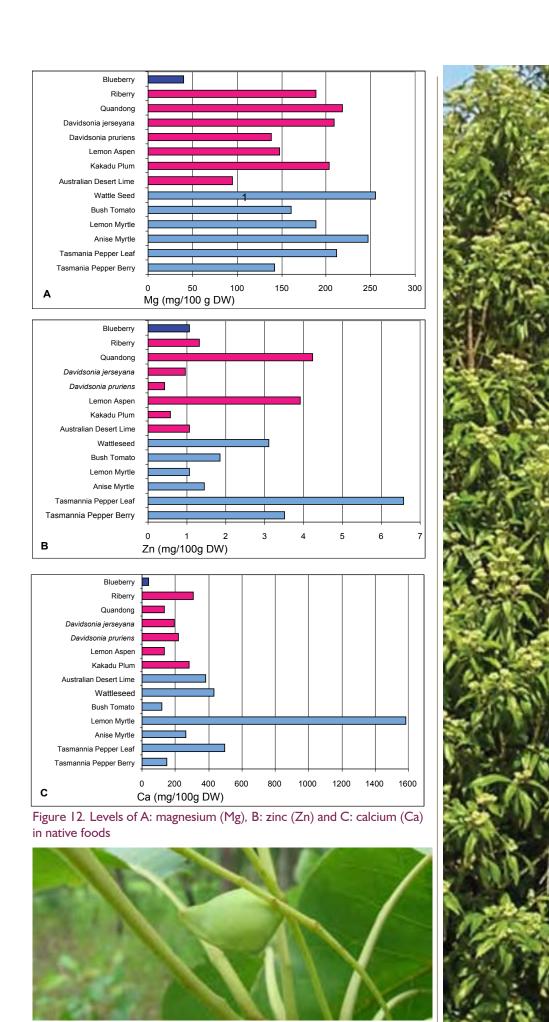
Among the fruits, Australian Desert Lime, Riberry and Kakadu Plum contained 384, 308 and 282 mg/100g DW of Ca, respectively. These levels are significantly higher than that found in Blueberry (39.6 mg/100gDW; Appendix Table A).

The Australian native herbs, spices and fruits evaluated in this study contained all three elements required for the genome health: Mg, Zn and Ca. Among the herbs and spices, Tasmannia Pepper Leaf and Wattleseeds can be considered as the richest sources of these minerals, with Lemon Myrtle being exceptionally rich in Ca and Anise Myrtle rich in Mg. Among the fruits, Quandong is the richest source of Mg and Zn, while Kakadu Plum provides Mg and Ca and Lemon Aspen – Zn.



Riberry





Kakadu Plum was in the group of fruits that had significantly higher calcium levels than Blueberry. (Photo: Robert Dean, Coradji, NT)

 ${\it Lemon Myrtle is exceptionally rich in calcium}$ 

#### Iron

Iron (Fe) functions in the hemoglobin in red blood cells, which transports oxygen from the lungs to the body's tissues, including the muscles and the brain. Iron can be accessed in many different types of food, but only about 10 to 15% is actually absorbed into the body [59]. The uptake of Fe from animal sources (derived from hemoglobine) reaches 15% to 35% [60] and is higher than that from plant foods (2 to 20%) [61]. Meat proteins and vitamin C improve the absorption of iron from plant sources [62]. Tannins (found in tea), calcium, polyphenols with galloyl groups, and phytates (found in legumes and whole grains) can decrease absorption of iron from plants [63, 64, 65]. Some phenolic compounds inhibit iron absorption. Among them are compounds that contain galloyl groups (tannic and gallic acids). Compounds that possess the phenolic catechol groups (e.g. catechin) do not interfere with Fe absorption [64]. Phenolic compounds that possess the catechol group display enhanced antioxidant properties [15]. Proteins found in soybeans may inhibit iron absorption [65]. According to the World Health Organisation, iron-deficiency affects about 48% of children and about 50% of women.

The recommended daily intake of Fe is 7 (men) and 12 – 16 mg (women) (Appendix Table B). Lentils, beans, tofu and spinach are among the richest sources of Fe and provide from 6 to 3 mg per serving [10]. In US diet fresh fruits and vegetables contribute about 19% of the RDI (recommended daily intake) [46].

Bush Tomato was identified as the richest Fe source with 26.5 mg/g DW and was followed by Tasmannia Pepper Leaf (11.3) mg/g DW) and Wattleseed (10.9 mg/g DW) (Table 6, Figure 13). Among fruits, Quandong was the richest source of Fe (16.5 mg/g DW) and was closely followed by Lemon Aspen (13.2 mg/g DW). With the exception of Davidsonia pruriens, the content of Fe in all evaluated sources was higher than in Blueberry (Figure 13).

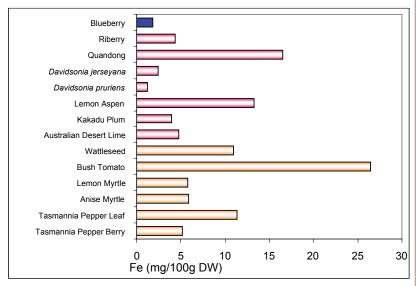


Figure 13. Levels of iron (Fe) in selected native fruits, herbs and spices

#### **Selenium**

Selenium (Se) salts are toxic in large amounts, but trace amounts of the element are necessary for cellular function in humans. Se forms the active center (coenzyme) of antioxidant enzymes, such as the glutathione peroxidise enzyme family, whose role is to protect the organism from oxidative damage. Subsequently, it provides antioxidant benefits and participates in the body's natural defences.

People dependent on food grown from selenium-deficient soil are at risk. In Finland where the levels of Se in soil are exceptionally low, supplementation of fertilizers with Se was found to be an effective and safe way to increase the Se intake nationwide [66]. Areas of Se deficiency were reported in the Northern Tablelands of NSW [67], South-eastern and coastal areas of Queensland [68], Southwestern areas of Western Australia [69], South-eastern areas of SA [70] and in pockets of high rainfall in Victoria & Tasmania [68]. Se toxicity is reported in some areas in Queensland. In North-western Queensland in Australia, soils with high levels of Se are associated with limestone shale.

Vegetation grown on soils receiving the run-off from these limestones produce acute toxicities in livestock [71].

Bush Tomato and Wattleseed were the only two sources that contained selenium at very low levels of 0.0067 and 0.0317 mg/100g DW, respectively (Table 6). The reported level of Se in Blueberry is  $0.66\mu g/100g$  DW or 0.066 mg/100g DW (Appendix Table A).

#### **Manganese**

Manganese (Mn) plays an essential part of proper bone and cartilage formation, and glucose metabolism. Manganese helps build and support strong bones in the body and prevent osteoporiosis. Mn is also involved in nervous and immune systems and regulates blood sugar levels. Overexposure to Mn causes alterations in Fe requiring enzymes present in the human brain (brain aconitase) and develops symptoms similar to those of Parkinson's diseases [72]. The adverse health effects of manganese (Mn) have been associated with organic Mn compounds (pesticides), mining and heavy air pollution. [72].

The recommended daily intake of Mn in the United States is 2mg [73]. Mn is present in milk, however its absorption is negatively affected by the presence of calcium [74]. Tea leaf contains 350–900  $\mu$ g/g DW and 1 litre of tea infusion contains approximately 115% of the average daily dietary intake of Mn. Under simulated intestinal conditions, the bioavailability of Mn from tea infusion reached 40% [75].

Tasmannia Pepper Berry was the richest source of Mn with the level of 33.8 mg/100g DW and was followed by *Davidsonia jerseyana*, Riberry and *Davidsonia pruriens* (30.15, 22.75 and 19.55 mg/100g DW, respectively (Table 6).

#### Molybdenum

Molybdenum (Mo) present in a number of various enzymes is a required element for life in higher organisms, including humans. The recommended daily intake is 75µg [73].

Deficiency of Mo leads to dysfunctional Mo-containing enzymes [76]. Lima bean seeds are the richest source of Mo (4.8 µg/g fresh weight), followed by green peas (3.5), eggs (0.49), spinach (0.26), carrot (0.08) and potatoes (0.03). Mo was not detected in cauliflower and onion [76].

With the exception of Quandong, that contained 0.055 mg (or 55  $\mu$ g) /100g DW, all samples of native Australian spices, herbs and fruits contained Mo at equal to or less than 0.02mg (or 20  $\mu$ g) /100g DW (Table 6).

#### **Potassium**

**Potassium (K)** is an electrolyte that interacts with sodium to conduct nerve impulses and many other functions in cells. In the past, high potassium

foods used to dominate, but through evolution, our diet has become higher in sodium. This reverses the high potassium/low sodium ratio and this reversal contributes to the increase of high blood pressure. Recently, investigations have been undertaken to replace Na with K in food in order to reduce hypertension.

The highest ratio of K:
Na among the evaluated fruit samples was found for Davidsonia pruriens (828),
Australian Desert Lime (575),
Davidsonia jerseyana (188) and Kakadu Plum (182). Bush Tomato contained 483-fold higher level of K than Na and in this respect was the most favourable 'spice' species (Table 6). This quality of native fruits and spices can be utilised in the development of low Na and high K level food products.



The highest ratio of K:Na among the evaluated fruit samples was found for Davidsonia pruriens

#### **Conclusions**

- The levels of antioxidant compounds, vitamins (vitamin E, folate) and minerals (Zn, Mg, Ca, Fe, Mn, Cu and Mo) in the majority of the evaluated sources exceed those in the Blueberry fruit that is recognised for health-enhancing properties.
- Phenolic compounds (hydrophilic compounds) were identified as the main sources of antioxidant activities. However, relatively high antioxidant activities have been detected in lipophilic fractions of Kakadu Plum, Lemon Aspen, Riberry, Davidson's Plum, Australian Desert Lime, Tasmannia Pepper Leaf, Tasmannia Pepper Berry and Lemon Myrtle. Presence of phytochemicals that provide antioxidant activity in both, the hydrophilic and lipophilic environment, suggests more comprehensive protection from oxidative stress and possibly more pronounced health benefits in comparison to commonly consumed fruits that are comprised predominantly of hydrophilic antioxidants.
- Kakadu Plum is the richest source of antioxidant compounds; in addition it contains an exceptionally rich mixture of phenolic compounds, high levels of vitamin C, E, folate, and lutein. It also contains essential minerals, e.g. Mg, Ca, and Zn required for synthesis and self-repair of human DNA, and Fe. The rich composition of phytochemicals and microelements indicates potential significant health properties of Kakadu Plum. Further research is required to identify possible specific health benefits arising from consumption of Kakadu Plum. Application for the development of functional foods and nutraceuticals is envisaged.
- Quandong has been identified as a rich source of phenolic-based antioxidants and in respect to antioxidant capacity it follows Kakadu Plum. Additionally, the fruit contains vitamin E, folate and minerals: Zn, Mg, Ca, Fe and Mo. In respect to the levels of the above mentioned compounds the fruit is superior to Blueberry. These qualities warrant further studies in relation to possible health-enhancing properties.
- Davidson's Plum contains high levels of anthocyanins, natural pigments that are strong antioxidants. It also contains lutein, vitamin E and folate, Zn, Mg, Ca and Mo. A high potassium:sodium (K:Na) ratio was identified.

- The fruit is suitable for processing and a possible application of Davidson's Plum as a source of natural food colorant that enhances health properties of foods could be considered.
- With respect to antioxidant capacity and composition of phytochemicals and essential minerals, Riberry appears to be similar to Blueberry.
- Australian Desert Lime has been identified to contain vitamin C, E and folate as well as lutein and can be promoted as source of these micronutrients.
- Lemon Aspen is a richer source of Zn, Mg, Ca, Fe and Mn than Blueberry.
- Tasmannia Pepper Leaf, Anise Myrtle and Lemon Myrtle are good sources of antioxidant compounds of hydrophilic and lipophilic nature, lutein, folate, vitamin E, vitamin C (Anise Myrtle) and vitamin A (Tasmannia Pepper Leaf). All contain minerals: Zn, Mg and Ca (with Lemon Myrtle being the richest source of Ca) that are required for synthesis and self-repair of human DNA.
- A majority of phenolic compounds present in Tasmannia Pepper Berry possess a catecholbased structure that indicates possible health-enhancing properties of this berry.
- Polyphenolic extract from Tasmannia
   Pepper Leaf is exceptionally rich in
   chlorogenic acid. Further research to identify
   potential health properties of the extract
   and possible application as a supplement
   in the food and/or the cosmeceutical
   industry should be considered.
- Bush Tomato and Wattleseed provide Se, which is necessary for antioxidant enzymes to function and can be promoted as a source of this essential mineral.



Kakadu Plum is the richest source of antioxidant compounds; in addition it contains an exceptionally rich mixture of phenolic compounds, high levels of vitamin C, E, folate, and lutein.

# Implications and recommendations

#### **Implications**

The results of this research yield a range of significant findings, suitable for either immediate or future use (pending confirmatory research), in the health-based marketing of native foods products. The data arising from the research results can be utilised by industry to promote the use of native fruits, herbs and spices for direct consumption and for the development of novel food products.

When products made of native fruits, herbs and spices are considered, further studies need to be conducted to confirm/validate the claims as technological processes may significantly alter the levels of evaluated compounds.

A quality assurance program to ensure the validity of any nutrient content claims across different batches of the same plant source, or significant additional confirmatory research would be required. In particular, research will be required for the further substantiation of results, where the nutrient or functional property was at an early stage of establishing its role in maintaining health, e.g. antioxidant capacity evaluated only in reagent-based assays.

Bioavailability of nutrients is a prerequisite of the health effect arising from its consumption. Further studies will be required to confirm accessibility and bioavailability of nutrients from food matrices.

Finally, disseminating the knowledge about healthenhancing properties of native species will change public perception towards consumption of native fruits, herbs and spices and their products and will create a market demand. This should strengthen the position of the Australian Native Food Industry in domestic and international markets.

#### Recommendations

Information obtained within this project can be used to promote and market products that are developed from the native plants evaluated in this study. Compositional nutrient data, levels of antioxidants (both hydrophilic and lipophilic), vitamins C, E and folate, provitamin A, as well as lutein, can be utilised by the industry to promote the evaluated plant species, provided that quality assurance across different batches of the same product follows. Additional research will be required for the further substantiation of possible health effects related to antioxidant testing data.

Kakadu Plum has been identified as the richest source of antioxidant compounds of hydrophilic and lipophilic nature. Moreover, it contains exceptionally high levels of vitamin C, as well as vitamin E, folate, and lutein. It is also a good source of minerals required for genome health (Mg, Ca, and Zn) as well as Fe. This rich composition

of phytochemicals and microelements in Kakadu Plum indicates possible significant health properties. Further research is recommended to explore these properties. If positive findings are made, application of Kakadu Plum as a component of functional foods and nutraceuticals might follow. Scientifically proven application of Kakadu Plum in functional food/pharmaceutical industries would place the industry at the forefront of the utilization of native plants to maintain the nation's health.

High antioxidant capacity of Quandong and Tasmannia Pepper Leaf combined with the presence of vitamin E, folate and essential minerals warrants further studies towards identification of potential health-enhancing properties.

Davidson's Plum is suitable for large scale processing. It is rich in anthocyanins. Potential evaluation of the plum as a source of natural food colorant that enhances antioxidant status of foods is recommended.

Finally, results obtained in this study should be utilised to create an internet deployed database on composition and biological effects of health-promoting phytochemicals and microelements present in native Australian plants. This database should be created and maintained by CSIRO or ANFIL and should be compatible with the European Food Information Resource Network (www.eurofir.net).

# Glossary

C 3-G	cyanidin 3-glucoside
СНА	chlorogenic acid
E number	number codes for food additives (found on food labels throughout the European Union. The numbering scheme follows that of the <b>International Numbering System</b> (INS) as determined by the <b>Codex Alimentarius</b> committee. E numbers are also encountered on food labelling in other jurisdictions, including the <b>GCC</b> , <b>Australia</b> , <b>New Zealand</b> and <b>Israel</b> . The "E" prefix is omitted in Australia and New Zealand. They are increasingly (though still rarely) found on North American packaging.
FRAP	Ferric Reducing Antioxidant Power
GA	gallic acid
HPLC	High Performance Liquid Chromatography
HPLC-DAD	HPLC equipped with diode array detector
LC-PDA-MS/MS	Liquid Chromatography Mass Spectrometry equipped with a photo-diode array detector
m/z	mass-to-charge ratio
MeOH	methanol
MS	mass spectrum (an intensity vs. m/z (mass-to-charge ratio) plot representing a chemical analysis)
MTBE	Methyl t-butyl ether
ORAC	Oxygen Radical Scavenging Capacity
PTFE	Polytetrafluoroethylene (PTFE) membrane (used for a variety of filtration applications)
R	rutin
ROS	reactive oxygen species
SRM	selected reaction monitoring
TFA	Triflouroacetic acid
ТР	Total Phenolics (the amount of phenolic compounds, expressed as gallic acid (GA), in 1 g dry (or fresh) weight of sample.
TPTZ	2,4,6-tripyridyl-s-triazine

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# Appendices

## Appendix Table A. Levels of selected micronutrients in Blueberry (Vaccinium spp.)

Nutrient and units	Blueberry, raw (100g FW)	Blueberry, raw (100g DW)*	Blueberry, frozen (100g FW)
Vitamin C (mg)	9.7	64.6	2.5
Vitamin A (µg retinol eq.)	3	19.8	2
Vitamin A (IU)	54	356.4	46
Lutein (µg)	80	528	68
Vitamin E (mg α-tocopherol equivalents)	0.57	3.762	0.48
Folate, total (µg)	6	39.6	7
Vitamin B-12 (mg)	0	0	0
Calcium (mg)	6	39.6	8
Iron (mg)	0.28	1.848	0.18
Magnesium (mg)	6	39.6	5
Phosphorus (mg)	12	79.2	11
Potassium (mg)	77	508.2	54
Sodium (mg)	1	6.6	1
Zinc (mg)	0.16	1.056	0.07
Copper (mg)	0.057	0.3762	0.033
Manganese (mg)	0.336	2.217	0.147
Selenium (µg)	0.1	0.66	0.1

<sup>\*</sup>Values recalculated into dry weight based on 15% average dry weight for Blueberry. Source: http://www.nal.usda.gov/fnic/foodcomp/search/ retrieved May 15, 2009.

# Appendix Table B. Recommended daily intakes (RDIs) for adults (expressed as mean daily intake) of micronutrients

(National Health and Medical Research Council, 1991).

Nutrient and units	Men	Women
Vitamin A (µg retinol equivalents)	750	750
Thiamin (mg)	1.1	0.8
Riboflavin (mg)	1.7	1.2
Niacin (mg niacin equivalents)	19	13
Vitamin B-6 (mg)	1.3-1.9	0.9-1.4
Folate (µg)	200	200
Vitamin B-12 (mg)	2.0	2.0
Vitamin C (mg)	40	30
Vitamin E (mg α-tocopherol equivalents)	10.0	7.0
Zinc (mg)	12	12
Iron (mg)	7	12-16
lodine (μg)	150	120
Magnesium (mg)	320	270
Calcium (mg)	800	1000
Phosphorus (mg)	1000	1000
Selenium (µg)	85	70
Sodium (mg)	920-2300	920-2300
Potassium (mg)	1950-5460	1950-5460

#### Ranking of plant species based on antioxidant capacity, content of phenolic compounds and vitamins

#### Herb/Spice category

#### Appendix Table C. Ranking of Australian herbs/spices based on antioxidant capacity, content of phenolic compounds and vitamins [Numbers represent the level of micronutrient per 100 gram dry weight (mg or $\mu$ g/100 g DW)].

Rank	Antioxida	nt capacity	Content	(per 1gDW )	Content (per 100 gDW)				
	ORAC	FRAP	TP	Anthocyanin (mg C3GEq/ gDW)	Lutein (mg)	Vit A (µg RI)	Vit C (mg)	Vit E (mg)	Folate (µg)
1	TPL	AM	TPL	TPB	AM	TPL	AM	AM	TPL
	4077.1*	2158.0	102.1	55.2	20.9	200	66.7	59.7	160
2	LM	TPL 1314.5	AM	В	LM	В	В	LM	AM
	3359.9		55.9	38.9	6.6	19.8	64.6	21.2	100
3	AM	LM	В	TPL	TPL 1.6			TPL	BT
	2565.8	1225.3	35.4	1.2				17.8	100
4	TPB	В	LM		В			WS	WS
	956.4	397.1	31.4		0.53			5.3	100
5	BT	TPB	TPB					BT	TPB
	931.3	332.9	16.9					4.6	87
6	В	BT	BT					В	LM
	436.8	206.2	12.4					3.76	71
7	WS	WS	WS					TPB	В
	61.54	17.8	0.8					1.2	39.6
RDI♦						750	40	10	200
Man									
RDI♦ Woman						750	30	7	200

ORAC -Oxygen Radical Absorbance Capacity (µmol TEq/gDW) FRAP -Ferric Reducing Antioxidant Power (µ mol Fe<sup>+2</sup>/g DW)

TP total phenolic compounds (mg GA Eq/g DW)

TPB -Tasmannia Pepper Berry TPL -Tasmannia Pepper Leaf

Anise Myrtle AM – LM -Lemon Myrtle **Bush Tomato** BT – WS -Wattleseed Blueberry B –

<sup>◆</sup> According to: National Health and Medical Research Council, 1991

<sup>\*</sup>Numbers represent the level of activity of micronutrient

#### **Fruit category**

#### Appendix Table D. Ranking of Australian fruits based on antioxidant capacity, content of phenolic compounds and vitamins Numbers represent the level of micronutrient per 100 gram dry weight (mg or µg/100 g DW).

	Antioxida	nt capacity	Content (	per 1gDW)	Content (per 100 gDW)					
Rank	ORAC	FRAP	TP	Anthocyanin (mg C3GEq/ gDW)	Lutein (mg)	Vit C (mg)	Vit E (mg)	Folate (µg)		
1	KP 2511.5	KP 4032.5	KP 158.6	DP <i>j</i> 98.7	KP, ADL 1.5	KP 7252.8	KP 6.1	ADL 420		
2	Q 2028.0	DPp 670.7	B, Q 35.0	DP <i>p</i> 47.8	DP <i>j</i> 1.4	ADL 962.3	Q 4.8	Q 120		
3	DP <i>p</i> 11928	DP <i>j</i> 599.8	DP <i>p</i> 18.2	R, B 35.3	DP <i>p</i> 1.15	B 64.6	ADL 4.0	KP, R, LA 110		
4	LA 1192.6	Q 454.9	R, DP <i>j</i> 14.0	Q 0.5	B 0.53		B 3.8	DP <i>p</i> 40		
5	DP <i>j</i> 900.3	B 397.1	LA 10.5				DP <i>j</i> 2.9	B 39.6		
6	R 817.2	R 376.9	ADL 9.4				R 2.6	DP <i>j</i> 34		
7	B 436.8	ADL 177.8					LA 1.9			
8	ADL 249.4	LA 90.2					DP <i>p</i> 1.2			
RDI♦ Man						40	10	200		
RDI♦ Woman						30	7	200		

ORAC -Oxygen Radical Absorbance Capacity (µ mol TEq/gDW) Ferric Reducing Antioxidant Power (µmol Fe<sup>+2</sup>/g DW) FRAP -

ADL -Australian Desert Lime

KP – Kakadu Plum LA – Lemon Aspen DPp – Davidsonia pruriens DPj – Davidsonia jerseyana

Q – Quandong R-Riberry Blueberry

<sup>\*</sup>According to: National Health and Medical Research Council, 1991

#### Ranking of plant sources based on content of minerals

#### Herb/Spice category

## Appendix Table E. Ranking of Australian herbs/spices based on content of minerals

Numbers represent the level of micronutrient per 100 gram dry weight (mg or µg/100 g DW).

Rank	Zn	Mg	Ca	Fe	Se	Р	Na	K	Mn	Cu	Мо
	(mg)	(mg)	(mg)	(mg)	(µg)	(mg)	(mg)	(mg)	(mg)	(mg)	(µg)
1	TPL	WS	LM	BT	WS 31.7	BT 256.5	AM	BT	TPB 33.8	TPB 0.85	WS
	6.56	255.1	1583.1	26.5			51.7	2251			25.1
2	TPB	AM	TPL	TPL	BT 6.65	WS	TPL	LM	AM	WS	BT
	3.50	247.4	495.1	11.4		227.5	47.3	1259	9.6	0.84	18.4
3	WS	TPL	WS	WS	В	TPB	WS	WS	WS	BT	LM
	3.10	212.1	434.4	10.9	0.66	126.0	47.3	1148	3.0	0.73	5.5
4	BT	LM	AM	AM		LM 114.1	TPB	TPB 1107	В	TPL	TPL
	1.85	188.4	261.5	5.9			27.4		2.2	0.62	3.3
5	AM	BT	TPB	LM		TPL	LM	TPL	BT	LM	AM
	1.44	160.3	147.8	5.8		106.3	19.2	838	1.32	0.47	2.6
6	B, LM	TPB	BT	TPB		AM 100.6	В	AM	LM	В	TPB
	1.05	142.2	117.1	5.2			6.6	773	1.30	0.38	2.3
7		В	В	В		В	BT	В	TPL	AM	
		39.6	39.6	1.8		79.2	4.7	508	ND	0.37	
RDI♦	12	320	800	7	85	1000	920-2300	1950-	320		75*
Man								5460			
RDI♦	12	270	1000	12-16	70	1000	920-2300	1950-	270		75*
Woman								5460			

TPB – Tasmannia Pepper Berry

TPL - Tasmannia Pepper Leaf

AM – Anise Myrtle

LM – Lemon Myrtle

BT – Bush Tomato

WS - Wattleseed

B - Blueberry

<sup>◆</sup> According to: National Health and Medical Research Council, 1991

<sup>\*</sup> According to: Pennington and Hubbard, 1997 [73]

#### **Fruit category**

## Appendix Table F. Ranking of Australian fruits based on content of minerals

Numbers represent the level of micronutrient per 100 gram dry weight (mg or  $\mu$ g/100 g DW).

Rank	Zn (mg)	Mg (mg)	Ca (mg)	Fe (mg)	P (mg)	Na (mg)	K (mg)	Mn (mg)	Cu (mg)	Mo (μg)
1	Q	Q	ADL	Q	LA	Q	Q	DP <i>j</i>	R	Q
	4.24	217.9	384.2	16.5	129.0	306.1	3456	30.15	1.14	55.6
2	LA	DPj	R	LA	ADL	R	KP	R 2	DPj	KP
	3.93	208.7	307.7	13.3	127.8	47.1	1905	2.8	0.92	18.5
3	R	KP	KP	ADL	R	LA	DP <i>j</i>	DPp	LA	DP <i>j</i>
	1.31	203.8	282.5	4.7	118.8	45.1	1857	19.55	0.83	13.7
4	ADL	R	DPp	R	DP <i>j</i>	KP	R	LA	ADL	LA
	1.06	189.0	217.4	4.3	105.5	10.5	1715	10.1	0.64	12.9
5	В	LA	DP <i>j</i>	KP	DPp	DP <i>j</i>	LA	KP	DPp	DPp
	1.05	147.6	193.6	4.0	94.5	9.9	1513	3.5	0.63	11.0
6	DP <i>j</i>	DPp	Q	DP <i>j</i>	Q	В	DPp	В	В	R
	0.96	138.1	133.3	2.4	96.6	6.6	1466	2.2	0.38	10.7
7	KP	ADL	LA	В	KP	ADL	ADL	ADL	KP	ADL
	0.57	94.5	133.4	1.8	52.5	2.2	1288	0.9	0.30	7.7
8	DPp	В	В	DPp	В	DPp	B 508	Q	Q	
	0.43	39.6	39.6	1.2	79.2	1.8		0.3	0.10	
RDI <b>♦</b> Man	12	320	800	7	1000	920-2300	1950-5460	320		75*
RDI <b>♦</b> Woman	12	270	1000	12-16	1000	920-2300	1950-5460	270		75*

ADL – Australian Desert Lime

KP – Kakadu Plum

A – Lemon Aspen

DPp – Davidsonia pruriens

DPj – Davidsonia jerseyana

Q – Quandong

R – Riberry

B - Blueberry

<sup>◆</sup> According to: National Health and Medical Research Council, 1991

<sup>\*</sup> According to: Pennington and Hubbard, 1997 [73]

#### **Health Benefits of Australian Native Foods**

An evaluation of health-enhancing compounds

by I. Konczak, D Zabaras, M Dunstan, P Aguas, P Roulle and A Pavan

RIRDC Pub. No. 09/133

This RIRDC research was developed to provide the native food industry with reliable information on the levels of health beneficial constituents and the antioxidant capacities of commercially significant native fruits, herbs and spices.

This report represents the first systematic evaluation of antioxidant capacity, and the identification of its sources; the presence of potentially bioactive phytochemicals (phenolic compounds and carotenoids) and selected vitamins. It also includes an evaluation of minerals, focusing especially on those that protect human DNA against mutations that can lead to the development of a range of chronic diseases.

Native species evaluated in this study exhibited superior antioxidant capacity as compared to the Blueberry standard, renowned worldwide as the 'health-promoting fruit.' In comparison to commonly consumed fruits that comprise predominantly hydrophilic antioxidants, native foods contained antioxidant activity in both hydrophilic and lipophilic fractions. This suggests more comprehensive protection from oxidative stress, and possibly more pronounced health benefits.

All of the evaluated plant species were found to contain vitamin E and folate. Rich sources of lutein, a compound essential for eye health are also present, as were magnesium, zinc and calcium, all important for the synthesis and selfrepair of human DNA. Sources of valuable selenium were also identified.



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