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Original article

Proximate and phenolic composition of selected native Australian food plants

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Summary Edible portions of *Leucopogon parviflorus*, *Arthropodium strictum*, *Carpobrotus rossii*, *Rhagodia candolleana*, *Typha orientalis*, *Correa alba*, *Dianella revoluta* and *Acacia longifolia* were collected in 2017 and 2019 from Warrnambool, Victoria Australia, and studied for phenolics and proximate composition. The plant species recorded consistent antioxidant activity with no nutritional alterations. Except for *A. longifolia*, total phenolic contents (TPC) were higher in 2019 than 2017 ($P < 0.05$). All the plant species contained Ca, Mg, Na and K. Except for betanin, isolated from *R. candolleana* (700 mg L^{-1}) and *C. rossii* (244 mg L^{-1}), higher quantities of polyphenols were extractable in methanol than water ($P < 0.05$). The plant fatty acid composition was predominantly palmitic, oleic and linoleic acids with *A. longifolia* containing 44% linoleic acid. While the plant nutrients and fatty acids were fairly stable, TPCs, some of the minerals and polyphenols varied between the years.

Keywords Antioxidants, functional properties, Indigenous Australians, mineral analysis, native plants, phenolic content.

Introduction

Australia is known for its rich biodiversity (Horwitz *et al*., 2008) and edible wild plant species that are endowed with remarkable ability to survive harsh environmental conditions (Cock, 2013). *Xanthorrhoea johnsonni* A. T. Lee, one of Australia's endemic species, for example, has been known to survive for more than five centuries (Cock & Kalt, 2012), while *Lomatia tasmanica* W.C. Curtis. is known as one of the world's oldest plant species (Lynch & Balmer, 2004) and *Terminalia ferdinandiana* Exell is reported as the world's richest source of vitamin C (Konczak *et al*., 2010; Njume *et al*., 2019). The country is known for changing climatic conditions with some regions unfavourable for human habitation and survival of its rich biodiversity. Despite these changes, many native food plant species relied upon by members of the Indigenous community have continuously thrived and survived (Clarke, 2007), with rich polyphenol and antioxidant contents, some of which have exhibited useful therapeutic properties *in vitro*; extracts of the native Australian bush fruit Illawarra plum (*Podocarpus elatus* Endl.) have been shown to reduce the proliferation of colon cancer cells by altering the cell cycle, increasing apoptosis and possibly inducing autophagy (Symonds *et al*., 2013). The active ingredients in Illawarra plum therefore may have the potential to provide an alternative chemopreventive strategy to conventional chemotherapy. Some native Australian plant species including cinnamon myrtle (*Backhousia myrtifolia* Hook. & Harv.), anise myrtle (*Syzygium anisatum* (Vickery) Craven & Biffen), lemon myrtle (*Backhousia citriodora* F. Muell) and Tasmannia pepper leaf (*Tasmannia lanceolata* R. Br.) with rich antioxidant and anti-inflammatory polyphenols are reported as potential sources of lead molecules for production of anti-inflammatory drugs (Guo *et al*., 2014; Rupesinghe *et al*., 2016). There is reason to believe therefore that such species that have survived unfavourable climatic conditions over the years and have accumulated a huge amount of bioactive compounds would likely have good nutritional and health benefits within Australian Indigenous communities. This philosophy is stimulated by the historical fact that native plant species have provided a primary source of food and general well-being for Australia's Aboriginal population for thousands of years (Brand-Miller & Holt, 1998).

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Some of the species include *Leucopogon parviflorus* (Andrews) Lindl (native currant), *Arthropodium strictum* R. Br. (chocolate lily), *Carpobrotus rossii* (Haw.) Schwantes (pig face), *Rhagodia candolleana* Moq. subsp. *candolleana* (seaberry saltbush), *Typha orientalis* C. Presl (water cumbungi or bulrush), *Correa alba* Andrews (white correa or cape barren tea), *Dianella revoluta* R. Br. (black anther flax lily) and *Acacia longifolia* subsp. *sophorae* (Labill.) Court (coast wattle). Knowledge regarding their application is still fresh in the minds of the elders of martang Djab Wurrung Country, an Indigenous community in the Western region of the state of Victoria in Australia.

With the coming of modernity and industrialisation, many communities in Australia have moved away from traditional staples to 'convenience energy-dense foods' in a nutrition transition era that is believed to be responsible for the high rate of noncommunicable diseases within the community (Naughton *et al.*, 2015). According to the Australian Institute of Health and Welfare (AIHW, 2016), 69% of adult Indigenous Australians are either overweight or obese (Vos *et al.*, 2009; Stevens *et al.*, 2018). Sedentary lifestyles and consumption of diets low in fruits and vegetables are some of the contributing factors to Australia's obesity problem (Njume *et al.*, 2019; Sevoyan *et al.*, 2019). This partly explains why Indigenous Australians who are overweight or obese may be more likely to also have type 2 diabetes mellitus (T2DM) than the rest of the population (de Abreu, Walker, & Gilbert, 2013). The economic, health and social effects of overweight and obesity including increased risk of cardiovascular disorders and T2DM accompanied by reduction in quality of life are becoming increasingly disturbing. Indigenous food plants are now being considered as possible sources of raw materials, nutrients and antioxidant polyphenols for the production of healthier food options. The species selected for this study are still of value to the Indigenous community, especially the older generation yet they are largely ignored by the younger generation due to changing social/dietary habits. The people of martang Djab Wurrung community continue to use and cherish these traditional foods despite popularity and availability of generic energy-dense convenience products. In this community, the white part of the underground stem and rhizomes of *T. orientalis*, a member of the family Typhaceae, is harvested in spring or summer, peeled, roasted, baked or boiled and consumed (Gott, 1999). Owing to its semiaquatic nature, *T. orientalis* is readily available from ponds, standing water and wetlands. The seeds of *A. longifolia*, fruits of *C. rossii*, berries of *L. parviflorus* and *R. candolleana* subsp. *candolleana* are harvested along the Victorian coastline and consumed directly while tubers of *A. strictum* are usually baked in hot ash or roasted before consumption (Ee & Yates, 2013). The round blue-purple fruits of *D. revoluta*, a popular ornamental and understory plant (Duncan *et al.*, 2004), are collected and consumed directly while the hairy thick leaves and shoots of *C. alba* are collected, dried and used as a tea substitute. Some members of this community believe that consuming these species is a healthier and more nutritious choice than fast foods. Despite such beliefs, a literature search of the nutritional and bioactive composition of many of the species generated scanty information, most of which has not been investigated scientifically except for studies conducted on the halophyte *C. rossii* by Pirie *et al.* (2013) and Pirie *et al.* (2014) which indicated that the plant crude extracts possess high *in vitro* antioxidant and *in vivo* low-density-lipoprotein-lowering activities. However, details of the plant bioactive compounds responsible for the above-mentioned characteristics remain unknown. This study therefore investigates the bioactive and nutritional quality of selected plant species in an attempt to highlight potential sources of raw materials for production of healthier food options within Australian Indigenous communities.

Materials and methods

Collection and preparation of samples

A permit to collect native plant species for this study was obtained from the Department of Environment, Land and Water Planning. In February 2017 and 2019, with the help of the elders of martang Djab Wurrung Indigenous community, edible portions of eight plant species were collected. The white part of the underground stems and rhizomes of *T. orientalis* were collected by uprooting the species from a group of the plants growing close together around a pond while tubers of *A. strictum* were harvested by digging up the plant from the ground and sniping the tubers from the stems with a pair of scissors. The tubers were collected from individual plants of the same species growing about a metre from each other. The black seeds of *A. longifolia* were collected from a group of individual plants of the same species growing next to each other along Warrnambool beach and fruits of *C. rossii* commonly known as karkalla were harvested from different clusters of the same plant species in the same area. The white-coloured berries of *L. parviflorus* were collected from a single mainland plant, 2 km from the beach while the greyish hairy leaves of *C. alba* and the glossy dark red berries of *R. candolleana* were collected from the Correa heath and sea berry salt bush, respectively, along the foot path of the beach of Warrnambool. The blue/purple berries of *D. revoluta* were collected from individual plants of the species growing about a metre from each other at Tower Hill, Warrnambool, Victoria Australia. About

300 g of edible plant material was collected for each species. It is important to note that approval to collect *D. revoluta* and *C. alba* was only granted in 2019 by the Indigenous Elders and even though we had a government permit to collect the samples, their approval was still required before collection. Berries of *L. parviflorus* were not collected in 2019 due to scarcity. Pictures of the plant species were also taken. Samples were collected in zip lock bags, labelled with date, name, parts and place of collection. The samples were transported on ice in a cooler box to Victoria University food laboratory, Werribee West Campus. The samples were identified using descriptions gathered at the time of collection and comparison of photographs and plant location to entries in the reference website of Flora of Victoria (https://vicflora.rbg.vic.gov.au/flora/search). The plant species were authenticated with assistance from a botanist at Victoria University and vouchers were prepared for future reference. Samples were cleaned by removing dirt, soil, residue and debris from other plant species and then washed with distilled water. Each of the plant material collected was subsampled while the rest of the material was stored at −80 °C for long-term storage.

Determination of moisture content

Moisture content was determined gravimetrically by oven- and freeze-drying according to standard procedures (AOAC, 2002; Donkor *et al.*, 2012; Al-Abdulkarim *et al.*, 2013). Fresh plant material, 25 g was weighed and placed in an oven (TED-66F; Thermoline Scientific, Wetherill Park, NSW, Australia) set at 60 °C until constant weight was obtained. The same amount of each fresh plant material was weighed, rapidly frozen in liquid nitrogen at −196 °C and freeze-dried (Dynavac FD 300; Airvac, Rowville, Vic., Australia) for 48 h. The procedure was performed in triplicate. Differences in weight between fresh and dried samples were recorded and used to compute the percentage moisture for each plant.

Determination of ash

Ash content of selected species was determined gravimetrically by burning the samples in a muffle furnace (N759; Labec, Marrickville, NSW, Australia) at 600 °C for 5 h according to the method of Ee & Yates (2013), with modifications. Exactly 15 g of plant material was separately weighed into heat-resistant crucibles. The crucibles were placed over a bunsen flame in a class 2 biosafety cabinet, and the samples were ignited and burnt until no smoke (removing as much soot as possible). Burning was then ceased and crucibles were allowed to cool at room temperature before placing in the muffle furnace. After 5 h, the furnace was switched off, allowed to cool for 2 h, and the samples weighed and recorded.

Determination of crude fat content

Crude fat content was determined by Soxhlet extraction following standard procedures (AOAC, 1984; Bhattacharjee *et al.*, 2013). Briefly, 3 g of dried plant material was ground into powder and placed in labelled thimble filters (MS CET; MicroAnalytix Pty Ltd, Taren Point, NSW, Australia). The filters were placed in 250-mL boiling flask (with known weight) containing 100 mL of petroleum ether (Sigma-Aldrich, Castle Hill, NSW, Australia). The flasks were heated at 50 °C and refluxed on Soxhlet apparatus for 5 h. The thimble filters were carefully removed. The extracts were concentrated to dryness using a rotary evaporator (EYELA; Tokyo Rikakikai Co., LTD, Japan), allowed to dry in a class 2 biosafety cabinet for 48 h with the fan on for complete evaporation of residual solvents and then weighed. The % fat for each sample was calculated as follows (Bhattacharjee *et al.*, 2013):

$$\text{Fat}\,(\%) = (\text{wt. of fat}/3) \times 100.$$

Determination of crude protein

The Kjeldahl method of protein determination involving digestion, distillation and titration (AOAC, 2002; Magomya *et al.*, 2014) was employed to determine the amount of protein in the selected species. Exactly 1 g of freeze-dried ground sample was wrapped in non-nitrogen containing filter paper and weighed into Kjeldahl digestion tube. This was followed by addition of copper sulphate ($CuSO_4$) tablets, (digestion catalyst) and 12.5 mL concentrated sulphuric acid (H_2SO_4). A few antibumping granules were also added into the tube, and digestion was performed at 420 °C for 1 h. A control tube with non-nitrogen containing filter paper, $CuSO_4$ tablets, 12.5 mL H_2SO_4 acid and antibumping granules only was used as a blank. After digestion, the tubes were allowed to cool at room temperature for 10 min. A total amount of 50 mL Milli-Q water was added to the tubes and connected to the distillation unit. Exactly 60 mL of 45% sodium hydroxide solution was added to the digestion tube, and ammonia was steam distilled for 5 min into a 250-mL conical flask containing 25 mL of 4% boric acid and indicator (0.1% methyl blue and 0.3% methyl red in ethanol). The resulting distillate (green in colour) was titrated against 0.1 M HCl acid with methyl red as an indicator. The volume of acid recorded for neutralisation was used to calculate percentage nitrogen and crude protein in the sample as per the equation below (Mæhre *et al.*, 2018):

$$\text{Percentage nitrogen (N)} = \frac{(\text{mLstandardacid} - \text{mLblank}) \times \text{molarityofacid} \times 1.4007}{\text{Weight of sample in grams}},$$

$$\text{Crude protein} = \% \text{nitrogen} \times 6.25.$$

Determinations were repeated twice; means and standard deviations (SD) were computed and recorded.

Determination of total dietary fibre

The determination of the total dietary fibre (TDF) of the species was performed in accordance with standard procedures of AOAC 985.29 with modifications (McCleary *et al.*, 2015). Briefly, duplicate dried and homogenised plant materials were weighed 1 g each into separate conical flasks containing 100 mL distilled water and 50 μL of heat stable α-amylase (Sigma-Aldrich) per flask. The flasks were then heated at 100 °C in a water bath (BTC 9090; Thermoline) for 15 min with intermittent shaking at 5-min intervals. The flasks were cooled to room temperature followed by addition of 10 mL 0.275 M NaOH solution and the pH was adjusted to 7.5. Exactly 100 μL of aminopeptidase (to breakdown proteins) was added to each flask and incubated at 60 °C for 30 min. The flasks were then cooled to room temperature and 10 mL of 0.325 M HCl was added to adjust the pH to 4.5. This was followed by the addition of 200 μL of amyloglucosidase (to convert starch to glucose) and incubated for 30 min at 60 °C in a shaker incubator (Victoria, Australia) at 10 ***g***. An aliquot of 280 mL 95% preheated ethanol at 60 °C was added to each flask to precipitate soluble fibre and remove depolymerised protein and glucose. The flasks were allowed to stand at room temperature for 1 h for precipitation to form. The precipitate was washed three successive times with 20 mL portions of 78% ethanol and filtered on a celite-fitted glass under suction/wash followed by two 10 mL portions of 95% ethanol washes and then finally with two 10 mL portions of 95% acetone. The residues were dried overnight at 105 °C in a hot air oven (SEM; Labquip Technologies Pty Ltd, Ferntree Gully, Vic., Australia), cooled in a desiccator and weighed. One residue was analysed for ash and another for protein. The TDF was calculated as the weight of the residue minus weight of protein plus ash.

Determination of total carbohydrates

Total carbohydrates were determined spectrophotometrically by using the anthrone method described by Ohemeng-Ntiamoah & Datta (2018), with slight modifications. Briefly, 0.1 g of dried ground plant material was weighed into boiling tubes and reducing sugars extracted with 2 × 5 mL aliquots of 80% ethanol. The mixture was filtered using filter paper No. 1 (pore size between 5 and 10 μm). The filtrate was reserved for analysis of simple sugars and to the residue was added 5 mL of 2.5 N HCl for extraction of starch. The tubes were heated in a water bath at 100 °C for 30 min, cooled to room temperature and neutralised by adding solid sodium carbonate until effervescence was ceased. The volume of each tube was made up to 100 mL by adding Milli-Q water and centrifuged at 4000 ***g*** for 5 min. A volume of 1.0 mL was collected from the supernatant and used for analysis of starch. A standard solution of glucose at 20, 40, 60, 80 and 100 mg L^{-1} was prepared for generation of a standard curve (plotting absorbance against concentration). Anthrone reagent (0.1%) was made fresh by adding 0.1 g of anthrone to 100 mL of ice cold 95% H_2SO_4. To each 1 mL sample, 3 mL of freshly prepared anthrone solution was added, vortexed for 3 s and heated for exactly 11 min at 100 °C in a water bath. The tubes were cooled rapidly to 0 °C by putting in ice bath for 5 min and diluted 10 times with Milli-Q water, and absorbance of the greenish-coloured solution was measured at 630 nm (against water) within an hour. Total carbohydrates were computed using the linear equation generated from the standard glucose curve and were the sum total of the value obtained from simple sugars and starch. The experiment was performed in triplicate.

Determination of mineral content

Total of ten minerals (Na, K, Ca, Mg, Fe, Zn, Cu, Cr, Se, and Mn) were determined in the plant samples using inductively coupled plasma spectrometry (ICP, ICPE-9000; Shimadzu Corporation, Kyoto, Japan) according to standard procedures (Antonious *et al.*, 2011). Exactly 1 g of sample ash was weighed into a conical flask, and 10 mL concentrated nitric (HNO_3) acid was added. The mixture was gently swirled, sealed with aluminium foil and allowed to digest overnight at room temperature. The mixture was heated for 1 h at 160 °C in an oil bath (Ratek; Ratek Pty Ltd, Boronia, Vic., Australia), cooled to room temperature and then diluted with 50 mL Milli-Q water. The mixture was filtered through cellulose filter paper No. 1 (pore size of between 5 and 10 μM). The filtrate was tenfold serially diluted and the minerals detected by spectrometry

using Shimadzu plasmic atomic emission spectrometer (ICPE-9000; Shimadzu Corporation). The sample minerals were tested and quantified by use of standard curves generated from a set of serially diluted reference standards of Ca, Mg, Na, K, Fe, Cu, Zn, Mn, Cr and Se (r-value = 0.999).

Extraction of phenolic compounds

Ten grams of homogenised freeze-dried plant material was soaked in 100 mL of 80% hexane in a conical flask. The flask was placed in a shaker incubator (MAXQ 4450; Thermo Scientific, Scoresby, Vic., Australia) set at 2 ***g*** for 24 h at 30 °C. Aqueous extracts were prepared by soaking the same amount of plant material in water and placed in the same incubator. The extracts were centrifuged (Avant J-26S XPI, Indianapolis, IN, USA) at 4000 ***g*** for 30 min. The supernatants of hexane were concentrated to dryness using a rotary evaporator (EYELA; Tokyo Rikakikai Co., LTD, Koishikawa Bunkyo) while the aqueous extract was rapidly frozen in liquid nitrogen at −196 °C and concentrated by freeze-drying. The procedure was repeated three times with new solvent before sampling the plant material a second time. Plant material used in the hexane extraction was spread in a biosafety class 2 cabinet for a few hours to allow the solvent to evaporate and then used for the methanol extraction following the same procedure (Massaud *et al.*, 2017). The resulting plant crude extracts of each solvent were combined and left in a biosafety class 2 cabinet with the fan on for 48 h to ensure complete evaporation of residual solvents while water was removed by freeze-drying.

Determination of antioxidant capacity by DPPH (2, 2-diphenyl-1-picrylhydrazyl) assay

This was performed according to the method of Sommano *et al.* (2013) with slight modifications. Briefly, 3.9 mL DPPH (Sigma-Aldrich) solution (0.075 mM in methanol) was added into test tubes containing 100 μL of twofold serially diluted methanol crude extracts beginning from 200 to 12.5 mg mL^{-1} and incubated for 30 min in the dark. The negative control was DPPH solution (Sigma-Aldrich) only. The optical density of all the tubes was read at 515 nm using Shimadzu UV-visible spectrophotometer (UV-1800; Shimadzu Corporation). In order to prepare a standard curve, the reduction in absorbance of DPPH solution with ascorbic acid at different concentrations over an incubation period of 30 min was measured and plotted. DPPH radical scavenging activities of the samples were expressed in ascorbic acid equivalent antioxidant capacity. The experiment was repeated twice to provide for $n = 3$, and mean ascorbic acid antioxidant capacity and standard deviation were computed and recorded.

Determination of antioxidant capacity by ABTS (2, 2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) assay

This was performed according to the method of Biskup *et al.* (2013) with slight modifications. Briefly, 7 mM solution of ABTS (Sigma-Aldrich) was mixed with 2.45 mM potassium persulphate as stock solution and allowed to stand in the dark for 12 h at room temperature to generate the ABTS radicals. The working solution was prepared by diluting ABTS stock solution with 50% ethanol to an absorbance of 0.8 at 734 nm. Exactly 0.1 g of Trolox (6-hydroxy-2,5,7,8-tetratmethylchroman-2-carboxylic acid) powder (Sigma-Aldrich) was dissolved in 100 mL of ethanol and twofold serially diluted in test tubes. Exactly 1 mL ABTS working solution was added in each tube. The tubes were incubated in the dark for 10 min, and the absorbance was read at 734 nm. A plot of the absorbance against concentration was used to generate the standard curve for Trolox. Approximately 1 g of methanol plant extract was dissolved in 3 mL of ABTS working solution and incubated in the dark for 10 min after which the absorbance was read at 734 nm. The experiment was repeated twice ($n = 3$) and mean antioxidant capacity and standard deviation were computed and recorded. The results were expressed as Trolox equivalent antioxidant capacity.

Determination of total phenolic content

This was performed according to the method of María *et al.* (2018) with slight modifications. Briefly, 0.005 g of methanol crude extract of each plant species was separately dissolved in 1 mL of methanol followed by addition of 0.5 mL Folin–Ciocalteu reagent. The mixture was vortexed for 1 min and incubated at room temperature for 3 min (Donkor *et al.*, 2012). Exactly 10 mL of sodium carbonate (75 g L^{-1}) and 5 mL of Milli-Q water was added to each tube and mixed. The tubes were incubated for 1 h at room temperature in the dark. The absorbance of the samples was read at 765 nm using Shimadzu UV-visible spectrophotometer (UV-1800; Shimadzu Corporation). In order to prepare a standard curve, changes in the absorbance of Folin–Ciocalteu reagent solution at different concentrations of gallic acid (GA) only after 1-h incubation were measured and plotted. The total phenolic content (TPC) was expressed as milligram GA equivalent. The experiment was performed in triplicate.

Analysis of fatty acids

The hexane crude extracts were used for fatty acids and gas chromatography/mass spectrometry (GCMS) analysis. The extracts were dissolved in concentrated hexane and analysed according to previously established procedures (Ee & Yates, 2013), with slight modifications.

Briefly, the crude extracts were mixed with concentrated hexane in a ratio of 1:5, respectively, and centrifuged at 2147 ***g*** for 10 min. The supernatant was removed, filtered through a 0.45-μm membrane filter and mixed with 0.5 mL of 0.2 M sodium methoxide (2.3 g of sodium in 200 mL anhydrous methanol). The mixture was vortexed for 15 s and left to stand for 10 min at room temperature. About 2–3 drops of bromothymol blue was added and mixed. Next, 0.4 mL of 1 M HCl was added dropwise until a yellow colour was detected. Approximately 600 μL of 1.5% (w/v) sodium carbonate in water was added drop wise until a blue colour was obtained. About 1500 μL of distilled water was added to bring the hexane layer to the top of the tube. The hexane layer was carefully removed and subjected to GCMS analysis using a Shimadzu MS detector (GCMS-QP2010 Plus). Composition of the oils was determined using a GC Phenomenex column (30 m × 0.25 mm id, ft. 0.50 μm). The detector was set at 350 °C and the injector port at 250 °C. Separation of fatty acids was achieved using the following column temperature conditions; 80 °C (held for 2 min), heated to 220 °C at 30 °C per min and held at 220 °C for 5.5 min with a total run time of 12.17 min. A mixture of nitrogen, hydrogen and air was used as carrier gas at a linear velocity of 3.5 mL s^{-1}. The compounds were identified by comparing their retention times with standard ester derivatives of fatty acids. The relative level and percentage of each fatty acid were estimated from the standard curve prepared for reference standards of palmitic, myristic, linoleic, oleic, stearic, arachidonic, behenic and erucic acids.

Determination of polyphenols

Detection of polyphenols in the various plant crude extracts was achieved by reverse-phase high-performance liquid chromatography (RP-HPLC) analysis as described by Donkor *et al*. (2012) with slight modifications using a photodiode array detector (PDA). Briefly, a Shimadzu HPLC system (Shimadzu-LC-2030C; Nishinokyo-Kuwabaracho, Nakagyo-Ku, Kyoto, Japan) equipped with a C18 Phenomenex Luna column (2.4 mm porosity, 250 × 4 mm, and an Ultra-Sep ES RP18 precolumn) was used with a 3-steplinear gradient for separation of compounds in the extracts. Two separate analyses were conducted at different times with different mobile phases as described below.

Isolation of betanin

The isolation of Betanin was performed with 10 mM sodium phosphate buffer pH 5.5 (A) and 100% HPLC grade methanol (B) as mobile phase. The gradient for separation was as follows: from 10% to 20% of B in 30 min, 20% to 60% of B in 20 min, then 60% to 90% of B for 20 min (flow rate of 1 mL per min, at 20 °C). The elution pattern was monitored with a PDA detector at 254 nm (Goncalves *et al*., 2012). The extracts were prepared in sodium phosphate buffer at pH 5.5 and filtered through 0.45-μm membrane filter and injected with a 10 μL injection volume. Betanin was identified by comparing with the retention time of reference standard (6.0 min) and quantified using a calibration curve generated from the standard. The column was flushed and auto purged with 100% methanol before and after the analysis.

Isolation of other polyphenols

The mobile phase for isolation of other components consisted of (A) 0.3% phosphoric acid and (B) 100% acetonitrile. The gradient used for separation of components was as follows: from 10% to 20% of B in 45 min, 20% to 60% of B in 20 min, then 60% to 90% of B for 20 min (flow rate: 0.7 mL min^{-1} at 20 °C). The elution pattern was monitored with a PDA detector at 220 nm. The extracts were prepared in methanol and filtered through a 0.45-μm membrane filter and injected with a 10 μL injection volume. The individual polyphenols were identified by comparing with the retention times of reference standards. Quantitative analysis was performed by calibration curves using the reference standards of GA, epigallocatechin (EPC), catechin (CH), epigallocatechin gallate (EPG), dihydroquinidine (DHQ), ferulic acid (FA), ρ-coumaric acid (PCA), luteolin (LT) and 2, 2-diphenyl-1-picrylhydrazyl (DP) (Sigma-Aldrich). Linearity was investigated in the range of 0–5 mg at five increasing concentrations. Intraday analyses of the same solution containing all phenolic compounds tested were used to validate the precision of the chromatographic system (Donkor *et al*., 2012).

Statistical analysis

All data including triplicate determinations for proximate analysis, antioxidant capacity, fatty acids, betanin, quantities of other polyphenols and TPCs of 2017 and 2019 were entered in Excel spreadsheet (WA, USA) and IBM SPSS statistic software version 25 (Chicago, IL, USA). Means and standard deviations were computed using both software, and multiple comparisons between means were performed by one-way analysis of variance test (ANOVA). Differences between means for proximate analysis, antioxidant capacity, fatty acids and TPC were considered significant at $P < 0.05$.

Results and discussion

Despite popular use, rich nutrient content and adaptability to harsh climatic conditions, there are limited scientific data for many useful native food plants to help reduce dependence on unhealthy food choices in

Australia. Before the coming of Westernisation, Indigenous Australians relied on their traditional staples composed mainly of uncultivated plant foods, nondomesticated animals, aquatic foods and insects, most of which were high in dietary fibre and slow digestible carbohydrates (Brimblecombe *et al*., 2014). Elders of the martang Djab Wurrung Aboriginal community in Victoria, Australia, still hold onto their beliefs and claims that their staples are relatively healthier than the predominantly wheat flour and sugar diets of modernity. This study was able to provide preliminary scientific evidence that underground stems and rhizomes of *T. orientalis*, berries of *L. parviflorus*, tubers of *A. strictum*, fruits of *C. rossii*, *D. revoluta* and *R. candolleana*, leaves of *C. alba* and seeds of *A. longifolia* used by the people of martang Djab Wurrung community do have nutritional properties that are worth preserving.

Proximate analysis

With the exception of seeds of *A. longifolia*, the moisture content of the plant species in 2017 was ≥66.8% for oven- and freeze-dried samples and dropped to ≥55.6% in 2019. Generally, all the plant species, except *T. orientalis* exhibited no alterations in moisture contents in 2019 (Table 1). The freeze-drying method revealed more water loss in almost all of the plant species tested than oven-drying in both years ($P < 0.05$). *Typha orientalis* which recorded the highest amount of moisture loss (93.6%) showed more moisture loss in 2017 compared to 2019 ($P < 0.05$). Freeze-drying has been reported as a less aggressive drying method (de Torres *et al*., 2010; Çoklar & Akbulut, 2017) due to its ability to prevent loss of volatile polyphenols and potential antioxidants, thus maintaining the TPC of the samples during processing (Mediani *et al*., 2014). Our results indicate that freeze-drying in addition to its less aggressiveness in dehydrating the samples is also an efficient moisture remover, consistent with the findings of Puranik *et al*. (2012) who after a comparative study of different drying techniques on the quality of garlic, concluded that freeze-dried samples had maximum water loss, than fluidised bed drying, oven-drying and microwave heating methods.

Apart from seeds of *A. longifolia*, leaves of *C. alba* and fruits of *D. revoluta* [with protein contents of 15.1 ± 0.05 g, 8.5 ± 0.05 g and 7.9 ± 0.02 g/100 g dry weight (DW), respectively], the rest of the plant species contained ≤6.8 g of protein per 100 g DW during both years of the study. Protein contents of individual species did not alter between 2017 and 2019 ($P > 0.05$), indicating that the people who depend on these species as a source of proteins are likely to obtain consistent amounts from them.

The ash content of the species ranged from 0.433 to 8.0 g/100 g DW in 2017 and 3 to 8.7 g/100 g DW in 2019. *Rhagodia candolleana* and *A. longifolia* produced more ash in 2019 than 2017 ($P < 0.05$) whereas the ash content of *T. orientalis*, *A. strictum* and *C. rossii* did not change within the years ($P > 0.05$). Seeds of *A. longifolia* recorded the highest crude fat content of 14 ± 0.2 g/100 g DW, followed by *L. parviflorus* with a crude fat content of 13 ± 0.05 g/100 g DW and *D. revoluta* (5.9 ± 0.6 g/100 g DW). The rest of the species recorded fat contents ≤3 g/100 g DW (Table 1), whereas no significant differences ($P > 0.05$) in crude fat contents were observed in plant samples for 2017 and 2019. *Typha orientalis* and *C. rossii* each produced 11 g/100 g DW for TDF. While the TDF of *A. longifolia*, *T. orientalis* and *C. rossii* was higher than the rest of the species ($P < 0.05$), those for *R. candolleana* and *L. parviflorus* were similar ($P > 0.05$). Apart from *R. candolleana*, TDF of the rest of the species did not change significantly between 2017 and 2019 ($P > 0.05$).

Tubers of *A. strictum* contained higher amounts of total carbohydrate ($P < 0.05$) whereas seeds of *A. longifolia* had lower carbohydrate content compared to the rest of the species ($P < 0.05$). The reducing sugar content of *R. candolleana* berries, *C. rossii* fruits and *T. orientalis* underground stems and rhizomes was consistently higher than their nonreducing sugar contents in both years sampled (Fig. 1a, b). *Leucopogon parviflorus* berries (Fig. 1a) and *C. alba* leaves (Fig. 1b) also contained higher amounts of reducing sugars than nonreducing sugars ($P < 0.05$) whereas *A. strictum* tubers and *A. longifolia* seeds contained higher amounts of nonreducing sugars ($P < 0.05$) (Fig. 1a, b). Seeds of *A. longifolia* have exhibited interesting characteristics (e.g. high amounts of proteins, high TDF and low amounts of total carbohydrate) which may be helpful in the management of overweight, obesity and possibly type 2 diabetes within the Indigenous community. Consumption of foods high in dietary fibre has been reported to modulate the absorption of carbohydrates into the blood and improve insulin sensitivity at the peripheral tissues (Galisteo *et al*., 2008). For people who are overweight or obese, dietary fibre supplementation may also enhance weight loss (Anderson *et al*., 2009). Furthermore, increased fibre intake is known to alleviate a number of gastrointestinal disorders including gastroesophageal reflux disease, duodenal ulcer, diverticulitis, constipation and haemorrhoids (Anderson *et al*., 2009). The high fibre content demonstrated by *A. longifolia*, *T. orientalis*, *C. alba* and *C. rossii* in this study highlights their usefulness as possible candidates for inclusion in foods with minimal fibre contents to meet the Australian recommended daily intake of 28 g and 38 g for women and men, respectively (Fayet-Moore *et al*., 2018).

Table 1 Changes in proximate composition (%w/w) of native plant species collected over 2-year period

	February 2017							
Factor/Test	**Rc (b)**	**Lp (b)**	**Dr (b)**	**Cr (f)**	**To (ur)**	**As (t)**	**Al (s)**	**Ca (l)**
Moisture OD	18.7 ± 0.2 (74.8)	18 ± 0.0 (72.0)	–	21.1 ± 0.3 (84.4)	22.7 ± 0.5 (90.8)	16.7 ± 0.2 (66.8)	1.5 ± 0.6 (6.0)	–
Moisture FD	18.4 ± 1.05 (73.6)	17.9 ± 0.3 (71.6)	–	21.2 ± 1.05 (84.8)	23.4 ± 0.9 (93.6)	17.4 ± 0.7 (69.6)	1.8 ± 0.2 (7.2)	–
Ash content	0.18 ± 0.005 (1.2)	0.42 ± 0.05 (2.8)	–	0.39 ± 0.03 (2.6)	0.1 ± 0.0 (0.67)	0.065 ± 0.0 (0.433)	1.2 ± 0.02 (8)	–
Total fats	0.06 ± 0.01 (2)	0.39 ± 0.05 (13)	–	0.03 ± 0.0 (1)	0.03 ± 0.0 (1)	0.02 ± 0.0 (0.7)	0.42 ± 0.2 (14)	–
Crude protein	0.054 ± 0.03 (5.4)	0.026 ± 0.0 (2.6)	–	0.039 ± 0.0 (3.9)	0.062 ± 0.03 (6.2)	0.045 ± 0.0 (4.5)	0.151 ± 0.05 (15.1)	–
Total dietary fibre	0.032 ± 0.0 (3.1)	0.042 ± 0.0 (4.2)	–	0.11 ± 0.06 (11)	0.11 ± 0.05 (11)	0.016 ± 0.0 (1.6)	0.172 ± 0.06 (17.2)	–
Total carbs	27.2 (59.1)	27.2 (59.1)	–	24.2 (52.6)	22.3 (48.5)	28.5 (61.95)	18.3 (39.7)	–

Values in brackets represent percentages. Data are mean ± SD of triplicate determinations of actual amount of each nutrient yield in grams.
–, not determined; %w/w, percentage weight by weight; Al, *Acacia longifolia* subsp. *sophorae*; As, *Arthropodium strictum*; b, berries; Ca, *Correa alba*; Carbs, carbohydrate; Cr, *Carpobrotus rossii*; Dr, *Dianella revoluta*; f, fruits; FD, freeze-drying; l, leaves; Lp, *Leucopogon parviflorus*; OD, oven-drying; Rc, *Rhagodia candolleana* subsp. *candolleana*; s, seeds; t, tubers; To, *Typha orientalis*; ur, underground stems and rhizomes.

Total phenolic content

In 2017, the TPC of *A. strictum* and *L. parviflorus* was higher than the other plant species (Fig. 2a) whereas in 2019 *C. alba* recorded higher TPC (Fig. 2b) than the rest of the species ($P < 0.05$). The TPC of *L. parviflorus* was not determined in 2019 due to unavailability of the berries. Except for *A. longifolia*, where TPCs did not change significantly in both years ($P > 0.05$), the rest of the species recorded higher TPCs in 2019 than 2017 ($P < 0.05$). Biosynthesis of phenolic components is reported to be influenced by agroclimatic conditions of sunshine, rainfall and temperature (Kumar *et al.*, 2017). The significant increase in TPC recorded in 2019 is therefore not surprising considering the reduced rainfall and increased sunshine reported in the study area from 2017 to 2019 (Australian government bureau of meteorology, 2019). A high TPC is likely to indicate richness in free radical scavenging polyphenols with different plant parts containing different amounts. While the leaves of *C. alba* recorded the highest in TPCs in 2019, berries of *D. revoluta* were the lowest, followed by the seeds of *A. longifolia* (Fig. 2a, b).

Polyphenol analysis

Figure 3a chromatogram depicts the standard mixture of nine reference compounds (GA, DHQ, EPC, CH, EPG, PCA, FA, LT and DP, whereas Fig. 3b, chromatogram depicts phenolic profile of *T. orientalis* aqueous extract with seven compounds (GA, DHQ, EPC, CH, EPG, PCA and DP).

The aqueous extract of *C. alba* also contained seven phenolic compounds (GA, CH, EPC, EPG, PCA, LT and DP), whereas the methanol extract contained five (GA, EPC, EPG, LT and DP). Overall, more components were detectable in aqueous extracts of five of the plant species studied (Table 2). However, except for BT (betanidin 5-*O*-β-D-glucoside), the methanol extracts produced higher quantities of extractable polyphenols than aqueous extracts ($P < 0.05$). GA was detected in all the species except *R. candolleana*, while DP was common to all eight species. FA was not detected in any of the species. *Rhagodia candolleana* recorded a very high amount of BT (700 mg L^{-1}) and together with *C. rossii* (244 mg L^{-1}) were the only two species that contained this pigment. It is important to note that samples of *C. rossii* collected in 2017 had a fresh green colour and did not contain BT. However, 2 years later in 2019, samples of *C. rossii* collected from the same spot were red-purple in colour and tested positive for BT. The production of BT in the 2019 samples of *C. rossii* may be attributed to changes in climatic conditions characterised by reduction in rainfall and increased sunshine in the state of Victoria from 2017 to 2019 (Australian government bureau of meteorology, 2019), which probably caused the plants to ripen faster and to produce more pigments in adaptation. BT is a photo labile betacyanin with strong antioxidant properties and useful compound in the food, beverage, cosmetic and pharmaceutical industries as a natural colorant (Goncalves *et al.*, 2012; Antigo *et al.*, 2018). It has antioxidative and anti-inflammatory properties, and to the best of our knowledge, extraction and isolation of this compound in *R. candolleana* and *C. rossii* are interestingly being reported for the first time.

The high betanin content of *R. candolleana* is not surprising, as this saltbush plant, adapted to harsh saline environments shares the same family (Chenopodiaceae) with *Beta vulgaris* L., the major source of betanin use for food-colouring purposes and contains

February 2019							
Rc (b)	Lp (b)	Dr (b)	Cr (f)	To (ur)	As (t)	Al (s)	Ca (l)
18.4 ± 0.3 (73.6)	–	19.8 ± 1.5 (79)	21 ± 0.2 (84)	22.5 ± 0.06 (90.1)	16.4 ± 0.2 (65.6)	1.5 ± 0.5 (6.0)	14.5 ± 1.5 (58.1)
18.2 ± 0.3 (72.7)	–	20.0 ± 0.5 (80.1)	21.2 ± 1.6 (84.8)	22.8 ± 0.05 (91.3)	17.1 ± 0.5 (68.4)	1.4 ± 0.02 (5.6)	13.9 ± 0.5 (55.6)
0.27 ± 0.0 (1.8)	–	0.15 ± 0.03 (1.0)	0.45 ± 0.04 (3)	0.18 ± 0.05 (1.2)	0.75 ± 0.5 (0.5)	1.3 ± 0.1 (8.7)	0.675 ± 0.2 (4.5)
0.06 ± 0.0 (2)	–	0.18 ± 0.6 (5.9)	0.03 ± 0.0 (1)	0.03 ± 0.0 (1)	0.02 ± 0.0 (0.7)	0.43 ± 0.3 (14.3)	0.45 ± 0.3 (3)
0.051 ± 0.05 (5.1)	–	0.079 ± 0.02 (7.9)	0.04 ± 0.0 (4.0)	0.068 ± 0.03 (6.8)	0.042 ± 0.02 (4.2)	0.15 ± 0.05 (15.0)	0.085 ± 0.05 (8.5)
0.046 ± 0.03 (4.6)	–	0.082 ± 0.02 (8.2)	0.112 ± 0.05 (11.2)	0.107 ± 0.05 (10.7)	0.015 ± 0.04 (1.5)	0.170 ± 0.02 (17.0)	0.101 ± 0.05 (10.1)
28.0 (62.2)	–	22 (48.9)	25.8 (57.3)	24.03 (53.4)	29.5 (65.5)	18.2 (40.4)	27 (60.0)

≥800 mg L^{-1} of the pigment (Goncalves *et al*., 2012; Wruss *et al*., 2015; Antigo *et al*., 2018; da Silva *et al*., 2019). At 700 mg L^{-1} betanin content, *R. candolleana* is therefore a likely potential source of the pigment for industrial application.

Apart from their food properties, the selected species also have cultural, medicinal and ornamental uses within the community (Knowles *et al*., 2014). It is not uncommon to find *R. candolleana*, *C. rossii* or *C. alba* propagated along beaches and foot paths with brightly coloured fruits or flowers. *D. revoluta* is also used as an ornamental plant in home and school gardens (Kjelgren *et al*., 2009). Whereas the gum of *A. longifolia* is used as glue especially in making Indigenous tools, the leaves of *C. rossii* are used to treat gastrointestinal upsets and sometimes topically applied to treat scratches and bites (Pirie *et al*., 2014). It is worth noting that even though most of these species are known to be resistant to diseases and pest, they can as well survive harsh environmental conditions. *Leucopogon parviflorus* was not readily accessible in the study area therefore domestication and further propagation may improve its availability.

The polyphenols detected in this study have been reported in other species including *Camellia sinensis* L. (Kuntze), *Malus prunifolia* (Willd.) Borkh, *Moringa Oleifera* Lam. and *Momordica charantia* L. (Hassan *et al*., 2011; Du *et al*., 2012; John *et al*., 2014; Oboh *et al*., 2015; Zhang *et al*., 2016; Perez *et al*., 2018). Most of the compounds (GA, EPC, LT, PCA, DHQ, CH and EPG) detected in this study have been reported to exhibit strong antioxidant activities and further claimed to have anticancer properties (Hassan *et al*., 2011; Du *et al*., 2012; John *et al*., 2014; Oboh *et al*., 2015; Zhang *et al*., 2016). The detection of LT, a flavonoid with strong antioxidative, antitumorigenic and anti-inflammatory properties in the methanol and aqueous extracts of *C. alba* is of particular interest as it is believed to have multiple cardioprotective effects (Luo *et al*., 2017). Furthermore, catechins are known to be inhibitors of enzymes involved in carbohydrate metabolism (He *et al*., 2007; Liu *et al*., 2016a), an important factor in the fight against type 2 diabetes. PCA has antioxidant, anti-inflammatory and antidiabetic properties and is known to lower blood glucose by interfering with the activity of glucose-6-phosphatase and fructose-1-6-bisphosphatase (Amalan *et al*., 2016). However, it is not obvious that such properties would have direct beneficial effects upon consumption of these species as there are likely to be changes during processing or cooking procedures.

Antioxidant capacity (DPPH, ABTS)

The antioxidant activity of methanol and aqueous extracts was similar for 2017 and 2019 ($P > 0.05$). However, PCA, BT and DHQ, were only detectable from the aqueous extracts or sodium phosphate buffer at pH 5.5 (Table 2) but did not change the overall antioxidant activity of the individual species (Fig. 4a, b). Both methanol and aqueous crude extracts of tubers of *A. strictum* exhibited the lowest antioxidant activities in 2017 and 2019 compared to the rest of the species ($P < 0.05$) (Fig. 4a, b). Even though it was expected that the aqueous extracts of the species will be more reactive because of the additional compounds, the difference was not significant ($P > 0.05$). The antioxidant activity of the aqueous extract of *C. alba* was stronger than *A. longifolia*, *D. revoluta* and *A. strictum* ($P < 0.05$), but was not significantly different from *T. orientalis* ($P > 0.05$) whereas the halophytic species (*C. rossii* and *R. candolleana* fruits and berries, respectively) had similar antioxidant capacities (Fig. 4a, b). The strong antioxidant content of most of

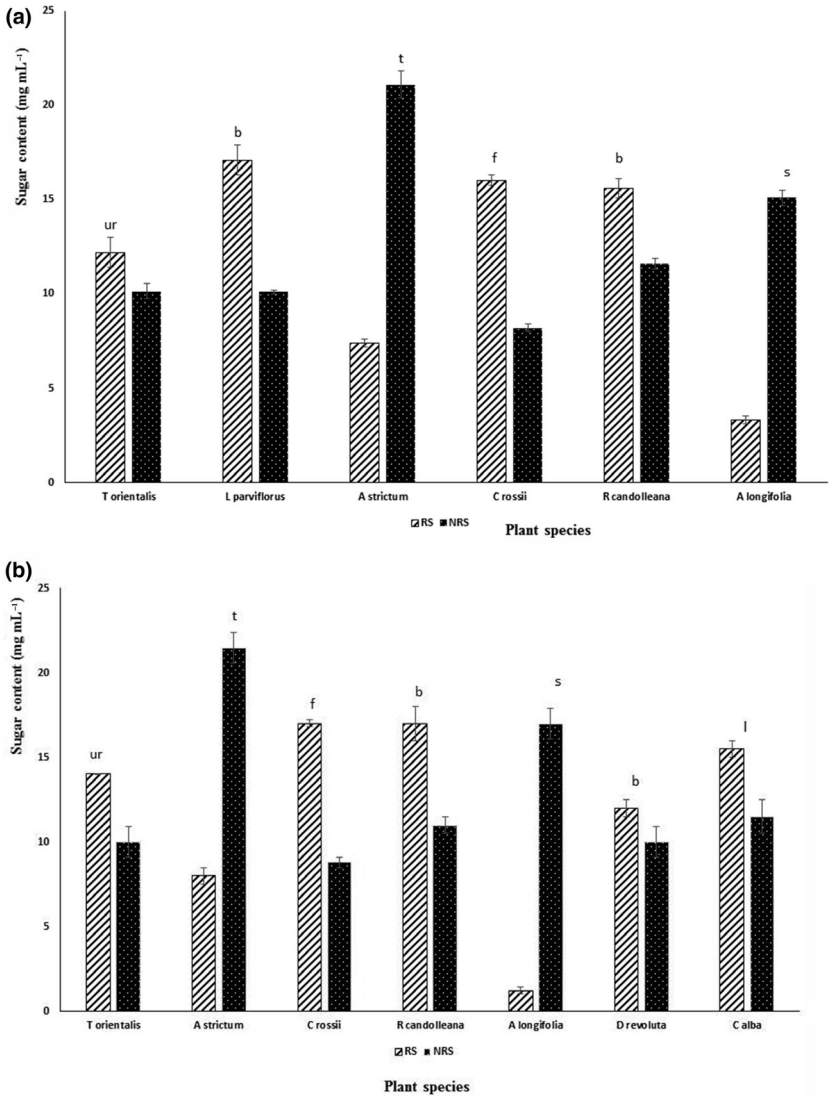

Figure 1 (a) Reducing and nonreducing sugar content of native plant species collected in 2017. Data are means ± SD of triplicate determinations. (b) Reducing and nonreducing sugar content of native plant species collected in 2019. Data are means ± SD of triplicate determinations. RS, reducing sugar; NRS, nonreducing sugar; ur, underground stems and rhizomes; b, berries; t, tubers; f, fruits; s, seeds; l, leaves.

the plant species especially *C. alba* and *T. orientalis* may indicate their natural adaptability in the wild Australian climate. Bunea *et al.* (2011) and Liu *et al.* (2016b) reported that species grown under different geographic/climatic conditions exhibited different TPC and antioxidant capacities. However, we did not record significant increase in antioxidant activity between 2017 and 2019 ($P > 0.05$). Further studies that would include longer time frames and more frequent sampling may help detect significant variability in antioxidant activity with the rest of the species.

With the rise in noncommunicable diseases (Vos *et al.*, 2009; de Abreu *et al.*, 2013; AIHW, 2016), consumption of plant species such as *C. alba*, *T. orientalis*, *L. parviflorus*, *C. rossii*, *R. candolleana* and *A. longifolia* which have demonstrated rich antioxidant potential and TPCs in this study is likely to be beneficial in curtailing alarming increases in overweight/obesity and oxidative stress-related diseases within the Australian Indigenous population. Coupled with their rich antioxidant content, crude extracts of *C. rossii* have been shown to possess hypolipidaemic effects in rats and it is believed that such properties may play a role in reducing cardiovascular risk (Pirie *et al.*, 2013, 2014).

It is worth mentioning that oxidative processes in the body result in production of reactive oxygen species or free radicals such as superoxide ions (O^{-2}), hydroxyl ions (OH^{-1}) and non-free radical species such as hydrogen peroxide (H_2O_2). These species have been known to play a role in the initiation and progression of degenerative pathological conditions such as cancer, diabetes mellitus, Alzheimer's and cardiovascular diseases (Nita & Grzybowski, 2016; Suzen *et al.*, 2017). Physiologically, the human defence mechanism makes provision

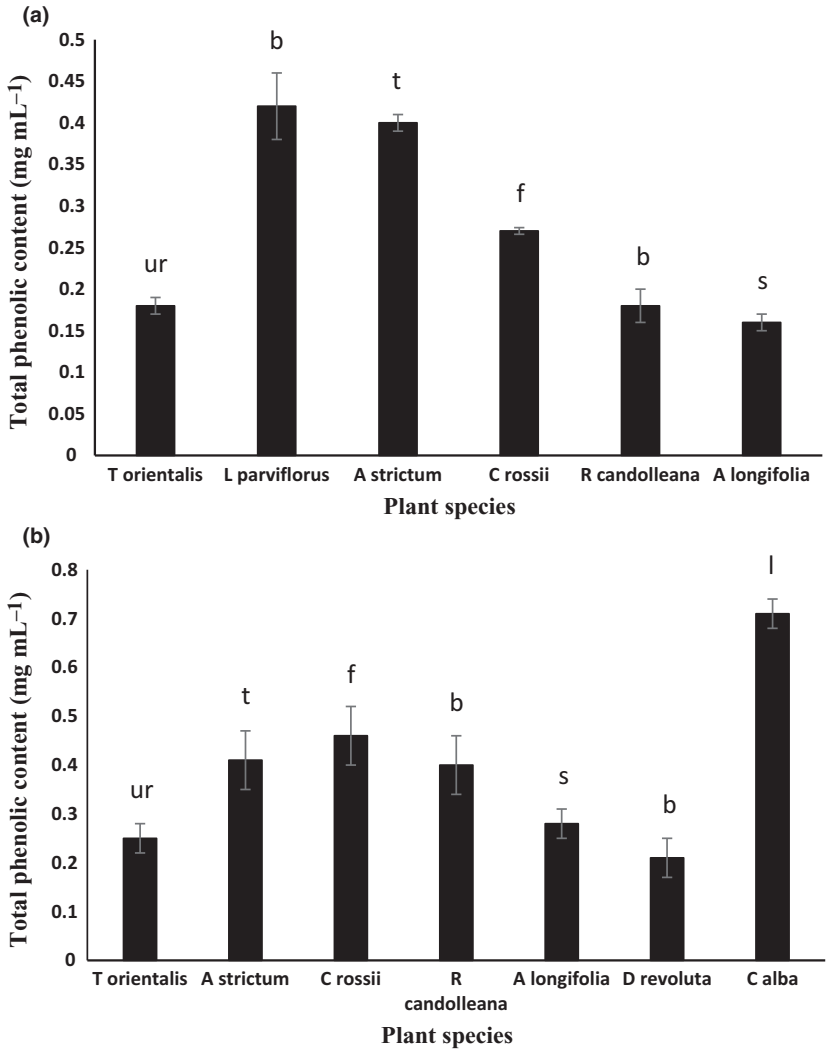

Figure 2 (a) Total phenolic content (TPC; mg mL^{-1}) of native plants collected in 2017. Data are means ± SD of triplicate determinations ($P < 0.05$). (b) TPC (mg mL^{-1}) of native plants collected in 2019. Data are means ± SD of triplicate determinations ($P < 0.05$). ur, underground stems and rhizomes; b, berries; t, tubers; f, fruits; s, seeds; l, leaves.

for the elimination of free radicals from the body. However, sometimes the physiological capacity of the human system is overwhelmed with free radicals, requiring therefore help from externally acquired antioxidants. These antioxidants are generally acquired from food plants with functional properties such as those described in this study. Synthetic antioxidants are readily available, yet their use is not without toxic effects (Saito *et al*., 2003; Yang *et al*., 2018) giving preference therefore to natural antioxidants with the advantage of low toxicity, low cost and stronger antioxidant capacity (Anbudhasan *et al*., 2014; Li *et al*., 2018). It is believed that the intake of antioxidant-rich foods is inversely related to the prevalence of degenerative diseases (Alissa & Ferns, 2012; Zhang *et al*., 2016), thus plant species such as *C. alba*, *T. orientalis*, *L. parviflorus*, *C. rossii*, *R. candolleana* and *A. longifolia* may be regarded as potential sources of antioxidants within the Indigenous community.

In a study conducted by Varpe *et al*. (2012), aqueous and methanol extracts of the pollen grains of *Typha angustifolia*, a member of the family Typhaceae, were shown to be effective against acute and chronic inflammatory conditions in rats owing to their antioxidant and rich phenolic content. However, none of the three main species of plants in Victoria: *Typha domingensis* Pers. (narrow leaf cumbungi), *T. latifolia* L. (lesser reed-mace) or *T. orientalis* C. Presl (broad leaf Cumbungi) have been reported to exhibit anti-inflammatory properties. The high antioxidant capacity of *T. orientalis* may indicate that most of the phenols in this plant may have strong antioxidant capacities. However, we did not record a significant increase in antioxidant capacity during both years of study and some volatile antioxidant compounds might have been lost during sample preparation, especially during drying (El-Ghorab *et al*., 2010).

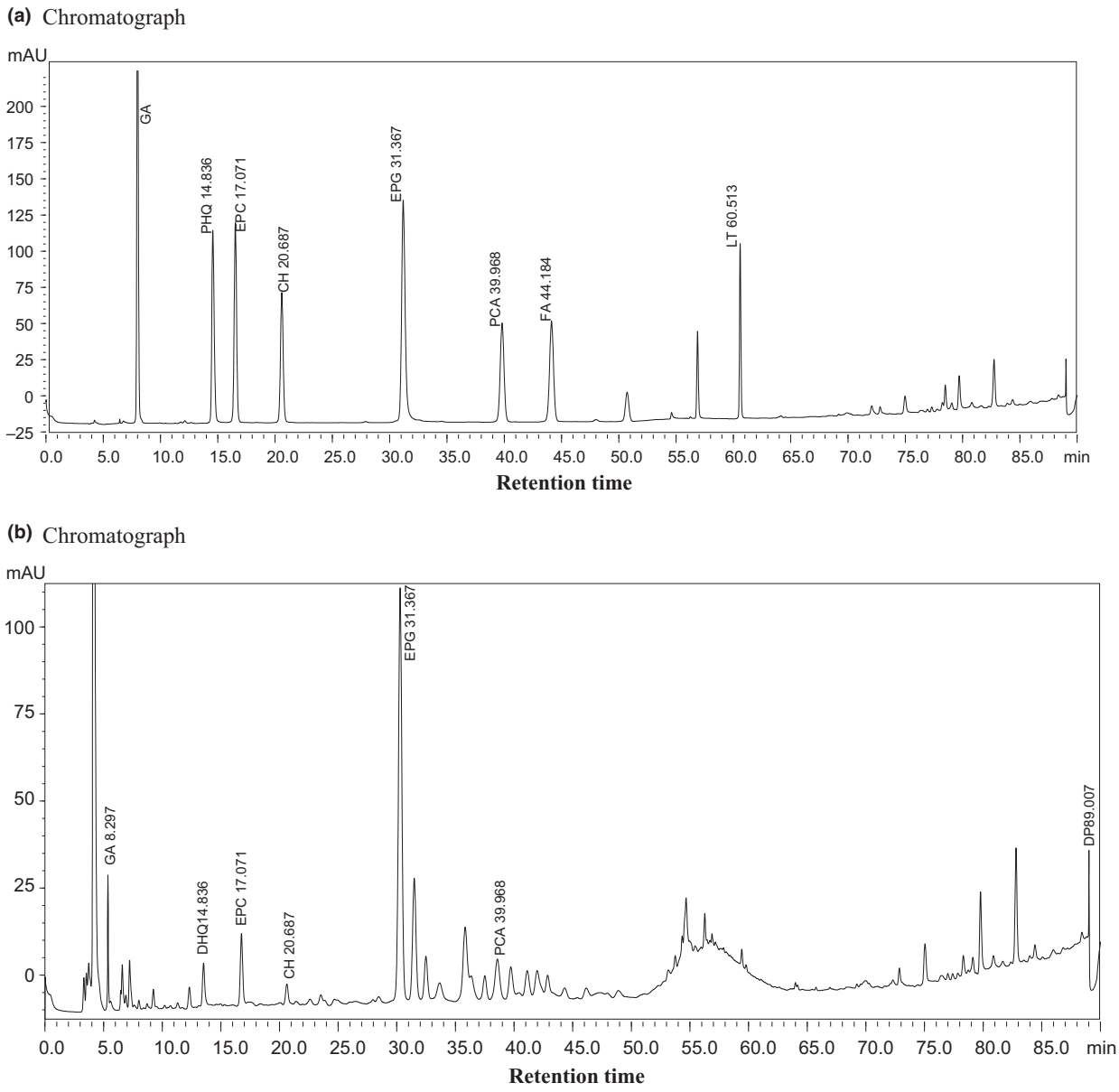

Figure 3 (a) RP-HPLC Analysis; elution profiles of standard mixture of polyphenols showing compounds together with their retention times. Chromatographic analysis was performed on a Shimadzu HPLC system equipped with a C18 Phenomenex Luna column (2.4 mm porosity, 250 × 4 mm, and an UltraSep ES RP18 pre-column) with a 3-steplinear gradient of solvent A 0.3% phosphoric acid and solvent B 100% acetonitrile. The elution pattern was monitored with a photodiode array detector (PDA) at 220 nm. (b) RP-HPLC analysis: phenolic profile of aqueous extracts of *T. orrientalis* showing 7 compounds and their retention times. Chromatographic analysis was performed on a Shimadzu HPLC system equipped with a C18 Phenomenex Luna column (2.4 mm porosity, 250 × 4 mm, and an UltraSep ES RP18 pre-column) with a 3-steplinear gradient of solvent A 0.3% phosphoric acid and solvent B 100% acetonitrile. The elution pattern was monitored with a PDA at 220 nm. Gallic acid (GA), dihydroquinidine (DHQ), epigallocatechin (EPC), catechin (CH), epigallocatechin gallate (EPG), ρ-coumaric acid (PCA), ferulic acid (FA), luteolin (LT) and 2, 2-diphenyl-1-picrylhydrazyl (DP).

Mineral composition

A total of eight out of ten and nine out of ten minerals were detected and quantified in 2017 and 2019, respectively (Table 3). *Arthropodium strictum* and *D. revoluta* were the only two species that showed traces of copper (0.05 and 0.04 mg L^{-1}) in 2017 and 2019, respectively, whereas only *T. orientalis*, *C. rossii* and *C. alba* were found to contain selenium. All the species contained Ca, Mg, K and Na (Table 3). *Typha*

Table 2 Phenolic compounds (mg L^{-1}) detected in methanol extracts and aqueous extracts of native plant species

Plant	GA	DHQ	EPC	CH	EPG	PCA	FA	LT	BT	DP
Methanol extracts										
To	127.3 ± 5.7	–	114.5 ± 19.1	115.4 ± 13	160.1 ± 26	–	–	–	–	19.6 ± 3.4
Lp	111.8 ± 28	–	19 ± 2.8	–	71 ± 28.3	–	–	–	–	21 ± 5.7
As	77 ± 21.2	–	–	–	59 ± 5.7	–	–	–	–	23 ± 4.2
Cr	91 ± 14.1	–	–	–	–	–	–	69 ± 22.6	–	23 ± 4.2
Rc	–	–	44 ± 14.1	–	–	–	–	83 ± 14.1	–	15 ± 6.4
Al	22 ± 5.7	–	81 ± 10	–	35.5 ± 9.2	–	–	–	–	18 ± 8.5
Dr	102 ± 8.5	–	26 ± 1.4	19 ± 2.8	–	–	–	41 ± 15.6	–	21 ± 10
Ca	25 ± 8.5	–	46 ± 14.1	–	52 ± 11.3	–	–	61 ± 18.4	–	20 ± 2.8
Aqueous extracts										
To	77.1 ± 5.8	17 ± 7.1	91.2 ± 8.5	93.5 ± 31.8	59.4 ± 25.5	93.5 ± 17	–	–	–	11.5 ± 17
Lp	99 ± 10	–	19.1 ± 7.8	–	65 ± 26.9	–	–	–	–	18.8 ± 7.1
As	59.5 ± 3.5	–	–	–	62.8 ± 5.7	–	–	–	–	18.5 ± 8.7
Cr	77.4 ± 14.1	–	–	–	–	–	–	51.5 ± 15.6	244 ± 8.5	21 ± 8.5
Rc	–	–	36 ± 5.7	–	–	–	–	76 ± 15.6	700 ± 70.7	17 ± 2.1
Al	22.8 ± 5	–	73.5 ± 14.1	–	33 ± 14.1	–	–	–	–	14.2 ± 1.3
Dr	88 ± 5.7	101 ± 5.7	22 ± 4.2	17 ± 1.4	–	–	–	33 ± 5.7	–	18.2 ± 5.7
Ca	17 ± 4.2	–	39 ± 10	31 ± 12.7	47 ± 7.1	34 ± 5.7	–	55 ± 4.2	–	20 ± 4.2

Data are mean ± SD of triplicate determinations.

–, tested but not detected; Al, *Acacia longifolia* subsp. *sophorae*; As, *Arthropodium strictum*; Ca, *Correa alba*; Cr, *Carpobrotus rossii*; Dr, *Dianella revoluta*; GA, gallic acid; Lp, *Leucopogon parviflorus*; Rc, *Rhagodia candolleana* subsp. *candolleana*; To, *Typha orientalis*.

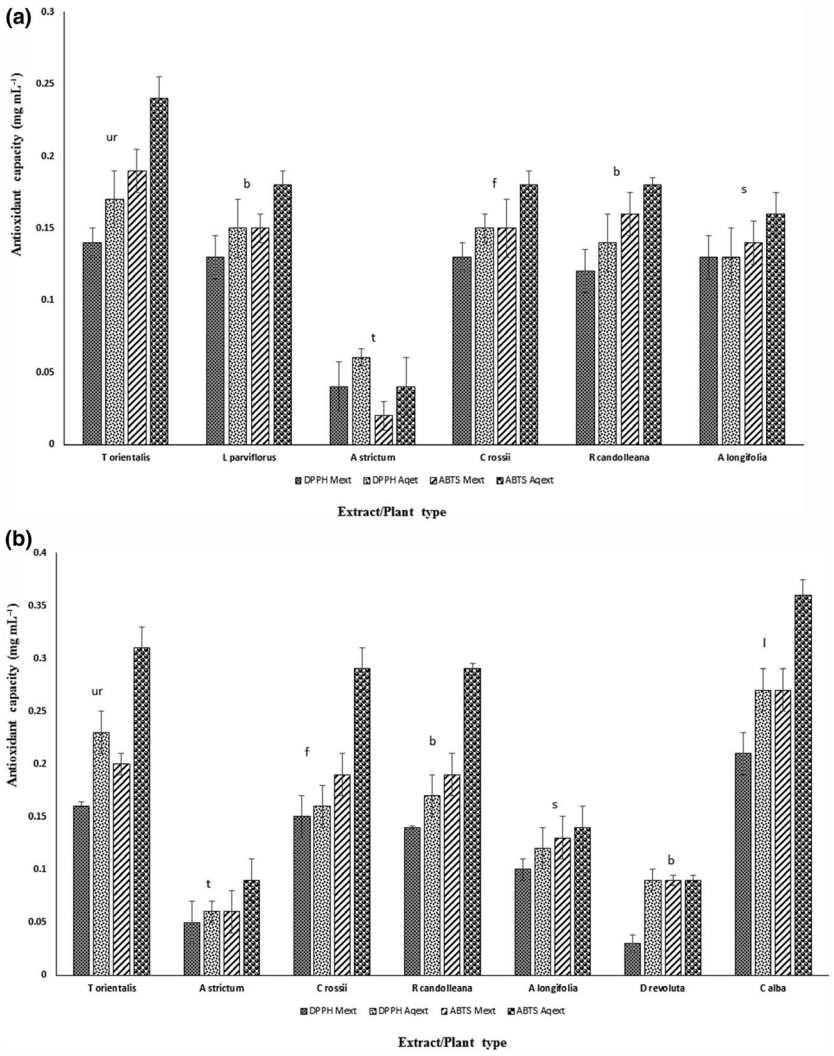

Figure 4 (a) Antioxidant activity (mg mL^{-1}) of native plants collected in 2017. Data are means ± SD of triplicate determinations ($P < 0.05$). (b) Antioxidant activity (mg mL^{-1}) of native plants collected in 2019. Data are means ± SD of triplicate determinations ($P < 0.05$). Mext, methanol extract; Aqext, aqueous extracts; ur, underground stems and rhizomes; b, berries; t, tubers; f, fruits; s, seeds; l, leaves.

orientalis and *A. strictum* recorded the highest detected number of minerals, seven out of ten tested. Zn was not detected in any of the species analysed. *Correa alba* recorded the highest amount of Ca whereas *R. candolleana* contained significantly higher amounts of Mg, Na, K and Fe than the rest of the species ($P < 0.05$). Samples of *A. longifolia* collected in 2019 were higher in Na, Ca and Fe than 2017 whereas samples of *T. orientalis* and *A. strictum* contained higher amounts of K ($P < 0.05$). The Na, Mg, Ca and Fe contents of *C. rossii* and *R. candolleana* were also higher in 2019 than 2017. Overall, five of the species exhibited higher mineral contents in 2019 than 2017. It is likely that the dry weather conditions and reduced rainfall in 2019 resulted in increased mineral concentrations in the plant species. Variations in mineral contents of some species due to weather conditions have been previously reported (Sud *et al.*, 1995). *Rhagodia candolleana* and *C. rossii* being coastal halophytic species collected from the coast of Warrnambool, Victoria, Australia, were among species with the highest mineral content (Table 3). It has been reported that coastal species and seaweeds may contain 10–20 times more minerals and trace elements than some inland species (Akhter *et al.*, 2014); thus, the high mineral content of *R. candolleana* and *C. rossii* is therefore not surprising. Considering that seawater is high in salt and minerals (Loganathan *et al.*, 2017), coastal species and seaweeds occasionally inundated with seawater are bound to be high in minerals.

Three of the species studied herein (*T. orientalis*, *C. rossii* and *C. alba*) also contained Se, a rare but important mineral in normal human physiology and function as part of the enzyme glutathione peroxidase involved in organic peroxide elimination (Trevisan *et al.*, 2014). Generally, minerals play an important

Table 3 Variation in mineral composition (mg L^{-1}) of selected native plant species

	Plant type							
Mineral 2017	**To**	**Lp**	**As**	**Cr**	**Rc**	**Al**	**Dr**	**Ca**
Ca	131.2 ± 1	21.4 ± 0.5	25.6 ± 0.5	444 ± 3.6	844.4 ± 1.5	20.1 ± 1.7	nd	nd
Cr	–	–	–	–	–	–	nd	nd
Cu	–	–	0.05 ± 0.04	–	–	–	nd	nd
Fe	5.84 ± 1	–	1.55 ± 0.6	6.47 ± 0.4	41.4 ± 2.1	–	nd	nd
K	385 ± 3	65.3 ± 6.4	112 ± 1	176 ± 3.5	730 ± 2.3	770.7 ± 2.9	nd	nd
Mg	61.8 ± 1.2	11.6 ± 0.8	11.3 ± 0.7	155.4 ± 5.5	565.8 ± 2.9	130 ± 2.6	nd	nd
Mn	0.11 ± 0.02	–	0.004 ± 0.002	–	0.03 ± 0.01	–	nd	nd
Na	97.8 ± 0.2	114 ± 2.9	5.14 ± 0.2	352 ± 2.5	855 ± 8.1	94.2 ± 0.9	nd	nd
Se	0.71 ± 0.09	–	–	1.41 ± 0.4	–	–	nd	nd
Zn	–	–	–	–	–	–	nd	nd

2019	**To**	**Lp**	**As**	**Cr**	**Rc**	**Al**	**Dr**	**Ca**
Ca	319 ± 1	nd	33 ± 3	615 ± 1	1004.7 ± 0.2	76.1 ± 1.7	8.9 ± 0.4	2010.3 ± 2.3
Cr	–	nd	–	–	–	–	0.08 ± 0.02	–
Cu	–	nd	–	–	–	–	0.04 ± 0.0	–
Fe	6.5 ± 0.8	nd	–	17.7 ± 0.8	61.4 ± 0.7	2.14 ± 0.1	–	25.8 ± 0.06
K	590.3 ± 0.5	nd	178 ± 1.5	191 ± 1	1389.3 ± 45.5	1070 ± 6	269 ± 1	763 ± 6
Mg	117.4 ± 2.5	nd	4.7 ± 0.3	349.3 ± 0.6	618.3 ± 1.5	128 ± 2.6	10 ± 0.09	374.3 ± 2.3
Mn	4.4 ± 0.2	nd	–	–	–	–	–	–
Na	150 ± 3	nd	9.4 ± 0.5	704.3 ± 4.5	1025 ± 5.6	167.3 ± 0.6	6.51 ± 0.06	256 ± 26.2
Se	0.28 ± 0.03	nd	–	5.5 ± 0.8	–	–	–	7.1 ± 0.2
Zn	–	nd	–	–	–	–	–	–

Data are mean ± SD of triplicate determinations.

–, not detected; Al, *Acacia longifolia* subsp. *sophorae*; As, *Arthropodium strictum*; Ca, *Correa alba*; Cr, *Carpobrotus rossii*; Dr, *Dianella revoluta*; Lp, *Leucopogon parviflorus*; Nd, not determined; Rc, *Rhagodia candolleana* subsp. *candolleana*; To, *Typha orientalis*.

role in the functioning of human physiology and may include structural, catalytic, signalling or osmotic functions. While Na and K ratios are important in the transmission of impulses in the central nervous system, maintenance of osmotic balance across membranes and regulation of blood pressure, Ca and Mg are important components of bones, muscle contraction and enzyme activities (Morris *et al*., 2010).

The daily intake for Se in Australia is 50 μg day^{-1} and 60 μg day^{-1} for women and men, respectively (NHMRC, 2014). Unfortunately, common foods may not always have adequate concentrations to meet dietary requirements (Multari *et al*., 2016), and so little amounts from native food plants within meals may be helpful. In a previous study, *Typha latifolia* L., a related species of *T. orientalis,* was found to contain Mn in the leaves and Zn and Fe in the roots (Parzych *et al*., 2016). However, this study detected small amounts of Mn and Fe but not Zn, in the underground stems and rhizomes of *T. orientalis* consumed by the Victoria Indigenous population. The importance of all three minerals is well established and cannot be overemphasised; Mn is a cofactor of arginase, glutamine synthetase and pyruvate carboxylase while Zn is a cofactor of lactate dehydrogenase, alkaline phosphatase, superoxide dismutase, retinene reductase and Fe, an important component of haemoglobin and cytochromes that function in cellular respiration (Soetan *et al*., 2010; Yamada *et al*., 2014; Chen *et al*., 2018).

Fatty acid composition

Fatty acids detected in the standard mix are displayed in Fig. 5, a whereas Fig. 5b depicts fatty acid profile of *A. longifolia* with linoleic acid (an omega-6-polyunsaturated compound), as the dominant fatty acid. Total of ten different fatty acids were identified in the selected species (Table 4); however, not all the fatty acids were found in any single species. The dominant fatty acids were palmitic, oleic and linoleic acids. Palmitic acid was the most common fatty acid, detected in six of the eight species studied. The fatty acid content of the species did not differ in 2017 and 2019 ($P > 0.05$). The detection of palmitic, linoleic and oleic acids was found to be ≥ 20%, 42.4% and 12.3%, respectively, in the seeds of *A. longifolia*. These results are consistent with the findings of Brown *et al*. (1987) who reported these as major fatty acids in Australian *Acacia* species. Also, while working on Tunisian *Acacia* species, Youzbachi *et al*. (2015) reported linoleic (>52%) and oleic (15–27%) acids as major

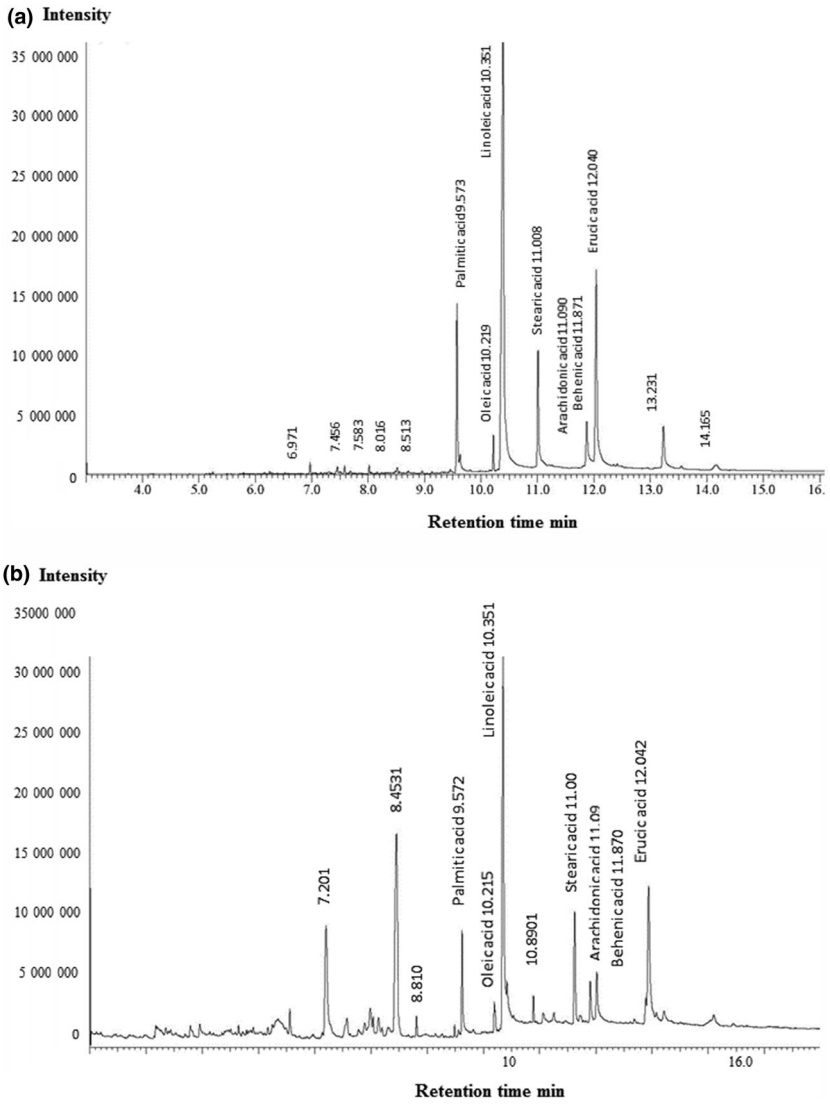

Figure 5 (a) GCMS analysis: elution profiles of standard mixture of fatty acids and their retention times. Palmitic acid, linoleic acid, oleic acid, stearic acid, arachidonic acid, behenic acid and erucic acid. Chromatographic analysis was performed on a Shimadzu GC system equipped with a Phenomenex column of 30 m × 0.25 mm id, ft. 0.50 μm and MS detector (GCMS-QP2010 Plus). The detector was set at 350 °C and the injector port at 250 °C. Separation of fatty acids was achieved using the following column temperature conditions; 80 °C (held for 2 min), heated to 220 °C at 30 °C per min and held at 220 °C for 5.5 min with a total run time of 12.17 min. A mixture of nitrogen, hydrogen and air was used as carrier gas at a linear velocity of 3.5 mL s^{-1}. (b) GCMS analysis: elution profiles of fatty acids from hexane extracts of *A. longifolia* and their retention times; palmitic acid, oleic acid, linoleic acid, stearic acid, arachidonic acid, behenic acid and erucic acid. Chromatographic analysis was performed on a Shimadzu GC system equipped with a Phenomenex column of 30 m × 0.25 mm id, ft. 0.50 μm and MS detector (GCMS-QP2010 Plus). The detector was set at 350 °C and the injector port at 250 °C. Separation of fatty acids was achieved using the following column temperature conditions; 80 °C (held for 2 min), heated to 220 °C at 30 °C per min and held at 220 °C for 5.5 min with a total run time of 12.17 min. A mixture of nitrogen, hydrogen and air was used as carrier gas at a linear velocity of 3.5 mL s^{-1}.

components in Tunisian *Acacia* seed oil. All three studies therefore have demonstrated that *Acacia* seeds could be regarded as a rich source of oil, mostly polyunsaturated and monounsaturated fatty acids with potential for wide industrial application.

Conclusion

All eight species (*T. orientalis*, *L. parviflorus*, *A. strictum*, *C. rossii*, *R. candolleana, D. revoluta*, *C. alba* and *A. longifolia*) were found to be sources of proteins, carbohydrates, TDF, minerals and water. Palmitic, oleic and linoleic acids were the dominant fatty acids isolated from the species in 2017 and 2019, and there were no differences for 2017 and 2019 samples. The aqueous and methanol crude extracts were positive for phenolic compounds and exhibited consistent antioxidant activities in 2017 and 2019, yet TPCs of the species were higher in 2019 than 2017 ($P < 0.05$). More components were detectable in aqueous extracts of five of the species studied, except for BT, the methanol extracts produced higher quantities of polyphenols than aqueous extracts ($P < 0.05$), indicating that both extraction methods are necessary to maximise the extraction of phytocomponents. *Rhagodia candolleana* to the best of our knowledge is being reported for the first time as a likely source of betanin, an antioxidant pigment with wide industrial application. All the species contained phenolic compounds with consistent antioxidant activities that are likely to be protective against diseases caused by free radical production in the body. However, the *in vivo* free radical scavenging

Table 4 Fatty acid composition of selected native plant species

Plant name	February 2017 collections	% estimate	February 2019 collections	% estimate
Typha orientalis	Palmitic acid	37 ± 4.2	Palmitic acid	39 ± 4.2
	Oleic acid	39 ± 6.4	Oleic acid	39 ± 3.5
	Myristic acid	29 ± 1.4	Myristic acid	33 ± 2.8
Leucopogon parviflorus	Palmitic acid	38 ± 5.0	nd	nd
	Oleic acid	15.6 ± 5.2	nd	nd
	Linoleic acid	41 ± 4.2	nd	nd
	Arachidonic acid	9 ± 1.4	nd	nd
	Myristic acid	tr	nd	nd
Arthropodium strictum	Palmitic acid	17 ± 2.8	Palmitic acid	21.5 ± 1.4
	Oleic acid	37 ± 3.5	Oleic acid	37 ± 4.2
	Linoleic acid	tr	Linoleic acid	tr
	Stearic acid	8.5 ± 2.8	Stearic acid	10 ± 4.2
	Myristic	8.2 ± 1.8	Myristic acid	9 ± 2.1
	Erucic	tr	Erucic acid	tr
Carpobrotus rossii	Palmitic acid	5.5 ± 0.7	Palmitic acid	7.5 ± 2.8
	Linoleic acid	10.1 ± 1.4	Linoleic acid	10 ± 0.7
	Myristic acid	tr	Myristic acid	tr
Rhagodia candolleana	Palmitic acid	17.6 ± 2.4	Palmitic acid	19.5 ± 3.5
	Oleic acid	31 ± 2.8	Oleic acid	31 ± 1.4
Acacia longifolia	Palmitic acid	20 ± 3.5	Palmitic acid	21 ± 0.7
	Oleic acid	12.3 ± 0.3	Oleic acid	15.2 ± 0.6
	Linoleic acid	42.4 ± 1.4	Linoleic acid	44 ± 4.2
	Stearic acid	24.5 ± 2.1	Stearic acid	22 ± 5.7
	Erucic acid	22 ± 2.8	Erucic acid	23 ± 4.2
	Arachidonic acid	11.2 ± 3.1	Arachidonic acid	13 ± 2.8
	Myristic acid	tr	Myristic acid	tr
	Arachidic acid	ndt	Arachidic acid	ndt
	Behenic acid	tr	Behenic acid	tr
Dianella revoluta	nd	nd	Linoleic acid	33.1 ± 3
	nd	nd	Linolenic acid	12.6 ± 2.8
	nd	nd	Myristic acid	15 ± 3.5
	nd	nd	Stearic acid	tr
	nd	nd	Erucic acid	tr
Correa alba	nd	nd	Oleic acid	27 ± 7.1
	nd	nd	Linoleic acid	13 ± 1.4
	nd	nd	Myristic acid	7.4 ± 1.1
	nd	nd	Arachidonic acid	21 ± 5.7
	nd	nd	Oxalic acid	tr
	nd	nd	Erucic acid	tr

Data are mean ± SD of triplicate determinations.
nd, not determined; tr, traces (<0.5%).

activity of most of these compounds is yet to be determined.

Whereas the nutritional contents and fatty acids of plant species were fairly stable between 2017 and 2019, some of the minerals, TPCs and some active components varied. Further studies would be required to determine greater degree of variability of different phytocomponents within a longer time frame.

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Data Availability Statement

Research data are not shared.

Ethical guidelines

A research permit to collect native species from Warrnambool, Victoria, Australia, was granted by the

Department of Environment, Land, Water and Planning (Permit number 10008221). Collections were carried out in collaboration with Indigenous Elders in Warrnambool after obtaining their blessing to conduct the study.

Conflict of interest

The authors declare no conflict of interest.

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