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Characterising the Physical, Phytochemical and Antioxidant Properties of the Tuckeroo (*Cupaniopsis anacardioides*) Fruit

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Abstract: The tuckeroo (*Cupaniopsis anacardioides*) is an Australian native plant that fruits over the summer months. There are very few studies that have characterised its fruit; consequently, this study aimed to delineate the physical, phytochemical and antioxidant properties of the tuckeroo fruit. The tuckeroo skin embodied the largest weight proportion with over 77% of the total fruit weight and it had the highest levels of total phenolic compounds (TPC; 151.36 mg GAE/g), total flavonoids compounds (TFC; 95.94 mg CAE/g), and proanthocyanidins (Proanth; 164.86 mg CAE/g) content, as well as the strongest antioxidant power. The seed and flesh accounted for 23% of the total fruit weight and they possessed significantly lower levels of TPC, TFC and Proanth. This study has demonstrated that the tuckeroo fruit skin is a rich source of phenolic compounds, which can be further isolated and identified for further utilisation in the food and pharmaceutical industries.

Keywords: antioxidant capacity; *Cupaniopsis anacardioides*; phenolic compounds; tuckeroo fruit; bio-active compounds

1. Introduction

Australia is a big island continent with diversified weather throughout the country, thus fruit bearing plants, especially native plants, have developed unique survival characteristics to adapt to such conditions [1]. As such, native Australian fruits can offer enormous opportunities for the discovery of preventive and/or therapeutic phytochemicals, such as novel anti-cancer agents [2]. *Cupaniopsis anacardioides* (A. Rich) Radlk (commonly called Tuckeroo, Carrotwood, Beach Tamarind or Green-leaved Tamarind) is an Australian native plant, which grows along the coastal regions of New South Wales, Queensland and Northern Australia. The Tuckeroo has dark green leaves, a short trunk, greenish white flower, and an especially orange capsuled round fruit. The fruit (Figure 1) is tapered at the base with three lobes, with one shiny black seed in each lobe enclosed by an orange aril. During ripening the colour of the fruit changes from yellow to orange and occurs between October and December. The Tuckeroo fruit is a food of many birds, such as the figbird, olive-backed oriole and pied currawongs [3]. Commonly, the fruits that are eaten by birds can be consumed by humans and these fruits may contain various bioactive compounds, which can be linked to human health benefits [2,4]. Therefore, this study aimed to characterise the physical, phytochemical and antioxidant properties of the Tuckeroo fruit and its major components, including skin, flesh and seed for further isolation and identification.

Figure 1. Tuckeroo (*Cupaniopsis anacardioides*) fruits.

2. Materials and Methods

2.1. Materials

Tuckeroo fruits (*Cupaniopsis anacardioides*) were collected from three regions including Ourimbah (33°21′13.19″ S 151° 22′ 23.99″ E), Terrigal Beach (33°26′52.8396″ S 151°26′40.0596″ E), and Avoca Beach (33°27′54″ S 151°26′6″ E), New South Wales, Australia in December 2015. After collection, the fruits were divided into two groups. One group was processed as the whole fruit while the second group was separated into its components, namely, skin, seed and flesh. Fruit materials were then immediately dried using a freeze dryer (FD3 freeze dyer, Thomas Australia Pty. Ltd., Seven Hills, NSW, Australia). The dried fruit materials were then ground into powder (1.4 mm in particle size) using a blender (HBB250S, John Morris Scientific, Chatswood, NSW, Australia) and stored at −20 °C for further analysis.

2.2. Methods for Characterisation of Tuckeroo (Cupaniopsis anacardioides) Fruits

2.2.1. Diameter of the Fruits

Thirty fresh fruits were selected randomly from the harvested samples. A thickness gauge (ε = 0.0001 mm) was used for measuring the fruit diameter and the values were represented as the mean (mm) ± standard deviation.

2.2.2. Weight of the Whole Fruit and Fruit Components

The weight of whole fruit or its skin, seed or flesh was measured using an analytical balance (ε = 0.0001 g) from 30 randomly collected fruits. The values were expressed as mean value (g) ± standard deviation.

2.2.3. Moisture Content

Moisture content was determined based on weight difference. Samples were dried using a conventional oven set at 110 °C for 8 h. Moisture values of the fruit were expressed as percentage ± standard deviation.

2.3. Extraction

To identify the impact of different solvents on phytochemical and antioxidant properties, the dried ground fruit was extracted using seven solvents including water, methanol, methanol 50%, ethanol, ethanol 50%, acetone, and acetone 50%. To further determine the phytochemical and antioxidant capacities of tuckeroo fruit and its different components (skin, flesh and seed), the best solvent (acetone 50%) was used as an extraction solvent. Extraction was done as described in a previous study with some modifications [5]. One gram of dried sample was extracted in 50 mL of solvent using an ultrasonic bath (Soniclean 1000HD, 220 V, 50 Hz and 250 W, Soniclean Pty Ltd., Thebarton, Australia) set at 40 °C, 150 W for 30 min. The extracted solution was then cooled on ice to room temperature and filtered using Whatman paper for further analysis.

2.4. Determination of Phenolic Content and Its Secondary Metabolites

2.4.1. Total Phenolic Content

Total phenolic content (TPC) was determined using a previously described method [6]. Briefly, 0.5 mL of diluted sample was mixed with 2.5 mL of Folin-Ciocalteu reagent 10% (v/v) and 2 mL of Na_2CO_3 7.5% (w/v), then left in the dark at room temperature for 60 min. Measurement was taken at 760 nm using a UV spectrophotometer (Varian Cary 50 Bio, Varian Australia Pty. Ltd., Melbourne, Victoria, Australia). Gallic acid was used as a standard curve and TPC was expressed as milligrams of gallic acid equivalents per gram of dried sample (mg GAE/g).

2.4.2. Flavonoids

Total flavonoid content was determined using the method described by Wu and Ng [7]. 0.5 mL of diluted sample was added into 2 mL of H_2O and subsequently with 0.15 mL of 5% (w/v) $NaNO_2$, then left at room temperature for 6 min. 0.15 mL of 10% $AlCl_3$ was then added and the mixture was left to stand for 6 min. Finally, 2 mL of 4% NaOH and 0.7 mL of deionised water were added and the mixture was left at room temperature for 15 min. Measurement was conducted at 510 nm. Catechin was used as a calibration standard curve and the values of total flavonoids were described as mg of catechin equivalents per g of dried sample (mg CAE/g).

2.4.3. Proanthocyanidins

Proanthocyanidins were estimated using the method described by Li et al. [8]. The ratio 1:6:3 ($v/v/v$) of diluted sample, 4% (w/v) vanillin, and concentrated HCl, respectively, were mixed and incubated at room temperature for 15 min. The absorbance was then measured at 500 nm and catechin was used as a calibration standard curve. The values of proanthocyanidins were expressed as mg of catechin equivalents per g of dried sample (mg CAE/g).

2.5. Determination of Antioxidant Capacity

Four antioxidant assays including 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), cupric-reducing antioxidant capacity (CUPRAC) and ferric antioxidant power (FRAP) were applied in the current study to determine antioxidant capacity of the samples.

2.5.1. ABTS Radical Scavenging Activity

The ABTS assay was applied based on the method reported by Thaipong et al. [9] with some modifications. The stock solution was prepared by mixing 7.4 mM ABTS and 2.6 mM $K_2S_2O_8$ with ratio 1:1 (v/v) and kept in the dark for 15 h. A working solution was then diluted from the stock solution by methanol to reach an absorbance of 1.1 ± 0.02 at 734 nm. 0.15 mL of diluted sample was added to 2.85 mL of working solution and left at room temperature in the dark for 2 h. Measurement

was taken at 734 nm and trolox was used to prepare the standard curve. Antioxidant capacity was expressed as mg trolox equivalents per g of dried sample (mg TE/g).

2.5.2. DPPH Radical Scavenging Activity

DPPH radical scavenging activity was determined as described by Thaipong et al. [9] with some modifications. A stock solution of DPPH in methanol was prepared, then diluted in methanol to obtain a working solution with an absorbance value 1.1 ± 0.02 at 515 nm. To 0.15 mL of sample, 2.85 mL of working solution was added and then left for 3 h in the dark at room temperature. Trolox was used as a standard curve and the DPPH results were expressed as mg trolox equivalents per g of dried sample (mg TE/g).

2.5.3. Cupric-Reducing Antioxidant Capacity

CUPRAC was measured using a UV spectrophotometer at 450 nm, as described by Apak et al. [10] with a few modifications. To 3 mL of a $CuCl_2$, neocuproine and NH_4Ac (1:1:1, $v/v/v$) mixture was added 1.1 mL of diluted sample, then left in the dark at room temperature for 90 min before measuring at 450 nm. Trolox was used as a standard curve and the CUPRAC value was expressed as mg trolox equivalents per g of dried sample (mg TE/g).

2.5.4. Ferric Antioxidant Power

FRAP was determined based on a previous method [9] with some modifications. A working solution was prepared by mixing 300 mM acetate buffer, 10 mM 2,4,6-Tripyridyl-S-triazine (TPTZ) in 40 mM HCl and 20 mM $FeCl_3$ with a ratio of 10:1:1 ($v/v/v$). Before analysis, the working solution was incubated until the temperature reached 37 °C. 0.15 mL of diluted sample was then added with 2.85 mL of working solution, then left in the dark at room temperature for 30 min. The absorbance was then measured at 593 nm and the FRAP values were expressed as mg trolox equivalent per g of dried sample (mg TE/g).

2.6. Extraction Yields

Extraction yields (EY) were determined according to a previously described method [11]. A total of 3 mL of crude extracts were transferred to a pre-weighed pottery cup and then dried under 110 °C for 8 h to completely remove all moisture using a hot-air oven (Ketong, Jiangsu, China). EY was calculated by the weight difference as shown in the formula (1):

$$\mathrm{EY} = \frac{W_1 - W_2}{3} \ (\mathrm{g/mL}) \quad (1)$$

where by W_1 = total weight of pottery cup and 3 mL extract solution before drying and W_2 = total weight of pottery cup and 3 mL extract solution after drying.

2.7. Statistical Analysis

SPSS software version 23 was used for statistical analysis (one-way ANOVA with Duncan's post hoc multiple comparisons test) of the data. Differences between the mean levels of the components in the different experiments were taken to be statistically significant at $p < 0.05$.

R^2 was calculated based on levels of TPC, total flavonoids compounds (TFC), Proanthocyanidins from whole fruit and its skin, seed, and flesh with their respective antioxidant capacity.

3. Results and Discussion

3.1. Physical Characteristics of the Tuckeroo Fruit

The physical characteristics of the Tuckeroo fruit are represented in Table 1. The mean whole fruit weight was approximately 3.2 g, including the skin, the seed and the flesh. The Tuckeroo skin accounted for 77% of the whole fruit weight, followed by the seed (~16%), while the flesh only accounted for ~7% of the whole fruit weight. The moisture content of the whole fresh fruit accounts for about 69%, whereas, the moisture of the skin, flesh and seed was 77%, 56%, and 43%, respectively. The shape of the fruit is spherical with a diameter and length of 17–20mm (Table 1). In comparison with other fruits, the Tuckeroo fruit is smaller than the macadamia (*Macadamia tetraphylla*) nut [5] but larger than the blue honeysuckle (*Lonicera caerulea* L.) [12]

Table 1. Physical characteristics of the Tuckeroo fruit.

Characteristics	Mean ± Standard Deviation
Whole fruit weight (g)	3.20 ± 0.08
Seed (%) of fruit weight	15.67 ± 0.76
Flesh (%) of fruit weight	7.03 ± 0.09
Skin (%) of fruit weight	77.31 ± 1.26
Fruit moisture (%)	68.94 ± 1.17
Seed moisture (%)	42.52 ± 1.98
Flesh moisture (%)	56.16 ± 0.68
Skin moisture (%)	76.48 ± 0.40
Diameter of fruit (mm)	20.56 ± 9.24
Length of fruit (mm)	17.34 ± 3.80

The values are expressed as the mean values ± standard deviation (n = 30).

3.2. Extraction Yields, Phenolic Content and Antioxidant Capacity of the Tuckeroo Fruits as Influenced by Extraction Solvents

The results in Table 2 showed the impact of different solvents on the yields of extraction, phenolic content and antioxidant capacity of the Tuckeroo fruit. Generally, the extraction yields, phenolic content and antioxidant capacity of the Tuckeroo fruit were significantly affected by solvents in which they were extracted. Extracting with methanol, 50% methanol, and 50% ethanol had the highest extraction yields with approximately 44 g/100 g of sample. Followed by 50% acetone, water, and absolute acetone with about 41, 36, and 19 g/100 g of sample, respectively. These findings revealed that more than half of the yields of extraction would be lost depending on the solvent used. These findings were supported by previous studies on almond hulls [13] or on lemon peels [14], which reported that extraction solvents also significantly affected the extraction yields. These results can be explained by the various solubilities of the components including hydrophilic and lipophilic compounds in the plant material.

Extraction solvents were also found to significantly affect phenolic content, flavonoid and proanthocyanidin levels extracted from the Tuckeroo fruit. Table 2 reveals that absolute solvents had a lower extraction efficiency of phenolic content and flavonoids in comparison with the 50% mixtures of the organic solvents with water. Of note, organic solvents had higher extraction efficiency for proanthocyanidins than that of water, and 50% ethanol was found to extract more proanthocyanidins than that of absolute ethanol, whereas absolute acetone extracted significantly higher levels of proanthocyanidins than 50% acetone. These findings were in agreement with results from previous studies on macadamia skin and lemon pomace [5,14]. These variations can be explained by the different polarities of the phenolic compounds as well as their secondary metabolites, flavonoids and proanthocyanidins. Absolute organic solvents or water can extract targeted hydrophobic and hydrophilic compounds, whereas 50% mixtures of organic solvents and water can extract both hydrophilic and hydrophobic compounds, thus these mixtures had higher extraction efficiency than that of absolute organic solvents or water.

Table 2. Effect of solvents on extraction yields (EY), phenolic content and antioxidant capacity of the Tuckeroo fruits.

Solvents	EY (g/100 g Sample)	TPC (mg GAE/g Dried Sample)	TFC (mg CAE/g Dried Sample)	Proanth (mg CAE/g Dried Sample)	FRAP (mg TE/g Dried Sample)	CUPRAC (mg TE/g Dried Sample)	DPPH (mg TE/g Dried Sample)	ABTS (mg TE/g Dried Sample)
Water	35.78 ± 0.10 ^{c}	49.14 ± 1.04 ^{f}	30.5 ± 0.75 ^{d}	20.47 ± 0.09 ^{f}	76.03 ± 0.63 ^{f}	165.16 ± 4.88 ^{f}	158.84 ± 2.53 ^{e}	182.4 ±1.58 ^{d}
Methanol	44.22 ± 0.77 ^{a}	95.99 ± 1.35 ^{d}	49.32 ± 1.06 ^{c}	58.18 ± 0.39 ^{c}	135.56 ± 0.20 ^{d}	324.97 ± 3.55 ^{d}	329.80 ± 6.64 ^{b}	268.7 ±5.51 ^{c}
Methanol 50%	44.1 ± 0.20 ^{a}	106.23 ± 0.97 ^{b}	67.67 ± 0.82 ^{b}	56.74 ± 1.9 ^{c}	176.30 ± 0.41 ^{b}	370.12 ± 4.00 ^{a}	263.78 ± 2.95 ^{d}	346.98 ±1.59 ^{a}
Ethanol	33 ± 0.72 ^{d}	53 ± 0.72 ^{e}	27.33 ± 1.03 ^{e}	33.93 ± 0.6 ^{e}	78.96 ± 1.98 ^{e}	187.47 ± 1.87 ^{e}	155.56 ± 3.01 ^{e}	136.57 ±5.54 ^{e}
Ethanol 50%	43.61 ± 0.39 ^{a}	103.85 ± 0.72 ^{c}	66.47 ± 0.68 ^{b}	51.54 ± 0.96 ^{d}	157.03 ± 2.57 ^{c}	352.23 ± 3.83 ^{c}	274.19 ± 7.13 ^{c}	331.15 ± 5.12 ^{b}
Acetone	18.72 ± 0.54 ^{e}	20.17 ± 0.2 ^{g}	8.86 ± 0.54 ^{f}	205.39 ± 2.4 ^{a}	33.23 ± 0.12 ^{g}	79.26 ± 2.9 ^{g}	77.03 ± 1.35 ^{f}	43.66 ±1.6 ^{f}
Acetone 50%	41.44 ± 0.26 ^{b}	119.15 ± 0.87 ^{a}	83.92 ± 1.03 ^{a}	76.55 ± 0.85 ^{b}	176.53 ± 1.45 ^{a}	360.54 ± 1.85 ^{b}	370.94 ± 1.12 ^{a}	353.13 ±8.97 ^{a}

The values are expressed as the mean values ± standard deviation (n = 3). Data in the same column, with different superscript letters (a–g), indicating significant differences ($p < 0.05$).

Among the 50% mixtures of organic solvents and water, 50% acetone had the highest extraction efficiency for total phenolic compounds and flavonoids with approximately 119 mg GAE/g and 84 mg CAE/g, respectively. These findings were supported by the results of previous studies on black tea and black mate tea [15] and on macadamia (*Macadamia tetraphylla*) skin waste [5], which also found that 50% acetone had the highest extraction efficiency for phenolic compounds and flavonoids. Although only 37% of proanthocyanidins could be extracted using 50% acetone, in comparison with absolute acetone, the level of proanthocyanidins (76.5 mg CAE/g dried sample) was significantly higher than those extracted by water, methanol, ethanol or their combinations (Table 2).

The antioxidant properties of the Tuckeroo fruit were also significantly affected by the extraction solvents (Table 2). Results from four antioxidant assays, including DPPH, ABTS, FRAP, and CUPRAC revealed that absolute solvents had a significantly higher antioxidant capacity than that of water, however, they had significantly lower antioxidant properties in comparison with those of the mixture of 50% of these organic solvents with water. Among the mixtures of 50% of selected organic solvents with water, 50% acetone had the highest antioxidant power. These finding were supported by a previous study on lemon pomace [14] and pink-flesh guava by Musa et al. [16]. In general, 50% acetone was found to be the best solvent for extraction of phenolic compounds, their secondary metabolites as well as antioxidant properties. Therefore, 50% acetone was used for further characterisation of phytochemicals in different parts of the Tuckeroo fruit.

3.3. Phytochemical and Antioxidant Characteristics of the Tuckeroo Fruit

The phenolic profile of the Tuckeroo fruit and their different components are shown in Figure 2. It is interesting to note that the skin, the most dominant portion of the fruit, had the highest level of phenolic compounds (151.36 mg GAE/g dried sample), followed by the whole fruit (106.23 mg GAE/g), the seed (13.44 mg GAE/g), and the flesh (5.96 mg GAE/g). Levels of phenolic compounds in the fruit skin was almost double the levels found in the whole fruit, 10 times higher than that in the seed and 25 times higher than in the flesh.

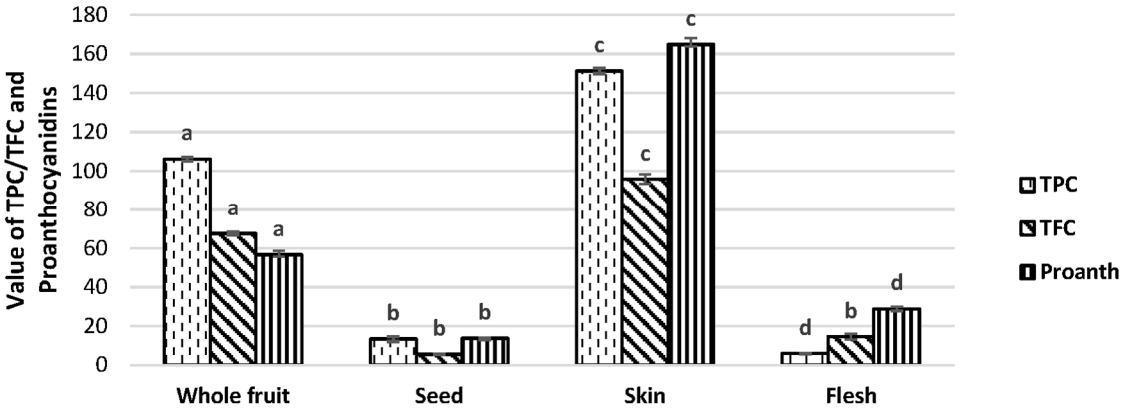

Figure 2. Phytochemical content of Tuckeroo whole fruits and its components. The vertical bars represent standard deviation ($n = 3$). Different superscript letters on top of each type of column indicate significant differences ($p < 0.05$).

Similarly, levels of flavonoids and proanthocyanidins in fruit skin (95.94 mg CAE/g, 164.86 mg CAE/g, respectively) were significantly higher than those in the whole fruit (67.67 mg CAE/g, 56.74 mg CAE/g , respectively), the seed (5.54 mg CAE/g, 13.77 mg CAE/g, respectively), or the flesh (14.61 mg CAE/g, 28.86 mg CAE/g, respectively). Flavonoid levels in the skin were 6.6 times and 17.5 times higher than those in the flesh and the seed, whereas levels of proanthocyanidins in the skin was 5.7 times higher than that in the flesh and approximately 12 times higher than the seed.

In comparison with other native Australian fruits, the Tuckeroo fruit skin had higher levels of phenolic compounds than those reported in the Illawarra plum (68 μM GAE/g fresh sample) [2,17,18] or the riberry (23.62 μM GAE/g fresh sample) [17]. As shown in Table 1, the Tuckeroo fruit skin accounted for approximately 77% of the whole fruit weight, therefore this skin is a great starting material for further extraction and isolation of phenolic compounds and their secondary metabolites; flavonoids and proanthocyanidins.

Antioxidant properties have been involved in the defense mechanisms of the organism against pathologies associated with the attack of free radicals [19]. The total antioxidant (ABTS), free radical scavenging (DPPH), and iron chelating properties (FRAP and CUPRAC) of Tuckeoo fruit were tested and the results are shown in Table 3. Tuckeroo whole fruit had a significant higher antioxidant capacity ABTS and DPPH than that of apricot fruit (*Prunus armeniaca*) [20] and guava fruit extracts [9]. The results showed that skin had the highest antioxidant capacity reflected from all four antioxidant assays. Antioxidant capacity of seed and flesh was not significantly different and was much lower than that of the skin. The different antioxidant capacities of the various parts of the fruit can be explained by the difference of total phenolic components and their secondary metabolites, which are the major contributors to antioxidant properties of plants. The strong positive correlations (Table 4) confirmed that antioxidant capacity of the Tuckeroo fruit extract was mainly driven from the total phenolics, flavonoids and proanthocyanidins

Table 3. Antioxidant capacity of Tuckeroo fruits.

Sample	FRAP (mg TE/g)	CUPRAC (mg TE/g)	DPPH (mg TE/g)	ABTS (mg TE/g)
Whole fruit	176.30 ± 0.41 ^{b}	370.12 ± 4.00 ^{b}	263.78 ± 2.95 ^{b}	346.98 ±1.59 ^{b}
Seed	19.84 ± 1.41 ^{c}	41.06 ± 0.76 ^{c}	34.98 ± 1.87 ^{c}	53.09 ± 2.16 ^{c}
Skin	287.22 ± 13.52 ^{a}	446.05 ± 11.87 ^{a}	641.13 ± 3.7 ^{a}	762.32 ± 0.45 ^{a}
Flesh	18.58 ± 0.1 ^{c}	29.32 ± 0.75 ^{c}	1.14 ± 0.18 ^{d}	21.05 ± 0.83 ^{d}

The values are expressed as the mean values ± standard deviation (n = 3). Data in the same column, with different superscript letters (a–d), indicating significant differences ($p < 0.05$).

Table 4. Correlations between phenolic compounds, flavonoids, proanthocyanidins and antioxidant properties.

Antioxidant Capacity	R^2 Values		
	TPC	TFC	Proanth
ABTS	0.9998	0.9823	0.9828
DPPH	0.9998	0.9802	0.9808
CUPRAC	0.9980	0.9841	0.9852
FRAP	0.9959	0.9901	0.9901

In comparison with other fruits, the antioxidant capacity in the Tuckeroo fruit skin was higher than that in either brown tea [21] or red grape fruit juice [22]. Therefore, future studies are recommended to extract and isolate phenolic compounds from the Tuckeroo fruit skin for further assessment of their biological properties due to their potent antioxidant capacity.

4. Conclusions

This study revealed that the Australian native Tuckeroo fruit is a rich source of phenolic compounds with potent antioxidant capacity. Among the fruit components, the Tuckeroo skin accounts for the largest weight proportion with over 77% of the total fruit weight and also had the highest levels of TPC, TFC and proanthocyanidins as well as the strongest antioxidant power. This study also found that organic solvents play a very important role in extraction of phytochemicals from the Tuckeroo fruit. Among the tested solvents, 50% acetone was the best solvent for the extraction of TPC, TFC and antioxidant properties, whereas absolute acetone was the best solvent

for extraction of proanthocyanidins. As the Tuckeroo skin has very high levels of phytochemicals and antioxidant capacity, future studies are recommended for further isolation and identification of phenolic compounds from this material for potential industrial applications.

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