Antioxidant Capacity and Bioactive Compounds of Selected Underutilized Fruit Species in Sri Lanka

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ABSTRACT

The aim of this study was to determine total antioxidant capacity (TAC), total phenolic content (TPC) and total flavonoid content (TFC) of six underutilized fruit species namely; *namnam (Cynometra cauliflora), lawulu (Pouteria campechiana), jambu (Syzygium aqueum), ambilla (Morus australis), billin (Averrhoa bilimbi)* and *veralu (Elaeocarpus serratus)* grown in Sri Lanka. Ferric reducing antioxidant power (FRAP) assay was used to evaluate TAC, whereas the TPC was determined by Folin-ciocalteu method and TFC was evaluated by a colourimetric method. Skin color, fruit fresh weight (FW), moisture content, total soluble solid (TSS) and pH of the six fruits were also determined. *Lawulu* had significantly the highest TAC (10.17±0.47 mgTE/gFW) and TPC (7.58±0.25 mgGAE/gFW) whereas, significantly the highest TFC (17.37±0.28 mgRE/gFW) was observed in *namnam* fruit. Total antioxidant capacity of *namnam* and *lawulu* were not significantly different. Significantly, the lowest TAC (0.49±0.01 mgTE/gFW) and TFC (0.17±0.04 mgRE/gFW) were recorded in *billin*, while *jambu* was recorded the lowest phenolic content (0.37±0.02 mgGAE/gFW). The flavonoid was not detected in *jambu*. Total antioxidant capacity showed positive significant correlations with the TPC (R^2=0.98, $p<0.001$) and TFC (R^2=0.66 $p<0.001$). *Lawulu* and *namnam* were suggested as good dietary antioxidant sources.

KEYWORDS: Antioxidant capacity, Flavonoids, Phenolics, Physical and chemical properties, Underutilized fruits

INTRODUCTION

Fruits and vegetables are a primary food source providing essential nutrients for sustaining life; they also contain a variety of phytochemicals such as phenolics, flavonoids, carotenoids and vitamin C, which provide important health benefits (Dragsted *et al.*, 1993; Hanasaki *et al.*, 1994; Wang *et al.*, 1996). Free radical induced oxidative stress has been associated with several cellular toxic processes including oxidative damage to protein and DNA, membrane lipid oxidation, enzyme inactivation and gene mutation that may lead to chronic diseases (Poulsen *et al.*, 1998).

Intake of sufficient amounts of antioxidants is necessary to prevent free radical induced oxidative stress. There is an abundance of evidence that regular consumption of fruits and vegetables is associated with reduced risks of chronic diseases, such as cancer, cardiovascular disease, diabetes and Alzheimer's disease (Doll, 1990; Dragsted *et al.*, 1993). It has been reported that the majority of the antioxidant capacity of fruits and vegetables may come from phenolics, antioxidant pigments and antioxidant vitamins (Eberhardt *et al.*, 2000).

Sri Lanka has over 60 varieties of underutilized fruit crops (Dahanayake, 2015). Unfortunately, most Sri Lankans are not aware about those underutilized fruits and their nutritional and health benefits. Even though fruit consumption is very important, Sri Lanka's per capita fruit consumption remains far below the required average daily intake of 40 g (Dahanayake, 2015).

Underutilized fruit consumption is one of effective solutions for this fruit shortage as well as prevention of such chronic diseases in Sri Lanka. It has been revealed that *katuanoda* (*Annona muricata*) is a type of unexploited fruit which has the ability to kill cancer cells and is 10,000 times more effective than strong chemotherapy drugs, all without any harmful side effects. 'Annonacin' is one compound with cancer healing properties in *katuanoda* (Dahanayake, 2015).

Recent researches reported that among commercialized fruits, blueberries, cranberries, blackberries, raspberries, strawberries, apple, cherries, blackplums, avocados and pears have higher amounts of antioxidants and categorized these fruits as top ten fruits in the world with high antioxidant activities. There may be more health benefits in underutilized fruits in Sri Lanka than commercialized fruits. However, studies on health benefits of underutilized fruits are very few so far.

Therefore, this study was carried out to determine the antioxidant capacity and bioactive compounds of underutilized fruit species grown in Sri Lanka.

MATERIALS AND METHODS

Location

The experiment was carried out in the Laboratory of Department of Plantation Management, Faculty of Agriculture and Plantation Management, Wayamba universityof Sri Lanka, Makandura, Gonawila (NWP) from January to May 2016.

Materials

Six species of underutilized fruits namely, *namnam*, *lavalu*, *jambu*, *ambilla*, *billin* and *veralu* were employed for this study (Table 1). Well ripen fruits of each species were harvested from home gardens and directly transported to the laboratory under cool condition. They were stored at -20 °C until analysis. A Completely Randomized Design (CRD) with three replicates was used in the experiment.

Chemicals and Reagents

Folin-ciocalteu reagent, Gallic acid, Ferric chloride ($FeCl_3$ $6H_2O$), 2,4,6-tripyridyl-2-try-azine (TPTZ), 6-hydroxy-2,5,7,8-tetra methyl-chroman-2-carboxilic acid (Trolox) and Rutin were purchased from sigma Aldrich Chemical Co. (St. Louis, Mo). All other chemicals used were of analytical grade.

Determination of Physical and Chemical Properties

Fresh weight and skin colour of each fruit species (n=10) were recorded separately. The moisture content of samples was determined by drying at 80±2 °C until they reached to a constant weight. As chemical properties, total soluble solid content (TSS) and pH of fruit juice were determined by using a digital refractometer (DR 103L, RF.5190, Holland) and a digital pH meter (995414, Romania, Europe) respectively.

Extraction of Phytochemicals

All fruit samples were extracted and analyzed in triplicate. Phenolic compounds were extracted by modifying previously published methods (Sun *et al.*, 2002) as described below. Briefly, 10 g of fresh weight of the edible part of fruits was weighed and cut into small pieces with a sharp knife. Then, the sample was homogenized with chilled 80% methanol using a fruit blender for 5 min under chilled conditions. The sample was then further homogenized using a homogenizer (Witeg, 0400189139T002, German) for an additional 1 min. The homogenates were filtered through no 1 Whatman filter paper on a Buchner funnel under vacuum. The filtrate was then recovered with chilled 80% methanol to a final volume of 50 mL.

Determination of Total Phenolic Content (TPC)

Total phenolic contents of the fruit extracts were determined according to the Folin-ciocalteu method as described by Abeysinghe *et al.* (2007). Briefly, 4 mL of distilled water and 0.5 mL of properly diluted fruit extract were placed in a 15 mL Teflon centrifuge tubes. Folin- ciocalteu reagent (0.5 mL) was added to the solution and allowed to react for 3 min. The reaction was neutralized with 1 mL of saturated sodium carbonate. Absorbance at 760 nm was read after 2 h, using a spectrophotometer (Shimadzu, UV Mini 1240, Japan). Gallic acid was used as the standard and data were expressed as mg Gallic acid equivalents (GAE)/g fresh weight (FW) of each fruit species.

Determination of Total Flavonoid Content (TFC)

Total flavonoid content (TFC) was determined by the colourimetric method (Liu *et al.*, 2002) with slight modifications. A volume of 0.5 mL of a known dilution of fruit extract was added to Teflon centrifuge tubes containing 3.5 mL of distilled water, mixed with 0.3 mL of 5% $NaNO_2$. After 6 min, 0.3 mL of 10% $Al(NO_3)_3.6H_2O$ solution was added. The mixture was allowed to stand for another 6 min, and then, 2 mL of 2M NaOH was added. The reaction mixture was diluted with 1.4 mL of distilled water and the absorbance of the mixture at 510 nm was measured immediately using a spectrophotometer (Shimadzu, UV Mini 1240, Japan). Total flavonoid content was calculated using the standard rutin curve and expressed as milligrams of rutin equivalents (RE) per g of fresh weigh.

Determination of Total Antioxidant Capacity (TAC)

Total antioxidant capacity was determined using ferric reducing antioxidant power (FRAP) assay as described by Benzie and Stain (1996). Briefly, the FRAP reagent was freshly prepared by mixing 25 mL of 300 mM sodium acetate buffer (pH 3.6), 2.5 mL of 10 mM TPTZ solution and 2.5 mL of 20 mM ferric chloride solution. Absorbance at 593 nm was measured 4 min after mixing of 100 μL of fruit extract with 900 μL of FRAP reagent, using a spectrophotometer (Shimadzu, UV Mini 1240, Japan). Total antioxidant capacity was expressed as mg trolox equivalents (TE)/gFW.

Statistical Analysis

Values shown in Tables and Graphs are the mean of three replicates±SD.

Table 1. Information on selected fruit species

Common name	Botanical name	Family	Date of collection
Namnam	*Cynometra cauliflora*	Fabaceae	07th February 2016
Lawulu	*Pouteria campechiana*	Sapotaceae	09th February 2016
Jambu	*Syzygium aqueum*	Myrtaceae	18th March 2016
Ambilla	*Morus australis*	Moraceae	26th March 2016
Billin	*Averrhoa bilimbi*	Oxalidaceae	06th April 2016
Veralu	*Elaeocarpus serratus*	Elaeocarpaceae	23th May 2016

Data were subjected to analysis of variance (ANOVA), and means were compared by Turkey Multiple Rang Test using SAS (Version 9). Correlation and regression analyses of total antioxidant versus total phenolics or total flavonoids were performed to show correlations and their significance.

RESULTS AND DISCUSSION

Physical and Chemical Properties

Fruit skin colour, fruit weight, water content, total soluble solid (TSS) and pH of selected six underutilized fruit species are presented in Table 2. Fruit skin colour of selected species varied from light green, yellow to reddish purple. The highest fruit weight (242.1±99.2 g) was observed in *lawulu* whereas, the lowest fruit weight (0.4±0.1 g) was observed in *ambilla*. Water content (%) varied from 68.5±0.8% to 95.3±0.3%. Acidic fruit juice was recorded in all selected underutilized fruit species except *lawulu*. *Billin* was the most acidic fruit (pH 1.92±0.02) recorded among the selected fruit species. *Lawulu* had the highest pH value (5.17±0.01) and TSS value (17.33±0.47) whereas the lowest TSS value (3.95±0.20) was observed in *veralu* fruit (Table 2).

Total Phenolic Content

The total phenolic content of the six selected underutilized fruit species is summarized in Figure 1. Among all fruit species tested, *lawulu* had the highest (7.58±0.25 mgGAE/gFW) phenolic content, followed by *namnam*, *veralu*, *ambilla*, *billin* and *jambu*. There was no significant variation in the contents of total phenolics observed between *billin* (0.38±0.05 mgGAE/gFW) and *jambu* (0.37±0.02 mgGAE/gFW) which had the lowest total phenolic contents among all selected fruit species (Figure 1). Wu *et al.* (2004) reported blueberry had the highest phenolic contents (7.95±0.96 mgGAE/gFW) among 24 commercial fruit species. According to results the phenolic content of *lawulu* is high as that of blueberry.

Total Flavonoid Content

There was significant variation in the contents of total flavonoids in all selected underutilized fruit species (Figure 2). The flavonoid was not detected in *jambu*. The highest total flavonoid content (17.37±0.28 mgRE/gFW) was observed in *namnam* among all the selected fruit species. *Billin* reported the lowest level of flavonoids (0.17±0.04 mgRE/gFW).

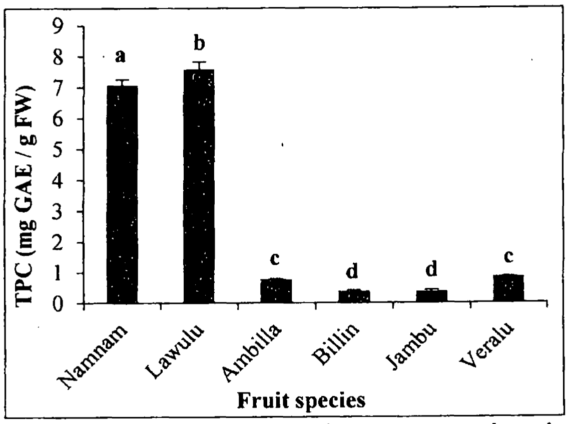

Figure 1. Total phenolic contents in six selected underutilized fruit species. *Means with the same letter represent non-significant differences (p<0.05). TPC- Total phenolic conent FW- Fresh weight, GAE- Gallic acid equivalents*

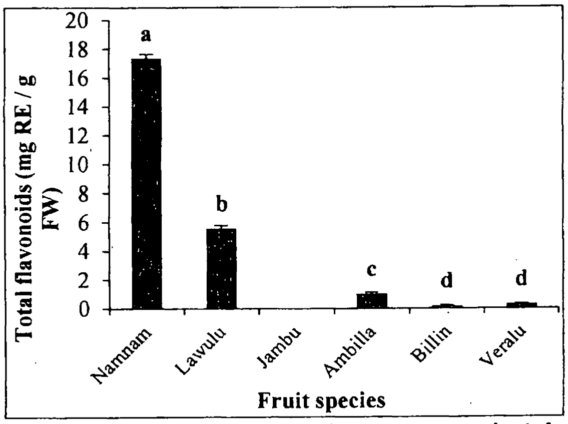

Figure 2. Total flavonoids content in six selected underutilized fruit species. *Means with the same letter represent non-significant differences (p<0.05). FW- Fresh weight; RE- Rutin equivalents*

Total Antioxidant Capacity (TAC)

The total antioxidant capacity varied significantly among selected underutilized fruit species (Figure 3). *Lawulu* (10.17±0.47 mg TE/ g FW) and *namnam* (9.66±0.28 mgTE/gFW) had significantly higher TAC than other selected fruit species.

Table 2. Physical and chemical properties of selected underutilized fruit species

Fruit species	Skin color	Fruit weight (g)	Water content (%)	pH	TSS
Namnam	Brownish yellow	28.2 ± 9.6	79.8 ± 0.8	3.16 ± 0.06	14.78 ± 0.65
Rawulu	Yellowish orange	242.1 ± 99.2	68.5 ± 0.8	5.17 ± 0.01	17.33 ± 0.47
Jambu	Reddish pink	6.3 ± 0.7	94.8 ± 0.1	2.92 ± 0.02	5.67 ± 0.21
Ambilla	Reddish purple	0.4 ± 0.1	88.7 ± 1.8	3.34 ± 0.03	9.68 ± 0.35
Billin	Light green	11.7 ± 1.6	95.3 ± 0.3	1.92 ± 0.02	5.21 ± 0.06
Veralu	Olive green	8.6 ± 0.9	75.1 ± 0.4	3.51 ± 0.04	3.95 ± 0.20

TSS- Total Soluble Solid

Billin fruit showed the lowest TAC (0.49±0.01 mgTE/gFW), however, it was not significantly different from the TAC values of *jambu* and *ambilla* fruits (Figure 3).

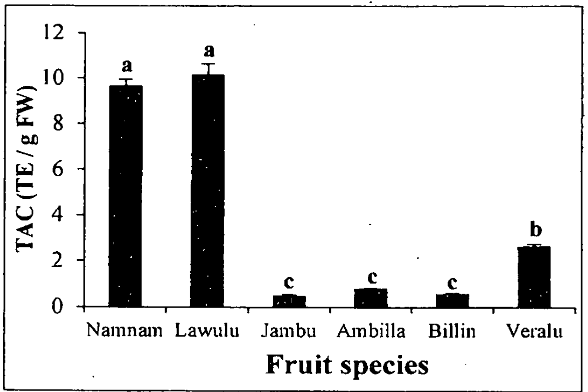

Figure 3. Total antioxidant capacities in six selected underutilized fruit species *Means with the same letter represent non-significant differences (p<0.05); TAC- Total Antioxidant Capacity; TE-Trolox Equivalents; FW- Fresh Weight.*

Correlations between TAC and Phenolics

In the selected underutilized fruit species, TAC showed positive significant correlations with the total phenolics (R^2=0.98; p<0.001) (Figure 4) and total flavonoids (R^2=0.66; p<0.001). These strong correlations suggest that the phenolic components contribute significantly to the fruit antioxidant capacity. A similar strong relationship between the phenolic content and TAC of fruit was observed by Sun *et al.* (2002).

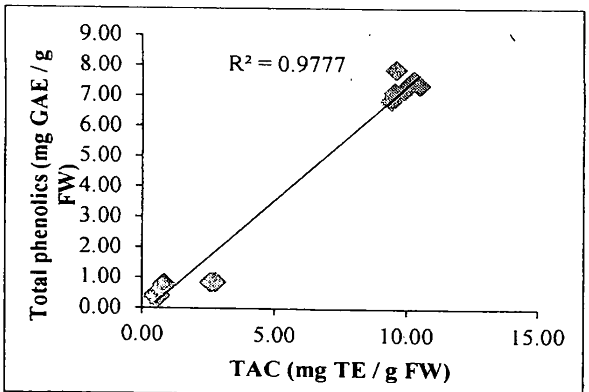

Figure 4. The correlation between TAC and total phenolic contents of selected fruits. *TAC-Total antioxidant capacity; FW- Fresh weight; TE-Trolox equivalents; GAE- Gallic acid equivalents*

CONCLUSIONS

In this study, selected underutilized fruit species showed different levels of phenolic content and antioxidant capacity. Significantly higher phenolic compounds and total antioxidant capacities were observed in *lawulu* and *namnam* fruits when compared to other selected fruits. Very low levels of phenolic compounds and antioxidant capacity were recorded in *billin* and *jambu* fruits. The flavonoids were not detected in *jambu*. A positive correlation existed between TAC and phenolic compounds indicating that the phenolic compounds contribute significantly to the fruit antioxidant capacity. It is revealed that *lawulu* and *namnam* are potential sources of potent dietary antioxidants.

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