

Phenolic Content and Antioxidant Capacity of Selected Underutilized Fruits Grown In Sri Lanka

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ABSTRACT: The present study was carried out to evaluate the total phenolic content and antioxidant properties of four selected underutilized fruits species: *Manilkara hexandra* (Roxb.) Dubard (*Palu*), *Salacia chinensis* L. (*Himbutu*), *Schleichera oleosa* (Lour.) Oken (*Koan*) and *Eugenia uniflora* (Cherry) grown in Sri Lanka. Antioxidative constituents were extracted into water and the antioxidant activity of fruit extracts was assessed using biochemical assays: 1,1-diphenyl-2(2,4,6-trinitrophenyl) hydrazine (DPPH) radical scavenging assay (n=3) and ferric reducing power assay (FRAP)(n=3). The total phenolic content was estimated using Folin-Ciocalteu's colorimetric assay and expressed as milligrams of gallic acid equivalents per 100 g of both fresh and dry weight of fruit. The fruit extracts tested exhibited a broad range of antioxidant activity as measured by DPPH radical scavenging and FRAP assays. The highest antioxidant activity was exhibited by *Manilkara hexandra* (*Palu*) in both assays performed. Furthermore, it was observed that the antioxidant activity as measured by the DPPH radical scavenging activity and FRAP assay strongly correlated ($R^2 = 0.997$) with total phenol content for extracts of all four underutilized fruit species tested. Therefore, the antioxidant activity of the fruits could be attributed to phenolic compounds present in the samples. This is the first time that antioxidant potential of some of the above fruits is reported.

Keywords: Antioxidant activity, DPPH, FRAP, Total phenolic content, Underutilized fruits of Sri Lanka

INTRODUCTION

Fruits are rich sources of phytochemicals especially phenolic antioxidants. Antioxidants from plant materials terminate the action of free radicals which are spontaneously generated in the body thereby protecting the body from a myriad of diseases (Lai *et al.*, 2001). Increased intake of fruits and vegetables can be considered an important means of increasing the concentration of antioxidants in plasma (Maurizio and Bruno, 2006). Fruit and vegetable consumption has been linked to reduced cardiovascular diseases and stroke. Furthermore, a new scientific base is emerging to support the protective role of fruits and vegetables in prevention of cataract formation, age related macular degeneration, chronic obstructive pulmonary disease, diverticulosis and other digestive disorders and hypertension. Phenolic compounds from plants possess strong antioxidant activity thus help protect the body cells against oxidative damage caused by free-radicals (Kahkonen *et al.*, 1999). Beneficial effects

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of phytochemicals are mainly attributed to their antioxidant potential thus it is of paramount importance to assess the antioxidant efficacy of fruit extracts. The total antioxidant potential (TAP) is a relevant tool for investigating the relationship between dietary antioxidants and pathologies induced by the oxidative stress (Pisoschi and Negulescu, 2011). This was confirmed by the data obtained from a population-based control study, proving that dietary TAP resulted in reduced risk of both cardiac and distal gastric cancer (Serafini *et al.*, 2002). The evaluation of the total antioxidant capacity (TAC) may be an appropriate tool to determine the additive antioxidant properties of plant foods (Pellegrini *et al.*, 2003).

It is estimated that there are more than 230 fruit species belonging to 57 plant families grown in Sri Lanka, most of which remain underutilized. Many of the underutilized species such as, *Nelli*, *Beli*, wood apple and *Annona* have long been used for their medicinal properties (Pusupakumara *et al.*, 2007). These fruit species are rich in phytochemicals such as carotenoids, sterols, saponins, terpenes, anthocyanins, phenolic acids, catechins and other polyphenolics. Phytochemical components such as phenolics, ascorbic acid and carotenoids may have direct influence over the radical-scavenging potential (Diplock *et al.*, 1998).

Manilkara hexandra (Roxb.) Dubard, *Palu* belongs to family Sapotacea, dominated in tropical dry mixed evergreen forests (Wadugodapitiya *et al.*, 2013), especially in Wanni district and southeastern part of the country. *M. hexandra* is considered as one of the most valuable timber species in the forest. The fruits are useful for treating bronchitis, urethrorrhea and leprosy (Longman, 2004). The bark is also recommended for fever, jaundice, helminthiasis and flatulence (Gopalkrishnan *et al.*, 2014). The roots are used to treat headache (Rao *et al.*, 2010) while seeds are used in pharmacology. However, there are no reports on antioxidant efficacy of fruit pulp of *M. hexandra*. The present study was carried out to evaluate the antioxidant properties of *M. hexandra* *Salacia chinensis* L. (*Himbutu*), *Schleichera oleosa* (Lour.) Oken (*Koan*) and *Eugenia uniflora* (Cherry). *Salacia chinensis* L. (*Himbutu*) belonging to the family Hippocrateaceae is a woody climbing shrub that thrives well in wetlands of Sri Lanka. All parts of the plant are traditionally used in Ayurveda: ripe fruits and roots are used in indigenous medicine to treat diabetes mellitus, amenorrhoea, dysmenorrhoea and sexually transmitted diseases. Stems of *S. chinensis* have been extensively used for carminative, emmenagogue, blood tonic, cardio tonic, anti-inflammatory, antidiabetic purposes and the treatment of rheumatism, leucorrhoea and stimulated lochial excretion (Govindaraj *et al.*, 2009). *Schleichera oleosa* (Lour.) Oken a member of family Sapindaceae is commonly known as *Koan* in Sri Lanka. It has potential of anticancer, antioxidant and antimicrobial activities and contains various polyphenolic compounds (Bhatiya *et al.*, 2013). The stem bark of *S. oleosa* is used as astringent and antipyretic, also useful in pruritus, adenitis, ripening boils, nostalgia, arthralgia, malaria, inflammations and ulcers (Shrinivas and Baboo, 2013). In 2015, Thatavong and Athipornchai concluded that presence of terpinoids, flavonoids, tannins and steroids in methanolic extracts of fruits of *S. oleosa* is evident for the antioxidant action of fruit. *Eugenia uniflora* L. family Myrtaceae commonly known as Brazilian cherry or Cherry, cultivated mostly in home gardens and small-scale agriculture farms in Sri Lanka. Besides being highly desirable for fresh consumption, the *E. uniflora* fruits are also used for juice and ice cream production (Celli *et al.*, 2011). Its leaves are used in popular medicine as infusion in the treatment of fever, rheumatism, stomach diseases and disorders of the digestive tract, hypertension, yellow fever, and gout. It may also reduce weight, blood pressure, and serve as a diuretic (Adebajo *et al.*, 1989).

The most commonly used methods for measuring antioxidant activity are those that involve the generation of free radical species which are then neutralized by antioxidant compounds

(Arno *et al.* 2001). The objective of the present study was to evaluate four selected underutilized fruit species grown in Sri Lanka as a source of natural antioxidants using in vitro biochemical assays.

MATERIALS AND METHODS

Samples

Manilkara hexandra (Roxb.), *Salacia chinensis* (Himbutu), *Schleichera oleosa* (Lour.) Oken (Koan), *Eugenia uniflora* (Cherry) fruits were collected from their natural habitats in Badulla and Kilinochchi districts. Fig. 1 illustrates the fruit species selected for the study.

Chemicals and reagents

Folin Ciocalteu's reagent, gallic acid, 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH), 2, 2-azobis (2-ethylbenzothiazoline-6-sulfonic acid), 2, 4, 6-tris (2-pyridyl)-1, 3, 5-triazine (TPTZ), ethyl acetate, methanol and concentrated sulfuric acid were purchased from Sigma Aldrich, USA. All chemicals were of analytical or higher grades.

Preparation of fruit extracts

The edible portion of the fruits (10 g) was homogenized with accurately measured distilled water (30 ml). The homogenate was placed on a reciprocating shaker at 150 rpm for 90 min at ambient temperature. The mixture was centrifuged at 4,500 g for 10 min and the supernatant was filtered through Whatman number 1 filter paper. The extraction was repeated twice with the residue and the supernatants were combined, lyophilized and stored at -20 °C until analysis.

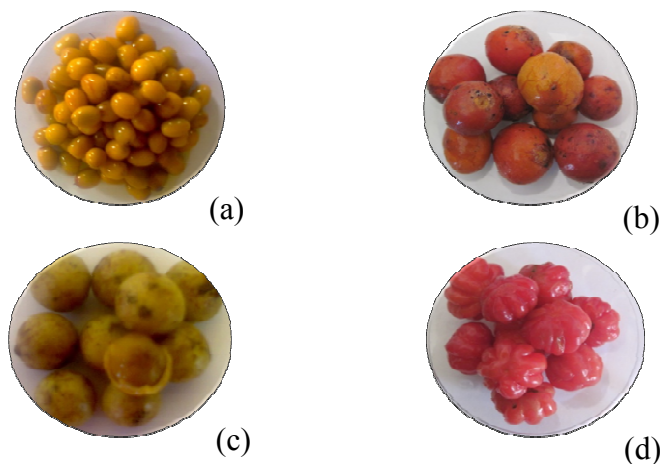


Fig. 1. Fresh fruits of *Manilkara hexandra* (a), *Salacia chinensis* (b), *Schleichera oleosa* (c) and *Eugenia uniflora* (d).

Total Phenolic Content of Fruit extracts

Total phenolic content of aqueous extracts was determined using Folin-Ciocalteu's colorimetric method explained by (Hassimotto *et al.*, 2009) using gallic acid as the standard. The absorbance was measured using spectrophotometer (mrc, UV-18, UK) at 765 nm and expressed as milligrams of gallic acid equivalents (mg GAE) per 100 g of fresh weight (FW) and dry weight (DW).

DPPH Radical Scavenging Activity of Fruit extracts

The radical scavenging activity of the aqueous extracts was assessed using modified 1, 1-diphenyl-2-picrylhydrazyl (DPPH) assay (Jagtap *et al.*, 2010). The stock solution was prepared by dissolving 10 mg of DPPH in 25 ml of methanol. The working solution was prepared by mixing 5 ml of stock solution with 60 ml methanol to obtain an absorbance of 0.348 ± 0.02 at 517 nm. Different dilutions of the extracts were prepared in triplicate. An aliquot of 800 μ L of 0.093 mM DPPH radical in methanol was added to a test tube containing 400 μ L, 200 μ L and 100 μ L of the sample separately. The reaction mixture was vortexed for 30 s and left to stand at room temperature (25 °C) in dark for 30 min. The percentage of remaining radical in medium is calculated as the absorbance of the sample divided by that of DPPH control at the same time multiplied by 100. The amount of sample needed to decrease the initial DPPH concentration by 50%, EC_{50} , was calculated.

$$EC_{50} = \{1 - (A_{\text{sample}}/A_{\text{control}})\} * 100$$

Where A_{sample} = Absorbance of sample at 517 nm
 A_{control} = Absorbance of Control at 517 nm

Ferric reducing antioxidant power of Fruit extracts

Ferric reducing antioxidant power (FRAP) assay was performed according to Benzie and Strain (1996) and Faria *et al.* (2005) with some modifications. Briefly, the working FRAP reagent produced by combination of 150 mM acetate buffer (pH 3.6), 5 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) and 10 mM $FeCl_3 \cdot 6H_2O$ at 10:1:1 ratio prior to use and heated to 37 °C in a water bath for 10 min. One milliliter of FRAP reagent at 37 °C was mixed with 50 μ L of sample and the solution was vortexed for 30s. After 4 min absorbance was read at absorbance 593 nm using UV-Visible spectrophotometer (Shimadzu, UV, Japan). The results were expressed as mM of Fe^{2+} /g dry weight using a Fe^{2+} (1.25- 20 mM) standard curve.

Statistical analysis

Data are represented as mean \pm SD (n=3). The results were analyzed using Minitab 15 software at significant level of $P < 0.05$.

RESULTS AND DISCUSSION

Total Phenolic Content of Fruit extracts

The TPC of fruits ranged from 136.4 ± 0.007 to 316.54 ± 0.042 mg gallic acid equivalents per 100 g fresh fruit with *Manilkara hexandra* showing the highest TPC (Table 1).

Table 1. Total phenolic content of fruits expressed on fresh and dry weight basis

Fruit species	mg of GAE/100g Fresh weight basis	mg of GAE/100g Dry Weight basis
<i>Palu (Manilkara hexandra)</i>	316.54 ± 0.04^d	1430.67 ± 0.03^d
<i>Himbutu (Salacia chinensis)</i>	291.73 ± 0.01^c	1376.09 ± 0.13^c
<i>Koan (Schleichera oleosa)</i>	223.67 ± 0.05^b	989.69 ± 0.04^b
Cherry (<i>Eugenia uniflora</i>)	136.4 ± 0.01^a	847.21 ± 0.08^a

Data are presented as Mean \pm SD (n=3)

Mean values in a column superscripted by different letters are significant at $p < 0.05$

The aqueous extracts of *Palu (Manilkara hexandra)* and *Himbutu (Salacia chinensis)* showed the highest total phenolic content while the lowest was observed in cherry (*Eugenia uniflora*). This implies that there is good antioxidative property in *palu (Manilkara hexandra)* and *Himbutu (Salacia chinensis)*. The phenolic content of *Palu* fruit has not been reported in literature, however, the TPC of stem bark, leaves and root extracts has been reported.

Chavan *et al.* (2013) reported that the TPC of aqueous extract of *Himbutu (S. chinensis)* was 122 ± 0.44 mg GAE/100g FW. According to Kubola *et al.* (2011), the TPC of methanolic extracts of freeze dried *Koan (S. oleosa)* was reported to be 739 mg GAE g/100g DW of fruit. Bagetti *et al.* (2011) reported that TPC of Brazilian cherry (*E. uniflora*) to be 210 ± 3 mg GAE/100g FW. The TPC of four fruit species studied, are higher than that of recorded in literature. These variations might be attributed to differences in growing environment, season, cultivar, soil and rainfall (Manach *et al.*, 2004). When compared with other major fruit crops, these underutilized fruits recorded high TPC. The TPC in mango ranged from 32.06 to 139.71 mg GAE/100g FW (Liu *et al.*, 2013), 47.9 mg GAE/100g FW in pineapple, 57.6 mg GAE/100g FW in papaya (Luximon-Ramma *et al.*, 2003). As Folin-Ciocalteu reagent method was employed for TPC measurement, other reducing agents such as ascorbic acid, sugars and aromatic amines may also affect the results (Magalhães *et al.*, 2008).

DPPH Radical Scavenging activity of Fruit extracts

DPPH radical scavenging activity was expressed as percent inhibition at different concentration (750 ppm, 1500 ppm, 3000 ppm) of each extract. The DPPH radical scavenging capacity was expressed as IC₅₀ (Table 2).

Table 2. DPPH radical scavenging ability expressed as IC₅₀ value

Plant extract	DPPH Radical Scavenging Activity (% inhibition)			
	750 ppm	1500 ppm	3000 ppm	IC ₅₀ value
<i>Palu (M. hexandra)</i>	54.94 ± 1.82	64.22 ± 0.40	68.93 ± 1.82	274 ± 1.34 ^d
<i>Himbutu (S. chinensis)</i>	48.41 ± 5.68	54.59 ± 2.43	62.93 ± 5.68	1260 ± 4.5 ^c
<i>Koan (S. oleosa)</i>	46.55 ± 2.23	51.53 ± 1.42	61.06 ± 2.23	1580 ± 1.96 ^b
Cherry (<i>E. uniflora</i>)	12.35 ± 2.64	34.77 ± 0.60	53.59 ± 2.64	2797 ± 1.96 ^a

Data are presented as Mean ± SD (n=3)

Aqueous extract of cherry (*E. uniflora*) showed the highest IC₅₀ value indicating the lowest antioxidant capacity while *Palu (M. hexandra)* showed the highest antioxidant capacity. There are no literature data available on the antioxidant capacity of fruit pulp of *Palu*.

Ferric Reducing Antioxidant Power of Fruit extracts

Standardization of FRAP assay was carried out by reacting TPTZ with FeSO₄. The ferric reducing antioxidant potential of the samples was calculated using Fe²⁺ standard curve and expressed as equivalent to that Fe²⁺ mM /g of fresh extract. FRAP values of the samples tested ranged from 0.50 ± 0.00 to 4.23 ± 0.01 Fe²⁺ mM /g of extract. Aqueous extract of *Palu (M. hexandra)* displayed significantly (p < 0.05) higher ferric reducing antioxidant potential than the other fruit extracts as shown in Table 3.

Table 3. - FRAP value of aqueous fruit extracts

Fruit	FRAP assay equivalent to Fe ²⁺ mM / g Fresh Weight basis
<i>Palu (M. hexandra)</i>	4.23 ± 0.00 ^a
<i>Himbutu (S. chinensis)</i>	3.13 ± 0.02 ^b
<i>Koan (S. oleosa)</i>	1.12 ± 0.01 ^c
Cherry (<i>E. uniflora</i>)	0.50 ± 0.00 ^d

Data are presented as Mean ± SD (n=3)

Correlation between Antioxidant activity and Total Phenol Content

Many studies have demonstrated a strong relationship between total phenolic content and antioxidant activities in different foods (Yang *et al.*, 2004; Kriengsak *et al.*, 2006). In the present study, a fairly strong correlation was observed between total phenolic content of *Palu (Manilkara hexandra)* and DPPH and FRAP of *Palu* extracts (Figures 2 and 3).

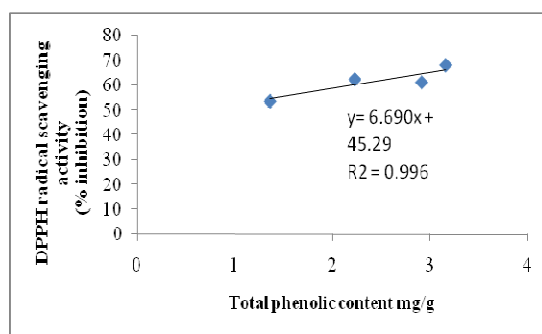


Fig.2. Correlation between DPPH radical scavenging activity and Total Phenol Content of each fruit extract in different concentration

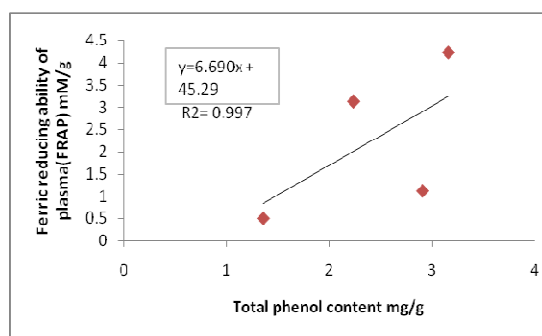


Fig. 3. Correlation between FRAP assay and Total Phenol Content of each fruit extract in different concentration

A fairly strong positive correlation is observed between TPC with DPPH radical scavenging activity ($R^2 = 0.996$) as well as a strong correlation ($R^2 = 0.997$) was observed for TPC and FRAP values obtained for fruit extracts. The strong correlation between TPC and antioxidant capacity as measured by DPPH radical scavenging capacity and FRAP indicate that the phenolic compounds present in the fruits are mainly attributed for the observed antioxidant properties.

CONCLUSIONS

Out of the four selected underutilized fruit species for the evaluation of antioxidant activities, *Manilkara hexandra* exhibited the highest antioxidant capacity. The phenolic content displayed strong positive correlation with antioxidant activity of the species namely, *Manilkara hexandra* followed by the *Salacia chinensis*. The differences in the antioxidant activities among the fruits could be attributed to their differences in phenolic contents and compositions present in the samples.

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