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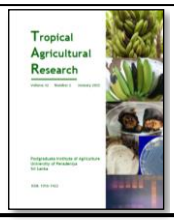

Antioxidant Efficacy of Selected Underutilized Fruit Species Grown in Sri Lanka

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ABSTRACT

The present study was carried out to determine the vitamin C content, total phenolic content (TPC), antioxidant efficacy and total monomeric anthocyanin content (TMAC) of twenty-one underutilized fruit species grown in Sri Lanka. Approximately 5 g of fruit homogenates was used to determine the vitamin C content while lyophilized aqueous extracts were used to perform in-vitro antioxidant assays. Folin-Ciocalteu's colorimetric assay was used to determine TPC. The 2,2-diphenyl-1-pycrylehydrazyl (DPPH) radical scavenging, 2,2'- azinobis- 3-ethylbenzothiazoline-6-sulphonic acid (ABTS) total antioxidant activity and ferric reducing antioxidant power (FRAP) assays were used to determine antioxidant efficacy of fruit extracts, while the pH differential method was used to determine TMAC. The highest TPC (104 mg of gallic acid equivalents/g on dry weight (DW) basis), reducing power (155 µmol of ferrous sulphate equivalents/g DW), and the lowest IC_{50} (0.0004 g of DW/mL) were recorded in *Phyllanthus emblica* and obtained the first rank in overall antioxidative properties among the tested fruit species. Fruits with the highest total antioxidant activity were *Flacourtia indica*, *Morus alba*, *Phyllanthus emblica* and *Syzygium caryophyllatum*. The highest TMAC was found in *S. caryophyllatum* (79 mg of cyanidin-3-glucoside (C3G)/g DW) and the lowest in *Elaeocarpus. serratus* (0.29 mg C3G/g DW). Results obtained in this study can be used as baseline data to carry out further research and formulation of food compositional tables. The underutilized fruit species tested could be used as sources of natural antioxidants in nutraceuticals and in novel food product development.

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INTRODUCTION

A mounting volume of epidemiological evidences exist to the effect that intake of fruits and vegetables is inversely related to the risk of chronic diseases such as cancer, coronary heart diseases, atherosclerosis (Brecht *et al*., 2008), inflammation, brain dysfunction and arthritis (Leong and Shui, 2001). This inverse relationship between fruit and vegetable consumption and disease incidences is believed to be due to the presence of a plethora of phytochemicals bearing antioxidative properties (Heinonen and Meyer, 2002), which are synthesized in plants as an adaptation to physical, biological and chemical challenges such as pest and disease incidences, water loss and extreme temperatures (Brecht *et al*., 2008). These antioxidative compounds present in plants such as flavonoids, phenolic acids, tocopherols, ascorbic acid and carotenoids protect plants from oxidative stress and various other stresses by acting as free radical scavengers, metal chelators, hydrogen donors, singlet oxygen quenchers and reducing agents (Rice-Evans *et al*., 1997). Plants being major parts of the human diet, these phytochemicals present in them contribute to protect human body from various free radical induced chronic diseases. Among thousands of phytochemicals present in plant matrix, phenolic acids and flavonoids are considered the most widely distributed antioxidants (Rice-Evans *et al*., 1997).

Anthocyanins, one of the major groups of flavonoids present in the vacuolar sap of the coloured plant parts and fruits (Manach *et al*., 2004). Those possess various health benefits including anti-inflammatory, anticancer, anti-diabetic properties, among others (Rossi *et al*., 2003). Among six types of anthocyanidins present in food, cyanidin is the most common (Manach *et al*., 2004).

Vitamins C, E and carotenoids are believed to have antioxidant activities via multiple mechanisms (Rutkowski and Grzegorczyk, 2007). Vitamin C reduces the risk of arteriosclerosis, cardiovascular diseases and some forms of cancer (Lee and Kader, 2000).

Being a tropical country, Sri Lanka is rich in plant diversity. Many of the plants are with food uses and also used in traditional medicine, while these fruit plants play a vital role in the diet of low-income groups. However, their antioxidant potential is not fully exploited, thus records are scanty in literature. In this light, the present study was conducted to evaluate antioxidant efficacy of twenty-one underutilized fruit species grown in Sri Lanka.

METHODOLOGY

Fruit Samples

Healthy and ripe fruits of twenty-one underutilized fruit species from fifteen plant families (Table 1) were collected from the Fruit Research and Development Institute, Horana, and home gardens in Upcountry Intermediate Zone of Sri Lanka (IU3). The collected fruit samples during the peak production period of the years of 2013 and 2015 were transported to the analytical laboratory of the Regional Agriculture Research and Development Centre, Bandarawela, under cold conditions, sorted for defects, washed with running tap water, rinsed with distilled water, drained at room temperature (RT) to remove surface water, and photographed. Their skin colour was determined using the RHS colour chart (sixth edition 2015) whereas the flesh colour was determined in *Aegle marmelos, Dialium ovoideum* and *Limonia acidissima*. One kilogram of each fruit species was taken to extract the edible portion and homogenized. Forty grams and five grams of each homogenate were taken to extract phenolic compounds and to determine vitamin C content, respectively.

Chemicals and Reagents

All chemicals and reagents used in the study were of analytical grade.

Sample Extraction

Forty grams of the homogenate of each fruit was extracted with 120 mL of distilled water for 90 minutes at 150 rpm on a reciprocating shaker, and centrifuged (2,750 g) for 10 minutes. Then the supernatants were collected separately, and the extraction was repeated twice more. Collected supernatants were combined, lyophilized at −50 °C, 24 mbar for 96 h and subsequently stored at −20 °C until further analysis.

Sample Preparation

To perform 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) radical scavenging assay six different concentrations of each sample ranging from 0.5 to 10 mg/mL were used. In order to carry out other *in-vitro* assessments for antioxidant efficacy (*i.e*., total phenol and anthocyanin content), samples were prepared to have a final concentration of 4 mg/mL. All assays were carried out in six replicates.

Table 1: Selected fruit species

	Selected Fruit Species	Plant Family	Local (S)/ English (E) Names	Fruit Colour
1	*Aegle marmelos* L. Corr. Serr.	Rutaceae	*S*: *Beli* *E*: Bael fruit	Orange group N25-D (flesh)
2	*Averrhoa bilimbi* L.	Oxalidaceae	*S*: *Bilin* *E*: Bilimbi	Yellow-green group 145-A
3	*Averrhoa carambola* L.	Oxalidaceae	*S*: *Kamaranga* *E*: Star fruit	Yellow-green group 154-C
4	*Baccaurea motleyana* (Mull. Arg.) Mull. Arg.	Euphorbiaceae	*S*: *Gaduguada* *E*: Rambai	Yellow-orange group 16-D
5	*Carissa carandas* L.	Apocynaceae	*S*: *Maha-karamba* *E*: Ceylon Damson	Grayed-purple group N186-B
6	*Cordia dichotoma* G. Forst.	Boraginaceae	*S*: *Lolu* (*Yolu*) *E*: Indian Cherry	Orange group 27-C
7	*Cynometra cauliflora* Linn.	Fabaceae	*S*: *Nami-nam* *E*: Nam Nam	Grayed-brown group N199-D
8	*Dialium ovoideum* (Kunth) Baehni	Fabaceae	*S*: *Gal-siyambala* *E*: Velvet Tamarind	Grayed-orange group 164-B (flesh)
9	*Diospyros discolor* (Willd.) nom. Illeg	Ebanaceae	*S*: *Thimbiri* *E*: Velvet apple	Red group 43-C
10	*Elaeocarpus serratus* Linn	Elaeocarpaceae	*S*: *Weralu* *E*: Ceylon olive	Yellow-green group N144-D
11	*Flacourtia indica* (Burm. f.) Merr.	Flacourtiaceae	*S*: *Ugurassa* *E*: Governor's plum	Red-purple group 59-A
12	*Garcinia quaesita* Pierre.	Clusiaceae	*S: Goraka* *E:* Garcinia	Deep yellow-pink group 39-C
13	*Limonia acidissima* L.	Rutaceae	*S*: *Diwul* *E*: Wood Apple	Gray-brown group 200-B (flesh)
14	*Morus alba* L.	Moraceae	*S*: *Mulberry* *E*: Mulberry	Yellow-blue group N92-A
15	*Muntingia calabura* L.	Muntingiacea	*S*: *Jam* *E*: Jamaican Cherry	Yellow-green group 144-B
16	*Phyllanthus acidus* L. Skeels	Euphorbiaceae	*S*: *Rata Nelli* *E*: Star gooseberry	Yellow green group 150-B
17	*Phyllanthus embilica* L.	Euphorbiaceae	*S*: *Nelli* *E*: Indian gooseberry	Yellow-green group 144-D
18	*Pouteria campechiana* (Kunth) Baehni.	Sapotaceae	*S*: *Lawulu* *E*: Canistel	Yellow-orange group 17-A
19	*Solanum nigrum* L.	Solanaceae	*S*: *Kalukamberiya* *E*: Black Nightshade	Violet-blue group N92-C
20	*Syzygium caryophyllatum* L. Alston	Myrtaceae	*S*: *Heen-Dan* *E*: -	Violet-blue group N92-C
21	*Syzygium cumini* L. Skeels	Myrtaceae	*S*: *Ma-dan* *E*: Java plum	Violet-blue group N92-D

Note: Local name in Sinhala (*S*) and in English (*E*)

Determination of Total Phenol Content (TPC)

The Folin-Ciocalteu's (FC) reagent assay (Yu *et al.*, 2002) was adapted with minor modifications. Briefly, 20 µL of sample was mixed with 100 µL of 2 N FC reagent and 1.58 mL of distilled water, vortexed, allowed to stand at RT for 8 minutes, incubated for 30 minutes at RT after adding 300 µL of 0.7 M sodium carbonate. The absorbance of the mixture was measured (765 nm, Helios Omega – UV – VIS spectrophotometer). Negative control was

performed by adding methanol except the sample. Results were expressed as mg of gallic acid equivalents per gram dry weight of sample (mg GAE/g DW) by comparing with the standard calibration curve constructed using values of absorbance at 765 nm for different concentrations of gallic acid.

Determination of 2,2-diphenyl-1-pycrylehydrazyl (DPPH)Radical Scavenging Activity

The radical scavenging activity of fruit extracts was assessed using 2,2-diphenyl-1-picrylhydrazylhydrate (DPPH) radical scavenging assay (Su *et al*., 2007) with some modifications. The sample (0.2 mL) was mixed with 1.8 mL of 0.1 mM methanolic DPPH radical, left for an hour in the dark at RT and absorbance was recorded at 517 nm. The negative control was prepared by adding 0.2 mL of methanol, instead of sample. Using the following equation, the radical scavenging activity (RSA) was calculated as percentage of discolouration of DPPH radical.

$$\text{RSA\%} = 1 - (A_{\text{sample}}/A_{\text{control}})\} \times 100$$

Where, A_{sample} is the absorbance of the sample and $A_{control}$ is the absorbance of the control.

The results were expressed as IC_{50} values that denote the concentration of the sample required (g of dry weight/mL) to scavenge 50% of DPPH radicals in the reaction medium.

Determination of Total Antioxidant Activity (TAA)

The ABTS cation radical scavenging assay (Zhou and Yu, 2004) was used to determine TAA. To perform the assay 1.96 mL of stock (containing 2.5 mM ABTS and 2 mM AAPH in 1:1 ratio) was mixed with 0.04 mL of the sample and absorbance was measured over six minutes at 1st, 3rd and 6th minute at 734 nm. In place of the sample, the stock was used as negative control. Results were expressed as percentage inhibition of ABTS radical cation over six minutes by calculating RSA using following formula.

$$\text{RSA\%} = \{1 - (A_{\text{sample}}/A_{\text{control}})\} \times 100$$

Where, A_{sample} is the absorbance of the sample and $A_{control}$ is the absorbance of the control.

Determination of Reducing Power (RP) of Fruit Extracts

The ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996) was used with minor changes to determine reducing power (RP). The freshly prepared, pre-heated (at 37 °C) 1.5 mL of FRAP reagent was mixed with 0.5 mL of sample, vortexed for 30 s, incubated for 4 min at RT and absorbance was measured (593 nm). The FRAP reagent was used as the negative control. The RP of samples was determined using the $FeSO_4.7H_2O$ (0 - 1 mM) calibration curve and results were expressed as µmol of ferrous sulphate equivalents (FSE) in gram of fruits on dry weight basis (µmol of FSE/g DW).

Determination of Total Monomeric Anthocyanin Content (TMAC)

The pH differential method was adapted with slight modifications to determine the TMAC of lyophilized extracts (Lee *et al*., 2005). In brief, 1.8 mL of potassium chloride buffer (pH 1) and sodium acetate buffer (pH 4.5) were added separately into test tubes containing 0.2 mL of extracts, kept for 15 minutes at RT and absorbance was measured (520 nm and 700 nm). The TMAC was calculated using the following formula and the results were expressed in milligrams of cyanidin-3-glucoside in gram of fruits on dry weight basis (mg C3G/g DW), as it is the most abundant anthocyanin pigment in the nature (Francis and Markarkis, 1989).

$$\text{Cyanidin}-3-\text{glucoside} = \frac{A \times MW \times DF \times 10^3}{\varepsilon \times L}$$

Where, A, MW, DF, L and Ɛ are absorbance values $\{(A_{520}-A_{700})_{pH1} - (A_{520}-A_{700})_{pH4.5}\}$, molecular weight of C3G (449.2 g), dilution factor, path length (cm) and molar extinction co-efficient (26,900), respectively.

Determination of Vitamin C Content

The titrimetric method 967.21 (AOAC, 2005), was performed to determine vitamin C content and expressed in mg of ascorbic acid equivalents (AAE) per 100 g of fruits in fresh weight (mg AAE/100 g FW).

$$\text{Vitamin C content} = \frac{B2 \times V1 \times Vt \times 50 \times 100}{B1 \times V1 \times 10 \times W}$$

Where, B1, B2, V1, Vt, 10, 50 and W are volumes of dye reacted with standard ascorbic acid solution, volume of dye reacted with sample, volume of standard ascorbic acid solution taken for titration, total volume of standard ascorbic acid solution,

volume of sample taken to titration, total volume of sample prepared and weight of sample, respectively.

Statistical Analysis

Data were statistically analysed using SAS 9.1 statistical software. Analysis of variance and least significant difference tests were conducted to identify significant difference of vitamin C, TPC, TMAC and antioxidant efficacy (DPPH, AP and TAA) among the species. For ranking of fruit species standardized means were used (Z values). Correlation among the studied variables was tested using Pearsons correlation coefficient. Clustering of the species was done using complete linkage method for standardised variables. Statistical significance was declared at $p< 0.05$.

RESULTS AND DISCUSSION

Total Phenol, Vitamin C and Total Monomeric Anthocyanin Contents

The TPC of selected fruit species significantly varied from 1.3 to 104 mg GAE/g of fruits on dry weight basis (DW) (Table 2). The highest TPC was observed in *P. emblica*, followed by *A. marmelos* and *M. calabura*, while *D. ovoideum* and *B. motleyana* showed the lowest TPC. In this study, it was found that TPC of extracts of coloured fruit species was higher than that of white fleshed fruits. It may be due to the presence of higher anthocyanin content in coloured fruits, which might have contributed to the TPC. The highest TPC of *P. emblica* could be due to high quantity of hydrolysable tannins in the fruit (Charoenteeraboon *et al*., 2010). Fruit extracts of *M. calabura*, *S. nigrum*, *C. carandus*, *S. caryophyllatum* and *S. cumini*, which are not commonly consumed by urban community possessed higher TPC values than some common underutilized fruit species namely *L. acidissima* and *E. serratus*.

Most of studied fruit species contained high TPC compared to commonly consumed fruits. The TPC of pineapple, mango, papaya and litchi were 0.479, 0.56, 0.576, and 0.288 mg GAE/g, respectively (Luximon-Ramma *et al.,* 2003), which was lower than the tested fruit extracts. The fruit extracts of *P. emblica*, *A. marmelos*, *M. calabura*, *P. campechiana*, *S. nigrum* and *C. carandus* exhibited high TPC than that of apple, which ranged between 2.11 and 3.41 mg GAE/g on fresh weight basis (Wu *et al*., 2004). The phenolic content varies with the cultivation practices, location, growing season and the maturity stage of the fruit (Kubola *et al*., 2011).

The highest vitamin C content was observed in *P. emblica* (523 mg AAE/100 g on fresh weight basis (FW) while the lowest was recorded in *D. ovoideum* (4.5 mg AAE/100 g on FW) (Table 2). Interestingly, all studied fruit species recorded higher vitamin C values than that of yellow pear (1.6 mg/100 g), red apple (2.2 mg/100 g) and green grapes (2.9 mg/100 g) (Tee *et al*., 1988). Some tropical fruits possess higher concentrations of bioactive compounds than temperate and sub-tropical fruits (Lule and Xia, 2012).

Anthocyanins are major phenolic compounds belonging to the flavonoid group and considered as one of the broadly distributed plant pigments with a wide range of colours (Elbe and Schwartz, 1996). There is an increasing interest in anthocyanin rich food as they possess anticancer, antioxidant, anti-inflammatory and anti-diabetic activities (Nile *et al*., 2015). The highest TMAC was recorded in *S. caryophyllatum* (79 mg C3G/g DW) followed by *M. alba*, *S. cumini*, *C. couliflora*, *C. carandas*, respectively while least was in *E. serratus, M. calabura* and *B. Motleyana* (Table 2). It was impossible to find published research on anthocyanin content of locally available underutilized fruit species.

Antioxidant Efficacy of Fruit Extracts

Due to the complex and multifunctional nature of phytochemicals, they act as free radical scavengers, reducing agents, metal chelators and absorbers of ultraviolet radiation (Lü *et al*., 2010). Therefore, the antioxidant efficacy of food cannot be judged using any single method, thus multiple numbers of *in-vitro* assays need to be used to obtain precise information on antioxidant efficacy of fruit extracts. Therefore, DPPH, ABTS and FRAP assays were used in this study to determine antioxidant efficacy of lyophilized aqueous extracts of selected fruit species.

The highest IC_{50} values denote the lowest RSA, and *B. motleyana* (7 g/mL) and *P. emblica* (0.0004 g/mL) possessed the highest and lowest IC_{50} values, respectively (Table 3). The fruit extract of *D. ovoideum* which possessed lowest TPC, was not significant with the values of *A. marmelos*, *F. indica*, *C. dichotoma* and *G. quaesita* which showed a comparatively low IC_{50} value. It could be due to the scavenging ability of non-phenolic compounds in the fruit extracts of *D. ovoideum*.

The FRAP assay measures the reducing power of fruit extracts, by producing coloured ferrous tripyridyltriazine (Fe^{2+}-TPTZ) from ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex (Benzie and Strain, 1996). The reducing power of

lyophilized aqueous fruit extracts ranged from the lowest in *D. ovoideum* (4.35 µmol FSE/g) to the highest in *P. emblica* (155 µmol FSE/g) (Table 3). The fruit extracts of *S. nigrum*, *M. calabura*, *F. indica*, *P. campechiana*, *G. quaesita* and *S. caryophyllatum* recorded the highest reducing power after *P. emblica*.

Table 2: Total phenolic content (TPC), total monomeric anthocyanin content (TMAC) and vitamin C (Vit C) content of selected fruit species

No.	Selected Fruit Species	TPC (mg GAE/g DW)	TMAC (mg C3G/g DW)	Vit C (mg AAE/100 g FW)
1	*A. marmelos*	21.5 ± 0.24^{b}	0.82 ± 0.04^{hij}	71.5 ± 0.93^{b}
2	*A. bilimbi*	11.8 ± 0.21^{f}	1.94 ± 0.19^{efgh}	49.3 ± 0.09^{m}
3	*A. carambola*	6.4 ± 0.07^{ij}	0.70 ± 0.14^{ij}	21.7 ± 0.01^{h}
4	*B. motleyana*	1.5 ± 0.01^{n}	0.29 ± 0.04^{j}	55.4 ± 0.56^{d}
5	*C. carandas*	10.0 ± 0.23^{g}	7.89 ± 0.51^{d}	10.1 ± 0.23^{lm}
6	*C. dichotoma*	5.9 ± 0.27^{ijk}	0.75 ± 0.05^{hij}	17.5 ± 0.07^{i}
7	*C. cauliflora*	4.6 ± 0.06^{l}	8.66 ± 1.68^{d}	21.8 ± 0.33^{h}
8	*D. ovoideum*	1.3 ± 0.02^{n}	2.56 ± 0.02^{efg}	4.5 ± 0.08^{o}
9	*D. discolor*	2.9 ± 0.12^{m}	0.45 ± 0.06^{ij}	39.2 ± 0.45^{g}
10	*E. serratus*	2.2 ± 0.03^{mn}	0.29 ± 0.03^{j}	46.5 ± 0.36^{f}
11	*F. indica*	14.4 ± 0.18^{e}	1.67 ± 0.23^{fghi}	13.4 ± 0.52^{k}
12	*G. quaesita*	5.1 ± 0.13^{kl}	2.94 ± 0.07^{e}	38.9 ± 0.43^{g}
13	*L. acidissima*	6.3 ± 0.17^{ijk}	2.67 ± 0.29^{ef}	6.6 ± 0.07^{n}
14	*M. alba*	5.2 ± 0.16^{jkl}	46.69 ± 0.14^{b}	15.4 ± 0.14^{j}
15	*M. calabura*	19.4 ± 0.14^{c}	0.31 ± 0.07^{j}	60.8 ± 0.15^{c}
16	*P. acidus*	8.3 ± 0.68^{h}	0.75 ± 0.13^{hij}	17.1 ± 0.41^{i}
17	*P. emblica*	103.7 ± 1.53^{a}	2.98 ± 0.37^{e}	523.1 ± 1.29^{a}
18	*P. campechiana*	15.7 ± 0.42^{d}	0.73 ± 0.05^{hij}	53.1 ± 0.54^{e}
19	*S. nigrum*	14.8 ± 0.71^{de}	1.44 ± 0.23^{ghij}	11.2 ± 0.15^{l}
20	*S. caryophyllatum*	8.0 ± 0.13^{h}	78.60 ± 0.59^{a}	38.2 ± 0.73^{g}
21	*S. cumini*	6.6 ± 0.06^{i}	12.71 ± 0.34^{c}	22.4 ± 0.33^{h}

Data are presented as Mean ± standard error, values with different letters in each column are significantly different at $p<0.05$, DW- dry weight basis, GAE – gallic acid equivalents, C3G – cyanidin-3-glucosode, AAE – ascorbic acid equivalents, FW- fresh weight basis

Table 3: Reducing power (RP) and the IC_{50} value of selected fruit species

	Selected Fruit Species	IC_{50} (g of DW/mL)	RP (µmol FSE/g DW)
1	*A. marmelos*	0.05 ± 0.01^{jklm}	33.59 ± 1.57^{fg}
2	*A. bilimbi*	5.18 ± 0.82^{b}	32.86 ± 3.89^{g}
3	*A. carambola*	0.74 ± 0.02^{e}	18.60 ± 2.29^{jk}
4	*B. motleyana*	6.92 ± 0.72^{a}	6.86 ± 0.76^{mn}
5	*C. carandas*	0.25 ± 0.01^{h}	38.89 ± 2.80^{ef}
6	*C. dichotoma*	0.09 ± 0.18^{jk}	10.13 ± 1.46^{jk}
7	*C. couliflora*	0.47 ± 0.03^{f}	25.07 ± 0.73^{hi}
8	*D. ovoideum*	0.08 ± 0.01^{jkl}	4.35 ± 0.14^{n}
9	*D. discolor*	1.06 ± 0.34^{c}	13.79 ± 0.37^{kl}
10	*E. serratus*	0.17 ± 0.02^{i}	10.20 ± 0.87^{lm}
11	*F. indica*	0.62 ± 0.01^{jklm}	62.10 ± 1.49^{c}
12	*G. quaesita*	0.10 ± 0.02^{jk}	46.51 ± 2.36^{d}
13	*L. acidissima*	0.24 ± 0.03^{h}	29.55 ± 2.39^{gh}
14	*M. alba*	0.25 ± 0.01^{h}	30.21 ± 0.62^{gh}
15	*M. calabura*	0.03 ± 0.01^{klm}	64.45 ± 0.87^{c}
16	*P. acidus*	0.91 ± 0.03^{d}	17.52 ± 2.38^{jk}
17	*P. emblica*	0.0004 ± 0.0^{m}	154.63 ± 0.14^{a}
18	*P. campechiana*	0.32 ± 0.04^{g}	47.59 ± 0.23^{d}
19	*S. nigrum*	0.29 ± 0.04^{gh}	71.80 ± 4.07^{b}
20	*S. caryophyllatum*	0.02 ± 0.02^{lm}	40.99 ± 1.88^{e}
21	*S. cumini*	0.11 ± 0.02^{ij}	21.51 ± 0.41^{ij}

Data are presented as Mean ± Standard error
FSE - Ferrous sulphate equivalents. Values with different letters in each column are significantly different at $p<0.05$

In ABTS assay, the combined antioxidant activities of all constituents were assessed through radical quenching ability, since it is soluble in both aqueous and organic solvents and therefore can assess the efficacy of both lipophilic and hydrophilic antioxidants (Apak *et al*., 2007). The fruit extracts of *F. indica*, *M. alba*, *P. emblica*, *S. caryophyllatum* showed more than 80% of RSA (Figure 1) while *B. motleyana* and *P. acidus* recorded the least after 6 mins (Figure 2). Within the first minute of the reaction more than 50% of $ABTS^{\bullet+}$ in the reaction medium was scavenged by extracts of *M. calabura*, *S. caryophyllatum*, *M. alba*, *P. emblica*, *F. indica*, *A. marmelos*, *E. serratus* and *D. discolour* (Figure 1). While fruit extracts of *P. campechiana* showed 9.5% activity at the first minute, it finally reached to a maximum of 76.1%. The extracts of *A. marmelos*, *S. cumini* and *D. discolor* showed equal antioxidant activities after six minutes (Figure 1). The extracts of *P. acidus* started to react with the radical after the first minute (Figure 2) and *G. quaesita*, one of the most common culinary agents in India and Sri Lanka

(Rasha *et al*., 2015), recorded 28.1% of RSA which is not significant with that of *A. bilimbi*.

Fruit extracts of *F. indica, M. alba, P. emblica, S. caryophyllatum, M. calabura, E. serratus, P. campechiana* and *D. discolor* (Figure 1) and *C. carandas, C. dichotoma, G. quaesita, A. bilimbi, L. acidissima, C. couliflora, P. acidus* and *B. motleyana* (Figure 2) showed the same pattern of rate of reaction while reaching fairly constant levels of reaction after the third minute with different RSA values, indicating that they completed the reaction within three minutes achieving maximum RSA of each extract whereas *P. campechiana* (Figure 1), S*. nigrum, A. carambola* and *D. ovoideum* (Figure 2) showed a further increase of the reaction rate without reaching a constant after the third minute. This may be due to the different classes of antioxidative compounds present in extracts starting to react with $ABTS^{\bullet+}$ radical after three minutes.

Both FRAP and ABTS assays rely on the hypothesis that redox reactions proceed very rapidly and complete the reactions within 4 and 6 minutes, respectively, however some polyphenols react slowly and require longer reaction time for detection (Prior *et al*., 2005). Therefore, underestimation of antioxidant capacity is possible with those assays. Thus single-point absorption endpoint is not sufficient to represent complete reaction. The DPPH and ABTS radical scavenging assays are based on the reduction of colour of DPPH and ABTS radical by the antioxidant, thus the presence of coloured compounds in the test material could interfere with absorbance. The FC assay measures total phenolics in the reaction medium, and the mechanism involved is oxidation-reduction. Thus interference of other non-phenolic organic substances (ascorbic acid, organic acids, proteins and sugars) by reacting with FC reagent results in an overestimation. The radical scavenging assays such as ABTS, FRAP and DPPH are simple, rapid, inexpensive and do not require specialized equipment, thus are widely used (Prior *et al*., 2005). In this study, pH differential method was used to estimate total monomeric anthocyanin content based on the reversible colour change of monomeric anthocyanin pigment at pH of 1.0 (coloured) and 4.5 (colourless), whereas polymeric forms resistant to colour change and therefore are not included in the measurements (Lee *et al*., 2005).

Ranking of Fruit Species, Clustering and Correlation Analysis

Ranking sequences of fruits in terms of overall antioxidative activity was carried out according to the standardized values (Z value) of TPC, DPPH, ABTS, FRAP, TMAC and vitamin C (Table 4). The highest mean value represents the highest overall antioxidant activity bearing the lowest rank (Table 4). Among the fruit extracts tested, *P. emblica* was characterized as the fruit with the highest overall antioxidative property (rank 1); this may be due to its higher antioxidative properties of all constituents. Interestingly, the second rank was obtained by the native fruit species (*S. caryophyllatum*) which has been red-listed by the World Conservation Monitoring Centre (1998). The extracts of *M. alba*, *M. calabura* and *F. indica* obtained the third, fourth and fifth ranks, respectively. The aqueous extracts of *B. motleyana* could be identified as the fruit species with the lowest overall antioxidant activity among selected fruit species.

To obtain a complete insight of the relationship among multiple variables, the use of multivariate analysis is more appropriate than using univariate statistical analysis and standard error values (Tomsone *et al*., 2012). Therefore, hierarchical cluster analysis was performed and a dendrogram was created based on the antioxidant efficacy (DPPH, ABTS and FRAP), TPC, TMAC and vitamin C values of selected fruit species. Figure 3 illustrates the dendrogram obtained with the Euclidean distances. The height of vertical line indicates the difference of similarity among clusters.

Based on the similarity levels from zero, it was possible to resolve four major clusters (Figure 3). The cluster one is simplifolious which consisted of only *P. emblica* which was dissimilar in all characteristics from other fruit species tested. This was due to its higher TPC, RSA (DPPH), reducing property (FRAP) and TAA of fruit extracts. Lyophilized extracts of *A. blimbi* and *B. motleyana* exhibited the least TMAC and antioxidative properties among tested fruit species were grouped in cluster 2 at 29.7% similarity level. The cluster 3 was formed with those having the highest anthocyanin content (*M. alba* and *S. caryophyllatum*) with 45.9% similarity. Within the cluster 4, two main sub-clusters could be identified. The first sub-cluster of cluster 4 formed from 8 leaves with the common features of total antioxidant activity and anthocyanin contents. The sub cluster 2 of cluster 4 was grouped based on the level of total phenolic content, with remaining 8 species. *A. carambola* and *C. dichotoma* possess similarity in both TMAC and TPC at 93.6% while *L. acidissima and C. cauliflora* grouped at 95.6% for similar ABTS levels.

The values of FRAP (r = 0.8999, p < 0.001) and vitamin C (r = 0.9597, p < 0.001) showed a strong correlation with TPC (Table 5). The values of ABTS showed a significant negative correlation with DPPH radical scavenging activity. FRAP showed significant positive correlation with ABTS. It was because, the redox potential of Fe^{3+}-TPTZ and $ABTS^{+\bullet}$ is comparable to each other, thus similar compounds react in both assays (Prior *et al.*, 2005). Vitamin C has significant positive correlation with FRAP and TPC. Values of TMAC did not correlate with any of the parameters tested.

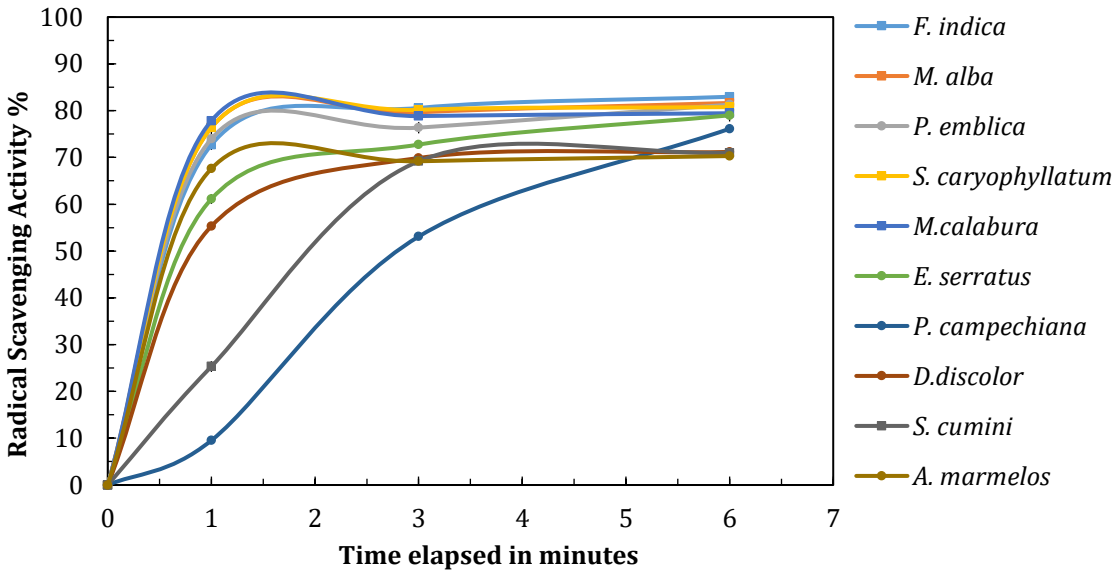

Figure 1: Radical scavenging activities over six minutes (fruit species possessing >70% of RSA at 6th minute)

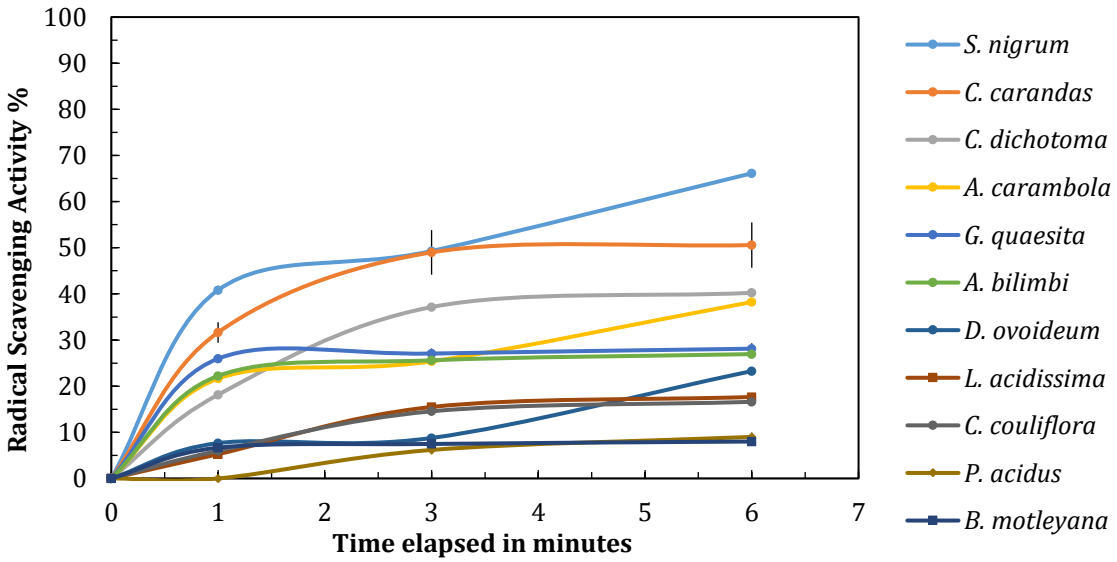

Figure 2: Radical scavenging activities over six minutes (fruit species possessing <70% of RSA at 6th minute)

Table 4: Standardized values (Z values) and ranks for antioxidant properties

	Selected Fruit Species	Z values of antioxidant properties						Mean	Rank
		Vit C	TPC	IC_{50}	FSE	TMAC	TAA		
1	*A. marmelos*	0.80	1.78	2.00	-0.50	-1.82	2.97	0.87	8
2	*A. bilimbi*	-1.80	-0.29	-11.19	-0.60	-1.55	-4.19	-3.27	20
3	*A. carambola*	-1.28	-1.43	0.21	-2.58	-1.85	-2.32	-1.54	15
4	*B. motleyana*	0.13	-2.48	-15.67	-4.21	-1.95	-7.31	-5.25	21
5	*C. carandas*	-1.76	-0.66	1.48	0.23	-0.12	-0.29	-0.18	10
6	*C. dichotoma*	-1.45	-1.53	1.89	-3.75	-1.84	-1.99	-1.45	14
7	*C. cauliflora*	-1.27	-1.81	0.93	-1.68	0.07	-5.89	-1.61	16
8	*D. ovoideum*	-2.00	-2.52	1.91	-4.56	-1.40	-4.79	-2.23	18
9	*D. discolor*	-0.55	-2.18	-0.59	-3.25	-1.91	3.11	-0.89	13
10	*E. serratus*	-0.24	-2.32	1.68	-3.74	-1.95	4.41	-0.36	11
11	*F. indica*	-1.62	0.26	1.96	3.45	-1.62	5.06	1.25	5
12	*G. quaesita*	-0.56	-1.71	1.88	1.29	-1.31	-3.99	-0.73	12
13	*L. acidissima*	-1.91	-1.46	1.49	-1.06	-1.37	-5.72	-1.67	17
14	*M. alba*	-1.54	-1.68	1.49	-0.97	9.24	4.84	1.90	3
15	*M. calabura*	0.36	1.33	2.05	3.78	-1.94	4.48	1.68	4
16	*P. acidus*	-1.47	-1.02	-0.22	-2.73	-1.84	-7.15	-2.41	19
17	*P. emblica*	19.68	19.28	2.12	16.29	-1.30	4.78	10.14	1
18	*P. campechiana*	0.04	0.55	1.30	1.44	-1.84	3.93	0.90	7
19	*S. nigrum*	-1.72	0.36	1.37	4.80	-1.67	2.28	0.90	6
20	*S. caryophyllatum*	-0.59	-1.08	2.08	0.53	16.93	4.69	3.76	2
21	*S. cumini*	-1.25	-1.38	1.84	-2.18	1.05	3.08	0.19	9

VitC - vitamin C, TPC - total phenolic content, IC_{50} - 50% of radical scavenging activity, FSE - reducing power in ferrous sulphate equivalent, TMAC - total monomeric anthocyanin content and TAA - total antioxidant activity.

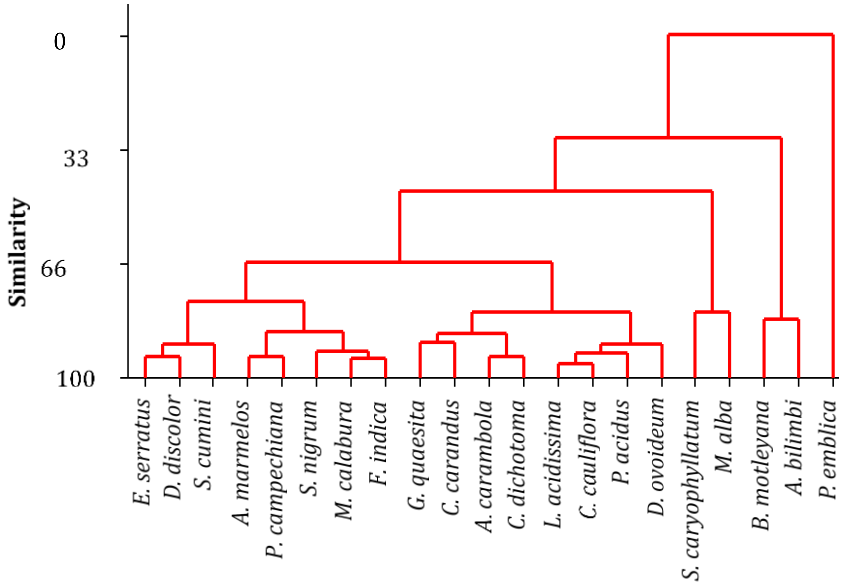

Figure 3: Dendrogram with complete linkage and Euclidean distance for values of TPC, IC_{50}, FSE, TAA, TMAC and vitamin C of selected fruit species

Table 5: Strengths of the relationships among TPC, DPPH, FRAP, ABTS, Vitamin C and TMAC

		TPC	DPPH	FRAP	ABTS	TMAC
TPC	*r*	1.0000				
	p					
DPPH	*r*	−0.1554	1.0000			
	p	0.5013				
FRAP	*r*	0.8999	− 0.2485	1.0000		
	p	<.0001	0.2773			
ABTS	*r*	0.3438	− 0.4746	0.4542	1.0000	
	p	0.1270	0.0297	0.0386		
TMAC	*r*	0.0933	− 0.1609	0.0021	0.3204	1.0000
	p	0.6876	0.4859	0.9929	0.1567	
Vitamin C	*r*	0.9597	−0.0146	0.7928	0.2319	− 0.1166
	p	<.0001	0.9499	<.0001	0.3118	0.6148

r - Correlation coefficient, p- probability value

CONCLUSIONS

There is a wide variation in the vitamin C, TPC, TMAC, and antioxidant efficacy of selected underutilized fruit species. *Phyllanthus emblica* has the highest overall antioxidant properties followed by *Syzygium caryophyllatum*, *Morus alba*, *Muntingia calabura* and *Flacourtia indica*.

The dendrogram obtained for tested fruit species showed that externally different fruit species are sharing same internal antioxidative characteristics. *P. emblica* cannot be substituted by any other fruit species as it dissimilar in all characteristics from other tested fruit species. The correlation analyses indicated that significant, positive, strong correlation between TPC, vitamin C and FRAP values and between ABTS and FRAP values. The knowledge disseminated through this study will promote the utilization, cultivation, and commercialization of these species.

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