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Antioxidative properties of 34 green leafy vegetables

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ABSTRACT

Green leafy vegetables available in Sri Lanka have not been fully exploited, although they are stipulated to be rich sources of natural antioxidant. This study examined the antioxidant properties of thirty four edible green leafy vegetables popular in Sri Lanka. Methanolic extracts of leafy vegetables were analysed for total phenolic, carotene and chlorophyll content, and were evaluated for total antioxidant capacity, reducing power, lipid peroxidation and DPPH radical scavenging assays. The results indicated that these leafy vegetables have remarkable variations in their antioxidant activities. Among the plant materials screened for their antioxidant properties, *Sesbania grandiflora*, *Cassia auriculata*, *Murraya koenigii* Spreng, *Passiflora edulis*, *Gymnema lactiferum* and *Olax zeylanica* showed high carotene content, antioxidant activities and polyphenolics compared to other leaf varieties tested. A good correlation was observed between antioxidant assays and polyphenolics of the leafy vegetables.

1. Introduction

Many chronic diseases such as cancer and cardiovascular diseases represent an increasing proportion of morbidity and mortality in the developing countries, including Sri Lanka. Various research findings have demonstrated that changes in oxygen utilization in the body and increased formation of reactive oxygen species (ROS) contribute to many chronic diseases (Kaliora, Dedoussis, & Schmidt, 2006; Madamanchi, Vendrov, & Runge, 2005). Although an organism is naturally equipped with antioxidant protection systems to cope with the harmful effects of ROS, the endogenous antioxidant defence system is not totally adequate to counteract the oxidative stress (Houston, 2010). Therefore, protection against oxidative stress depends partly on the adequacy of dietary antioxidants (Kaliora et al., 2006). Evidence suggests that phytochemicals from fruits and vegetables, including leafy vegetables, are capable of providing protection against free radicals. Therefore, a greater deal of research has been focused on natural antioxidants and it is necessary to screen the natural sources for their antioxidant potential.

Green leafy vegetables constitute a major part of any balanced diet and contain significant amounts of minerals and

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antioxidant vitamins (Subhasree, Baskar, Keerthana, Susan, & Rajasekaran, 2009). Various research findings have suggested that leafy vegetables have medicinal properties such as anticarcinogenic (Rajesh Kumar et al., 2002), antibacterial (Kubo, Fijita, Kubo, Nehei, & Gura, 2004) and antidiabetic (Kesari, Gupta, & Watal, 2005) effects. These health benefits of green leafy vegetables are attributed, at least in part, to their antioxidants. Recently, research has indicated that green leafy vegetables are rich sources of functional components. The major active antioxidant compounds are polyphenolic constituents and carotenoids, among others (Andarwulan et al., 2012; Deng et al., 2013; Khanam, Oba, Yanase, & Murakami, 2012; Subhasree et al., 2009). Lutein is one of the major carotenoids in green leafy vegetables which shows a marked antioxidant activity (Chandrika, Basnayake, Athukorala, Colombagama, & Goonetilleke, 2010). However, most of the green leafy vegetables available in Sri Lanka represent a class of underexploited plants that are stipulated to be rich sources of natural antioxidants.

A great number of *in vitro* assay methods have been developed to evaluate the efficiency of natural antioxidants. These methods include measurement of phenolic content using Folin–Ciocalteu assay, reducing power assay, total antioxidant capacity assay, lipid peroxidation assay and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging assay. The above *in vitro* methods can be used for the screening of green leafy vegetables commonly available in Sri Lanka. The purpose of this study was to examine the antioxidant characteristics of thirty four edible green leafy vegetables popular in Sri Lanka. The results of this study can be useful for food industry and in preventive medicine in the development of "natural antioxidants"/nutraceuticals from plant sources.

2. Materials and methods

2.1. Materials

Thirty four types of fresh green leafy vegetable samples were collected from various places in Colombo, Negombo and Makandura areas of Sri Lanka. The plant specimens were taxonomically identified by a botanist (Dr. H.D.D. Bandupriya) and the voucher specimens of the samples were deposited in the herbarium of the Department of Food Science and Technology of Wayamba University of Sri Lanka.

2.2. Reagents

Gallic acid, 2,2-diphenyl-2-picrylhydrazyl, Folin–Ciocalteu reagent, ethanol, methanol, sodium phosphate, ammonium molybdate, potassium ferricyanide, phosphate buffer, trichloroacetic acid, ferric chloride, ferrous sulphate, acetic acid, thiobarbituric acid, sodium dodecyl sulphate, butanol and sodium carbonate were obtained from Sigma Aldrich, St. Louis, MO, USA through Analytical Instrument Pvt Ltd, Colombo, Sri Lanka. All other chemicals used were of analytical grade.

2.3. Preparation of crude extracts

Edible portions of the leaves were cleaned with distilled water and air dried at room temperature (30 ± 2 °C) for 2 hours. The leaves samples were then oven dried at 45 °C to a constant weight. One gram of air dried sample from each of these thirty four leafy vegetables was mixed with 20 mL of methanol and vortexed at high speed for five minutes and then centrifuged (Hettich, EBA 20) for 10 min at 792 g. The extracts were subsequently filtered through a filter paper (Whatman No. 42; Whatman Paper Ltd, Maidstone, UK) and the prepared extracts were stored at –18 °C until assayed within 1 week.

2.4. Determination of total phenolic content

The total phenolic content was determined using Folin–Ciocalteu assay of Singleton, Orthofer, and Lamuela-Raventos (1999) with some modification, as described by Gunathilake and Rupasinghe (2014). About 0.5 mL of extract and 0.1 mL of Folin–Ciocalteu reagent (0.5 N) were mixed and incubated at room temperature for 15 min in the dark. Then 2.5 mL 7.5% sodium carbonate was added to the mixture and further incubated for 2 hours in the dark at room temperature and then the absorbance was measured at 760 nm using a UV/VIS spectrometer (Optima, SP-3000, Tokyo, Japan). The concentration of total phenols was expressed as μg gallic acid equivalents (GAE) per g dry weight of leaf. Gallic acid was used in the construction of standard curve and the linear range used for the calibration curve was 1000–15,000 μg GAE/L.

2.5. Total chlorophyll and carotene contents

The chlorophyll and carotene contents were analysed according to the method described by Türlerinde, Klorofil-A, and Saptanması (1998). The weighed samples were mixed with 96% methanol (50 mL for each gram) for one minute using a vortex. The homogenate was filtered through a filter paper (No: 42 Whatman) and centrifuged using the centrifuge (EBA20) for 10 min at 245 g. The supernatant was separated and the absorbance was read at 470, 653, and 666 nm on UV/VIS spectrometer (SP-3000).

The concentration of each pigment was calculated according to the formulas of Kichtenthaler and Wellburn (1983) and was reported as μg per g dry weight of sample.

Chlorophyll a = 11.75 (A662) – 2.350(A645).
Chlorophyll b = 18.61 (A645) – 3.960 (A662).
Carotene = 1000 (A470) – 2.270 (C_a) – 81.4 (C_b)/227.

where C_a is chlorophyll a and C_b is chlorophyll b.

2.6. DPPH radical scavenging assay

The capacity of prepared extracts to scavenge the 'stable' free radical DPPH was monitored according to the method of Hatano, Kagawa, Yasuhara, and Okuda (1988) with slight modifications. Extracts (100 μL) were dissolved in 3.9 mL freshly prepared methanolic solution of DPPH (1 mM, 0.5 mL). The mixture was vortexed for 15 seconds and then left to stand at room temperature for 30 min in the dark. The absorbance of the resulting solution was read spectrophotometrically (UV/VIS spectrometer (SP-3000, OPTIMA INC, Japan) at 517 nm. The percentage inhibition of the radicals due to the antioxidant activity of leaf extracts was calculated using the following formula.

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

$A_{control}$ is the absorbance of the DPPH solution with nothing added (control).

2.7. Reducing power assay

The reducing power of the prepared extracts was determined according to the method of Oyaizu (1986). Briefly, each extract (1 mL) was mixed with 2.5 mL of a 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of a 1% (w/v) solution of potassium ferricyanide. The mixture was incubated in a water bath at 50 °C for 20 min and then 2.5 mL of 10% (w/v) trichloroacetic acid solution was added and the mixture was then centrifuged for 10 min at 352 g. A 2.5 mL aliquot of the upper layer was combined with 2.5 mL of distilled water and 0.5 mL of 0.1% (w/v) ferric chloride solution. Absorbance of the reaction mixture was read using UV/VIS spectrometer (SP-3000) at 700 nm. Mean values from three independent samples were calculated for each extract.

2.8. Lipid peroxidation assay

A modified thiobarbituric acid reactive substances (TBARS) assay was used to measure the level of lipid peroxide formed in egg homogenates as lipid-rich media as described in Ohkowa, Ohisi, and Yagi (1979). Briefly 0.5 mL egg homogenate (10%, v/v) was mixed with 0.1 mL leaf extract, 0.4 mL distilled water and 0.05 mL ferrous sulphate (0.07M), and the mixture was incubated at room temperature for 30 min. After incubation, 1.5 mL 20% acetic acid (pH = 3.5), 1.5 mL 0.8% (w/v) thiobarbituric acid (in 1.1% sodium dodecyl sulphate) and 0.05 mL 20% trichloroacetic acid were added and the mixture was mixed and was heated at 95 °C for 60 min. After cooling to room temperature, 5.0 mL butanol was added and centrifuged at 528 g for 10 min. Absorbance of the mixture was read using UV/VIS spectrometer (SP-3000) at 532 nm. Percentage inhibition of lipid peroxidation by leaves extracts was calculated.

2.9. Total antioxidant capacity

The total antioxidant capacity of leaf extracts was analysed according to the method described by Prieto, Pineda, and Aguilar (1999). The tubes containing leaf extract (0.3 mL) and 3 mL reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were incubated at 95 °C for 90 min. After the mixture had cooled to room temperature, the absorbance of each solution was measured at 695 nm spectrophotometrically (UV/VIS-SP-3000) against a blank. The antioxidant capacity was expressed as ascorbic acid equivalents (AAE).

2.10. Statistical analyses

All data from the study were presented as mean ± standard deviation of three replications. The assumptions of normality were tested using the Anderson–Darling test and constant variance using residual versus fits, and the independent assumptions were achieved through randomizations. Samples were analysed in triplicate and one way analysis of variance (ANOVA) was performed, using general linear model (SAS 9.2, Cary, NC, USA). Differences were considered to be statistically significant if the probability values were less than 0.05 ($p < 0.05$). When there were significant differences, multiple mean comparisons were carried out using LSD method. To evaluate the relationship between the antioxidant activities and phenolic and carotene contents, the Pearson correlation coefficient analysis was performed using MINITAB 17.

3. Results

Total phenolics, chlorophyll and carotene contents of 34 leafy vegetables were analysed and the results are summarized in Table 1. The total phenolic content of the methanolic extracts of leafy vegetables was within the range of 0.92.0–11.03 mg GAE/g dry weight of leaf. Of the leafy vegetables studied, *Gymnema lactiferum* and *Sesbania grandiflora* leaves showed the highest total phenolic contents (11.03 and 10.98 mg GAE/g dry weight of leaf respectively), followed by *Cassia auriculata* leaves (10.70 mg GAE/g dry weight). Other leafy vegetables with relatively high antioxidant activities were *Passiflora edulis* (9.23 mg GAE/g dry weight), *Murraya koenigii* Spreng (8.71 mg GAE/g dry weight) and *Hemidesmus indicus* (8.18 mg GAE/g dry weight). However, among the leaves studied, *Asteracantha longifolia* (0.92 mg GAE/g dry weight) and *Alternanthera sessilis* (0.98 mg GAE/g dry weight) contained a significantly lower phenolic content.

Chlorophyll content in leafy vegetables ranged from 9.20 to 37.10 μg/g dry weight. *Boerhavia diffusa* (9.20 μg/g), *Alternanthera sessilis* (9.55 μg/g), *Allium cepa* (9.45 μg/g) and *Amaranthus caudatus* (11.37 μg/g) showed significantly lower chlorophyll content, whereas *Asteracantha longifolia* (37.10 μg/g) had the highest chlorophyll content. Carotene content of studied leafy vegetables was within the range of 0.47–4.17 mg/g dry weight. Among the leafy vegetables analysed, *Passiflora edulis* had the highest carotene content (4.17 mg/g), followed by *Cassia auriculata* (3.45 mg/g), *Solanum nigrum* (3.18 mg/g) and *Syngonium angustatum* (3.13 mg/g). The lowest carotene content was observed in *Alternanthera sessilis* (0.47 mg/g), *Allium cepa* (0.53 mg/g), *Boerhavia diffusa* (0.67 mg/g), *Osbeckia octandra* (0.79 mg/g) and *Bacopa monnieri* (0.89 mg/g).

The antioxidant activity of leafy vegetables was systematically assessed with reducing power assay, lipid peroxidation assay, DPPH radical scavenging assay and total antioxidant capacity assay, and the results are shown in Table 2. The percent inhibition of DPPH radical due to leaf extracts ranged from 3.70 to 52.2%. Among the leafy vegetables evaluated, *Olax zeylanica* and *Ipomoea batatas* show higher DPPH radical scavenging activities compared with other leaf extracts studied. *Murraya koenigii* Spreng, *Hemidesmus indicus*, *Cassia auriculata* and *Sesbania grandiflora* extracts also show higher DPPH radical scavenging activity compared to other leaves tested. However, *Asteracantha longifolia*, *Alternanthera sessilis*, *Amaranthus lividus*, *Amaranthus caudatus* and *Syngonium angustatum* displayed significantly lower DPPH radical scavenging activity.

The reducing power of the leaf extracts was within the range of 0.55–10.90 mg ascorbic acid equivalents (AAE/g dry weight

Table 1 – Total phenolic, chlorophyll and carotene content of selected green leafy vegetables in Sri Lanka.[a]

Common names	Scientific name	Total phenolic (mg GAE/g dw)	Total chlorophyll (a + b) (μg/g dw)	Total carotene (mg/g dw)
Koppa kola/shield aralia	*Polyscias scutellaria*	3.19 ± 0.15	21.03 ± 0.21	1.99 ± 0.01
Kurignan/ceylon cow tree	*Gymnema lactiferum*	11.03 ± 0.42	23.03 ± 1.22	1.77 ± 0.09
Wel kohila/five fingers	*Syngonium angustatum*	2.65 ± 0.10	31.01 ± 1.22	3.13 ± 0.04
Pitasudu sarana/spreading hogweed	*Boerhavia diffusa*	1.59 ± 0.92	9.27 ± 1.06	0.67 ± 0.02
Cassava	*Manihot esculenta*	2.94 ± 0.11	26.06 ± 3.23	2.19 ± 0.01
Sarana/giant pigweed	*Trianthema monogyna*	2.96 ± 0.18	27.06 ± 5.03	1.49 ± 0.02
Nivithi/spinach	*Spinacia oleracea*	3.81 ± 0.16	14.05 ± 0.23	1.96 ± 0.02
Thampala	*Amaranthus caudatus*	1.56 ± 0.61	12.04 ± 2.42	1.18 ± 0.05
Pumpkin	*Cucurbita maxima*	2.70 ± 0.07	20.06 ± 2.04	2.05 ± 0.01
Sweet potato	*Ipomoea batatas*	5.68 ± 0.13	27.06 ± 1.05	2.26 ± 0.04
Kathurumurunga	*Sesbania grandiflora*	10.98 ± 0.19	26.20 ± 1.16	2.29 ± 0.01
Mukunuwenna/joyweed	*Alternanthera sessilis*	0.98 ± 0.08	10.03 ± 6.16	0.47 ± 0.02
Gotukola	*Centella asiatica*	1.55 ± 0.05	25.32 ± 1.04	2.16 ± 0.08
Passion fruit	*Passiflora edulis*	9.21 ± 0.14	33.01 ± 3.04	4.17 ± 0.01
Hathawariya/wild asparagus	*Asparagus racemosus*	1.47 ± 0.09	24.00 ± 4.27	1.98 ± 0.01
Kankung/water spinach	*Ipomoea aquatica*	2.46 ± 0.08	25.01 ± 5.30	2.11 ± 0.02
Heen bowitiya	*Osbeckia octandra*	5.18 ± 0.41	13.02 ± 4.00	0.79 ± 0.01
Thebu/spiral ginger	*Costus speciosus*	4.60 ± 0.08	17.02 ± 4.00	1.89 ± 0.02
Ranawara	*Cassia auriculata*	10.70 ± 0.12	27.25 ± 7.07	3.46 ± 0.03
Wel penela/balloon vine	*Cardiospermum halicacabum*	2.16 ± 0.46	12.13 ± 2.07	1.28 ± 0.01
Neeramulliya	*Asteracantha longifolia L*	0.92 ± 0.08	37.12 ± 4.21	2.78 ± 0.02
Karapincha/curry leaves	*Murraya koenigii* Spreng	8.71 ± 0.02	27.05 ± 4.02	1.99 ± 0.00
Koora thampala/slender amaranth	*Amaranthus viridis*	3.25 ± 0.03	19.08 ± 6.02	1.91 ± 0.00
Rathu thampala/wild amaranth	*Amaranthus lividus*	2.37 ± 0.07	15.09 ± 2.02	1.23 ± 0.03
Anguna/green milkweed climber	*Wattakaka volubilis*	5.47 ± 0.07	24.04 ± 0.02	2.34 ± 0.01
Thumba	*Leucas zeylanica*	3.29 ± 0.19	15.04 ± 6.01	1.10 ± 0.01
Onion leaves/spring onion	*Allium cepa*	3.99 ± 0.16	10.01 ± 2.11	0.53 ± 0.01
Kowakka kola/ivy gourd	*Coccinia grandis*	2.29 ± 0.13	14.01 ± 0.22	1.64 ± 0.02
Asamodagam	*Trachyspermum involucratum*	2.24 ± 0.11	11.02 ± 0.12	1.01 ± 0.01
Lunuwila/water hyssop	*Bacopa monnieri*	5.47 ± 0.09	15.06 ± 2.33	0.89 ± 0.01
Kalukamberiya/black nightshade	*Solanum nigrum*	3.97 ± 0.45	27.14 ± 0.13	3.18 ± 0.01
Polpala/mountain knot grass	*Aerva lanata*	2.15 ± 0.12	11.05 ± 0.27	1.17 ± 0.04
Mella kola	*Olax zeylanica*	6.91 ± 0.64	22.14 ± 3.07	2.15 ± 0.04
Iramusu	*Hemidesmus indicus*	8.18 ± 0.31	25.02 ± 9.35	2.61 ± 0.01

Values are presented as mean ± SD, n = 3.
[a] mg gallic acid equivalent per g dry weight (dw) leaves.

of leaf) and *Passiflora edulis* (10.90 mg AAE/g dry weight) showed significantly higher ($p < 0.05$) reducing power compared with other leaves. Leaves of *Olax zeylanica* and *Allium cepa* also have shown higher reducing properties. *Boerhavia diffusa* (0.55 mg AAE/g dry weight), *Trianthema monogyna* (0.59 mg AAE/g dry weight), *Syngonium angustatum* (0.64 mg AAE/g dry weight), *and Asteracantha longifolia* (0.61 mg AAE/g dry weight) showed lower reducing power comparatively.

The percentage inhibition of lipid peroxidation by leaf extracts ranged from 34.3% (*Boerhavia diffusa*) to 89.8% (*Asparagus racemosus*). *Polyscias scutellaria*, *Manihot esculenta*, *Cucurbita maxima*, *Centella asiatica*, *Costus speciosus*, *Cardiospermum halicacabum*, *Murraya Koenigii* Spreng, *Amaranthus spp.*, *Leucas zeylanica* and *Solanum nigrum* also showed higher lipid peroxidation inhibition ability. The total antioxidant capacity of the leaf extracts was within the range of 4.42–25.70 mg AAE/g dry weight. *Murraya Koenigii* Spreng showed the highest antioxidant capacity (25.70 mg AAE/g dry weight), followed by *Hemidesmus indicus* (19.43 mg AAE/g dry weight) and *Cassia auriculata* (18.16 mg AAE/g dry weight). Other leafy vegetables with relatively high antioxidant capacity were *Passiflora edulis*, *Olax zeylanica* and *Costus speciosus*.

Fig. 1 shows the regression analysis to correlate the four antioxidant assays relevant to the total phenolic content of different leaf extracts. The highest correlation coefficient ($r^2 = 0.903$) was exhibited between the DPPH assay and the total phenolic content of leafy vegetable extracts (Fig. 1b), followed by antioxidant capacity assay and the total phenolic content ($r^2 = 0.890$) (Fig. 1a), reducing power assay and total phenolic ($r^2 = 0.846$) (Fig. 1c), and between lipid peroxidation and total phenolic content ($r^2 = 0.769$) (Fig. 1d). As lower correlation values were observed in four antioxidant assays with reference to total carotene and chlorophyll contents, they are not summarized here.

4. Discussion

The antioxidative potential of plant extracts can be measured using various *in vitro* assays and each assay is based on at least one feature of antioxidant activity. However, total antioxidant properties of plants cannot be evaluated by any single

Table 2 – DPPH radical scavenging activity, reducing power, lipid peroxidation activity and total antioxidant capacity of selected green leafy vegetables in Sri Lanka.

Common name	Scientific name	DPPH radical scavenging activity (% inhibition)	Reducing power assay (mg AAE/g dw)[a]	% Inhibition of lipid peroxidation	Total antioxidant capacity (mg AAE/g dw)[a]
Koppa kola	*Polyscias scutellaria*	13.71 ± 3.21	1.93 ± 0.02	81.51 ± 5.10	11.70 ± 0.71
Kurignan	*Gymnema lactiferum*	14.22 ± 0.11	4.47 ± 0.07	73.92 ± 10.41	12.62 ± 0.47
Wel kohila	*Syngonium angustatum*	5.21 ± 1.62	0.64 ± 0.04	55.66 ± 9.11	6.62 ± 0.12
Pitasudu sarana	*Boerhavia diffusa*	4.74 ± 0.28	0.55 ± 0.04	34.37 ± 1.71	6.28 ± 0.10
Cassava	*Manihot esculenta*	22.02 ± 0.90	3.79 ± 0.06	81.77 ± 5.46	4.42 ± 0.46
Sarana	*Trianthema monogyna*	9.01 ± 1.21	0.59 ± 0.06	70.78 ± 6.75	7.02 ± 0.26
Nivithi	*Spinacia oleracea*	10.01 ± 0.21	1.79 ± 0.16	56.71 ± 4.89	7.24 ± 0.06
Thampala	*Amaranthus caudatus*	4.53 ± 0.80	0.70 ± 0.04	79.21 ± 1.78	6.92 ± 0.06
Pumpkin	*Cucurbita maxima*	16.95 ± 5.53	2.08 ± 0.01	87.72 ± 1.48	12.72 ± 0.13
Sweet potato	*Ipomoea batatas*	51.29 ± 2.22	1.46 ± 0.07	59.12 ± 7.65	10.36 ± 0.10
Kathurumurunga	*Sesbania grandiflora*	44.78 ± 3.32	3.79 ± 0.04	81.56 ± 2.80	10.98 ± 0.20
Mukunuwenna	*Alternanthera sessilis*	4.37 ± 0.51	0.92 ± 0.02	71.36 ± 3.46	4.44 ± 0.14
Gotukola	*Centella asiatica*	8.42 ± 0.33	1.06 ± 0.02	80.34 ± 7.23	6.76 ± 0.03
Passion fruit	*Passiflora edulis*	45.91 ± 0.85	10.90 ± 0.10	75.25 ± 5.33	16.08 ± 0.04
Hathawariya	*Asparagus racemosus*	6.81 ± 1.13	1.38 ± 0.10	89.81 ± 2.53	6.66 ± 0.07
Kankung	*Ipomoea aquatica*	6.40 ± 0.28	1.57 ± 0.06	52.82 ± 6.00	9.46 ± 0.29
Heen bowitiya	*Osbeckia octandra*	13.43 ± 3.04	4.61 ± 0.14	73.93 ± 12.00	6.84 ± 0.16
Thebu	*Costus speciosus*	22.51 ± 0.87	2.58 ± 0.12	80.94 ± 7.80	14.46 ± 0.12
Ranawara	*Cassia auriculata*	47.21 ± 0.49	4.78 ± 0.02	37.02 ± 7.18	18.16 ± 0.18
Wel penela	*Cardiospermum halicacabum*	6.29 ± 0.30	5.18 ± 0.30	86.72 ± 4.37	6.68 ± 0.05
Neeramulliya	*Asteracantha longifolia L*	3.71 ± 0.33	0.61 ± 0.02	74.81 ± 5.94	5.28 ± 0.18
Karapincha	*Murraya koenigii* Spreng	48.80 ± 0.67	2.11 ± 0.01	83.52 ± 4.72	25.70 ± 0.37
Koora thampala	*Amaranthus viridis*	24.30 ± 1.55	4.40 ± 0.12	84.48 ± 6.32	15.82 ± 0.36
Rathu thampala	*Amaranthus lividus*	4.25 ± 3.20	1.29 ± 0.04	84.19 ± 10.93	5.67 ± 0.29
Anguna	*Wattakaka volubilis*	12.73 ± 0.60	1.78 ± 0.12	64.10 ± 22.54	10.33 ± 0.34
Thumba	*Leucas zeylanica*	14.36 ± 0.41	1.79 ± 0.02	83.30 ± 9.94	9.12 ± 0.17
Onion leaves	*Allium cepa*	7.71 ± 0.51	5.42 ± 0.02	75.54 ± 13.75	14.52 ± 0.11
Kowakka kola	*Coccinia grandis*	13.21 ± 0.38	1.99 ± 0.15	71.43 ± 5.17	7.24 ± 0.12
Asamodagam	*Trachyspermum involucratum*	6.80 ± 1.35	1.60 ± 0.01	79.62 ± 9.60	7.16 ± 0.31
Lunuwila	*Bacopa monnieri*	24.57 ± 5.24	2.14 ± 0.09	25.71 ± 14.41	15.46 ± 0.07
Kalukamberiya	*Solanum nigrum*	26.21 ± 0.73	3.98 ± 0.09	84.41 ± 5.31	8.59 ± 0.12
Polpala	*Aerva lanata*	5.51 ± 1.12	1.02 ± 0.04	65.01 ± 6.42	7.54 ± 0.17
Mella kola	*Olax zeylanica*	52.25 ± 0.56	5.76 ± 0.31	70.69 ± 11.22	15.86 ± 0.11
Iramusu	*Hemidesmus indicus*	48.34 ± 0.34	4.97 ± 0.08	55.63 ± 5.66	19.43 ± 0.75

Values are presented as mean ± SD, n = 3.
[a] mg ascorbic acid equivalent per g dry weight leaves.

method because of their complex nature of phytochemicals (Chu, Chang, & Hsu, 2000). Therefore, two or more methods should always be employed in order to evaluate the total antioxidative effects of plant extracts such as green leafy vegetables. The present study showed remarkable variations in antioxidant activities among the green leafy vegetables studied. The leaves were analysed in total phenolic, carotene and chlorophyll contents, and in four different antioxidant assays, DPPH radical scavenging assay, reducing power assay, total antioxidant assay and lipid peroxidation assay.

Phenolics are one of the most important natural antioxidants because of their important bioactivities (Chandrasekara & Shahidi, 2011). Phenolic substances have been reported for most of the examined leafy vegetables, and *Gymnema lactiferum*, *Sesbania grandiflora*, *Passiflora edulis*, *Murraya koenigii* Spreng, *Cassia auriculata* and *Hemidesmus indicus* have shown higher phenolic content. Table 3 shows the major bioactives in some leafy vegetable extracts. In Sri Lanka these leafy vegetables are also used in medicinal formulations to control various chronic diseases. However, there is not much published research available on antioxidant properties of leaves of *Gymnema lactiferum*, although it has higher phenolics compared with other leafy vegetables tested. *Gymnema* leaf possesses hypoglycaemic properties (Bandara et al., 2009) and the antidiabetic components of this plant have been identified as gymnemic acid, a phenolic triterpene (Kanetkar, Singhal, Laddha, & Kamat, 2006; Surveswaran, Cai, Corke, & Sun, 2007). *Sesbania grandiflora*, *Passiflora edulis* and *Murraya koenigii* Spreng are well known green leafy vegetables in Asia. Leaves of *Passiflora edulis* is a rich source of bioactive polyphenols, and in a previous rat study it was mentioned that leaf extract could be an option to enhance the supply of antioxidants to safeguard against oxidative stress (da Silva et al., 2013). Further, flavonoids present in *Passiflora edulis* leaves were identified by a high-performance liquid chromatography–diode array detection–tandem mass spectrometry method by Ferreres et al. (2007), and they have characterized sixteen apigenin or luteolin derivatives such as vitexin, isovitexin, orientin and isoorientin. Major antioxidant

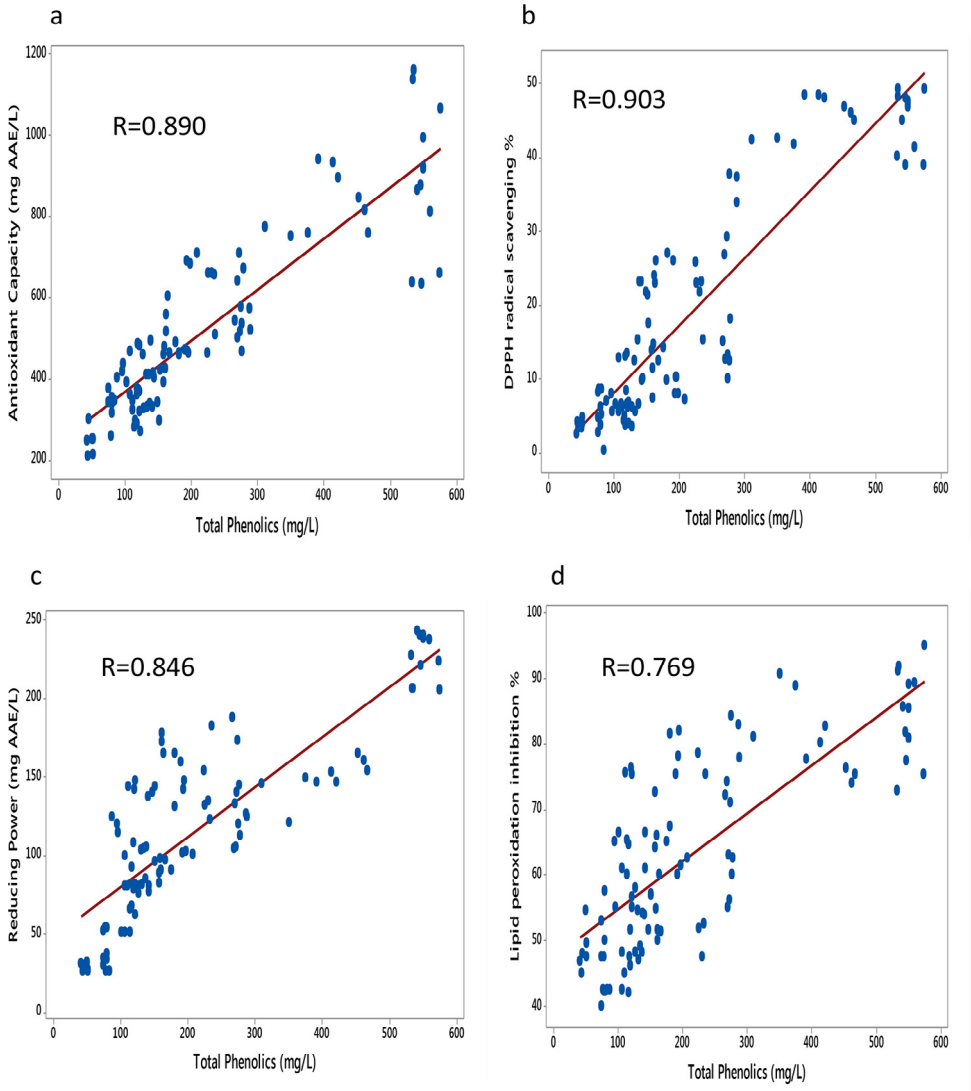

Fig. 1 – Correlation between total phenolics and four antioxidant activities: antioxidant capacity (a), DPPH radical scavenging activity (b), reducing power (c), and lipid peroxidation assay (d).

constituents of *Cassia auriculata* leaves are kaempferol-3-*O*-rutinoside together with kaempferol, quercetin and luteolin (Juan-Badaturuge, Habtemariam, and Thomas (2011). *Hemidesmus indicus* is a twining shrub and known to have wide pharmacological actions. 2-Hydroxy-4-methoxybenzoic acid (HMBA) and a number of pregnane glycosides are believed to be responsible for its various bioactivities, and cinnamic acid, coumaric acid, gentisic acid, and gallic acid are main phenolics reported in this plant (Jayaram & Dharmesh, 2011). Major phenolics in *Murraya koenigii* Spreng leaves are myricetin-3-galactoside, quercetin-*O*-pentohexoside, quercetin-3-diglucoside, quercetin-3-*O*-rutinoside, quercetin-3-glucoside, quercetin-3-acetylhexoside, quercetin-*O*-xylopentoside, kaempferol-*O*-glucoside, and kaempferol-aglucoside (Singh et al., 2011).

Carotenoids are pigments that play a role in the protection of plants against photo-oxidative processes and they are much effective antioxidant scavenging singlet molecular oxygen and peroxyl radicals (Stahl & Sies, 2003). Lutein is a major carotenoid in leafy vegetables which showed marked antioxidant activities (Nicolle et al., 2004). Chandrika et al. (2010) have quantified the carotenoid content in selected leafy vegetables. They have reported that leaves of *Ipomoea batatas* showed the highest carotene content and *Syngonium angustatum* leaves contained the highest amount of lutein as major carotenoid. In another study, carotenoid composition of green leafy vegetables was analysed by HPLC and reported that *Solanum nigrum* was found to contain higher levels of both lutein and β-carotene in the range of 84–187 and 50–115 mg/100 g dry weight, respectively (Raju, Varakumar, Lakshminarayana, Krishnakantha, & Baskaran, 2007). In our study, we also found that the leaves of *Passiflora edulis*, *Cassia auriculata*, and *Solanum nigrum* also contain higher carotene contents and they may have higher singlet oxygen radical scavenging ability. However, in this study, we have not evaluated singlet oxygen radical scavenging assay for the leaf extracts.

Radical scavengers may protect cell tissues from free radicals, thereby preventing diseases such as cancer (Nakayama, Yamada, Osawa, & Kawakishi, 1993). The radical scavenging

Table 3 – Major bioactive content in selected leafy vegetables.

Bioactives	Leafy vegetables	Content in mg/g dry weight	References
Gymnemic acid	*Gymnema lactiferum*	10–54	Kadiragamanathar, Geethika, Premakumara, and Balasubramanium (2009)
2-Hydroxy-4-methoxybenzoic acid	*Hemidesmus indicus*	0.10	Jayaram and Dharmesh (2011)
Gentisic acid		1.35	
Ferulic acid	*Hemidesmus indicus*	0.25	Jayaram and Dharmesh (2011)
	Amaranthus tricolor	0.003[a]	Khanam and Oba (2013)
Caffeic acid	*Hemidesmus indicus*	0.12	Jayaram and Dharmesh (2011)
	Ipomoea batatas	2.17	Islam et al. (2002)
Kaempferol-3-*O*-rutinoside	*Cassia auriculata*	Not given	Juan-Badaturuge et al. (2011)
Myricetin-3-galactoside	*Murraya koenigii* Spreng	0.33	Singh et al. (2011)
Quercetin-*O*-pentohexoside		0.71	
Quercetin-3-diglucoside		0.06	
Quercetin-*O*-xylopentoside		0.70	
Kaempferol-*O*-glucoside		2.41	
Quercetin-3-glucoside	*Murraya koenigii* Spreng	1.44	Singh et al. (2011)
	Cassia auriculata	0.31[a]	Rao, Kumar, Dhandapani, Krishna, and Hayashi (2000)
Lutein	*Polyscias scutellaria*	0.89	Chandrika et al. (2010)
	Gymnema lactiferum	0.93	
	Syngonium angustatum	1.73	
	Manihot esculenta	1.66	
	Trianthema monogyna	0.76	
	Spinacia oleracea	0.64	
	Amaranthus caudatus	0.49	
	Cucurbita maxima	1.53	
	Ipomoea batatas	1.69	
	Sesbania grandiflora	1.08	
	Alternanthera sessilis	0.29	
	Centella asiatica	0.98	
	Murraya koenigii Spreng	0.33	Zhang et al. (2011)
β-carotene	*Polyscias scutellaria*	0.17	Chandrika et al. (2010)
	Gymnema lactiferum	1.63	
	Syngonium angustatum	0.26	
	Manihot esculenta	0.52	
	Trianthema monogyna	0.56	
	Spinacia oleracea	0.30	
	Amaranthus caudatus	0.40	
	Cucurbita maxima	0.66	
	Ipomoea batatas	0.74	
	Sesbania grandiflora	0.40	
	Alternanthera sessilis	0.28	
	Centella asiatica	0.26	
	Murraya koenigii Spreng	0.11	Zhang et al. (2011)
Acylated cyanidins and peonidins	*Ipomoea batatas*	Not given	Islam et al. (2002)
3-*O*-caffeoylquinic acid	*Ipomoea batatas*	Not given	Islam et al. (2002)
3,4-di-*O*-caffeoylquinic acid			
3,5-di-*O*-caffeoylquinic acid			
4,5-di-*O*-caffeoylquinic acid			
3,4,5-tri-*O*-caffeoylquinic acid			
Carbazole alkaloids	*Murraya koenigii* Spreng		Tachibana et al. (2003)
Quercetin	*Sesbania grandiflora*	0.03[a]	Andarwulan et al. (2012)
	Cassia auriculata	Not given	Juan-Badaturuge et al. (2011)
Kaempferol	*Sesbania grandiflora*	0.18[a]	Andarwulan et al. (2012)
	Cassia auriculata	Not given	Juan-Badaturuge et al. (2011)
Anthraquinone glycosides	*Cassia auriculata*	Not given	Surveswaran et al. (2007)
Terpenoid glycosides protoanthocyanidin			
Phenolic acids			
Salicylic acid	*Amaranth tricolor*	0.02[a]	Khanam and Oba (2013)
Vanillic acid		0.01[a]	
Vitexin	*Passiflora edulis*	0.40	da Silva et al. (2013); Ferreres et al. (2007)
Isovitexin		0.50	
Isoorientin		1.05	
Rutin (quercetin-3-*O*-rutinoside)	*Murraya koenigii* Spreng	2.36	Zhang et al. (2011)
	Centella asiatica	1.92	
	Amaranth tricolor	0.003[a]	Khanam and Oba (2013)
	Cassia auriculata	0.77[a]	Rao et al. (2000)

[a] mg/g fresh weight.

activity of methanolic extracts of leafy vegetables was tested using DPPH assay as this is a commonly employed method in antioxidant studies and offers a rapid technique in which to screen the radical scavenging activity of commodities (Amarowicz, Pegg, Rahimi-Moghaddam, Barl, & Weil, 2004). In this process, polyphenolics have the ability to quench DPPH radicals by providing hydrogen atoms or by electron donation and convert them to a colourless product. Hence, the higher the percentage of inhibition of free radical activity, the more potent the antioxidant activity of the extract in terms of hydrogen atom-donating capacity (Amarowicz et al., 2004). The radical scavenging activity of leaf extracts of *Olax zeylanica* and *Ipomea batatas* was far superior to any of the other extracts investigated, and this is the first reported results about DPPH radical scavenging ability of *Olax zeylanica*. Deng et al. (2013) also reported that leaves of *Ipomea batatas* showed good antioxidant activity, although they have used different assays to evaluate antioxidant properties. *Ipomoea batatas* leaves are an excellent source of bioactive anthocyanin and polyphenolic constituents. The main anthocyanins reported in *Ipomoea batatas* leaves are acylated cyanidins and peonidins whereas major phenolic constituents were caffeic acid, 3-*O*-caffeoylquinic acid, 3,4-di-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid, 4,5-di-*O*-caffeoylquinic acid and 3,4,5-tri-*O*-caffeoylquinic acid, and also these phenolics were positively correlated with radical scavenging activities of *Ipomoea batatas* leaves (Islam, Yoshimoto, Ishiguro, & Yamakawa, 2002).

Leaves of *Murraya koengii*, *Cassia auriculata* and *Sesbania grandiflora* also have shown higher free radical scavenging activity, and this may be due to the presence of higher bioactive constituents. *Cassia auriculata* is a medicinal plant widely used in traditional medicine for treating diabetes and various other disease conditions, and the methanolic extract of this leaf displayed potent DPPH radical scavenging activity (Juan-Badaturuge et al., 2011). Other studies have shown that oleoresin of *Murraya koenigii* Spreng extracted using acetone (Rao, Ramalakshmi, Borse, & Raghavan, 2007) and alcohol (Ningappa, Dinesha, & Srinivas, 2008) has been reported to have higher radical-scavenging activities. Carbazole alkaloids were identified as main bioactives in *Murraya koenigii* Spreng, and it was suggested that an arylhydroxy substituent on the carbazole rings plays a role in stabilizing the thermal oxidation and rate of reaction against DPPH radical (Tachibana, Kikuzaki, Lajis, & Nakatani, 2003). *Sesbania grandiflora* contains high levels of kaempferol and this may be the main contributor to the antioxidant activity (Andarwulan et al., 2012; Mustafa, Hamid, Mohamed, & Bakar, 2010). Further, scavenging ability towards DPPH is increased by the length of the effective conjugated double bonds of a compound (Jiménez-Escrig, Jiménez-Jiménez, Sánchez-Moreno, & Saura-Calixto, 2000). The major bioactives of *Cassia auriculata* leaves reported were anthraquinone glycosides, terpenoid glycosides, protoanthocyanidin and phenolic acids, and have shown higher DPPH radical scavenging activities (Surveswaran et al., 2007). The extract of *Asteracantha longifolia*, *Alternanthera sessilis*, *Amaranthus lividus*, *Amaranthus caudatus* and *Syngonium angustatum* contained the lowest amount of polyphenolics compared with other leaves. Therefore, they exhibited lower radical scavenging activities. However, *Alternanthera sessilis* plant extracts were studied for its antioxidant activity by Borah, Yadav, and Unni (2011) and reported that methanolic extract has shown the highest radical scavenging activity.

Reducing power is associated with antioxidant activity and serves as a significant reflection of the antioxidant property of a commodity (Oktay, Gülçin, & Küfrevioğlu, 2003). Reducing power assay is also widely used in evaluating antioxidant activity of plant polyphenols and the reducing power of leaf extracts was evaluated by measuring the absorbance 700 nm after mixing the extracts with ferric compounds. The samples with higher reducing power show higher absorbance. The presence of reductants like antioxidants in the leaf extracts causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form, indicating that they are electron donors and can reduce the oxidized intermediates of lipid peroxidation process. Therefore, they can act as primary and secondary antioxidants (Amarowicz et al., 2004). It was reported that the reducing power of polyphenolics is probably due to the presence of hydroxyl group, which might act as electron donors (Shimada, Fujikawa, Yahara, & Nakamura, 1992). Leaves of *Passiflora edulis* and *Olax zeylanica* have shown higher reducing potential. This could be attributed to the high phenolic content in these leaf extracts, which can act as free radicals scavenger by donating an electron or hydrogen. In our study, we found that the phenolic contents of leafy vegetables extracts strongly correlate with reducing power assay ($r^2 = 0.846$).

Oxidation damages to the tissues through lipid peroxidation could result in changes in cellular biomembranes (Skrzydlewska, Ostrowska, Farbiszewski, & Michalak, 2002). We used three types of Amaranthus in the present study – *Amaranthus caudatus*, *Amaranthus lividus* and *Amaranthus viridis* – and they have shown higher lipid peroxidation inhibition activity. Ozsoy, Yilmaz, Kurt, Can, and Yanardag (2009) have reported that Amaranth leaves contained naturally occurring antioxidant components and possessed antioxidant activity which may be attributed to its lipid peroxidation inhibitory activities. Isoqercetin and rutin were the most abundant flavonoids, and salicylic acid, syringic acid, gallic acid, vanillic acid, ferulic acid, p-coumaric acid and sinapic acid are the most common phenolic acids in amaranth cultivars (Khanam & Oba, 2013). Although *Boerhaavia diffusa* exhibited lower antioxidant properties compared with other leafy vegetables in this study, in a previous *in vivo* study, ethanolic extracts of *Boerhaavia diffusa* leaves have shown antioxidant and hepatoprotective properties such as inhibition of acetaminophen induced lipid peroxidation (Olaleye, Akinmoladun, Ogunboye, & Akindahunsi, 2010). Further, in another *in vitro* study, the antioxidant potency of methanolic extract of *Boerhaavia diffusa* was studied and showed significant scavenging activity against hydroxyl and superoxide radicals and also it inhibited the lipid peroxidation in linoleic acid emulsion system (Prathapan et al., 2011). The antioxidant properties of leaves of *Hemidesmus indicus* (L.) were evaluated by Murali, Ashok, and Madhavan (2011) using an *in vivo* model (Wistar rats) and found that leaf of *H. indicus* var. *pubescens* possesses equipotent antioxidant effects with the standard antioxidant drug, silymarin. Lipid peroxidation is the net result of any free radical attack on membrane and other lipid constituent present in the system and this can be enzymatic or non-enzymatic. Since we have used egg-yolk as a substrate for this assay, it could be suggested that leaf extracts are active against non-enzymatic oxidation and the

inhibition of lipid peroxidation may be either due to chelation of Fe or by free radical trapping (Pandey, Chaurasia, Tiwari, & Tripathi, 2007). Lipid peroxidation inhibition activity may be due to the presence of polyphenolics and showed a good correlation with phenolic contents of leaf ($r^2 = 0.769$).

A spectrophotometric method described by Prieto et al. (1999) was used for the quantitative determination of antioxidant capacity. This assay is based on the reduction of MO(VI) to MO(V) by the sample analyte and subsequent formation of a green phosphate/MO(V) complex at acidic medium. Measuring a total antioxidant capacity of a sample might be more useful than determining that of specific antioxidant species in a sample. The total antioxidant capacity of leafy vegetables such as *Murraya koenigii* Spreng, *Hemidesmus indicus*, *Cassia auriculata*, *Passiflora edulis* and *Olax zeylanica* was studied and these have shown higher antioxidant activities including higher reducing potentials. In another study, Gupta and Prakash (2009) reported that antioxidant capacities were in the order of *Murraya koenigii* > *Amaranthus* sp. > *Centella asiatica*, and they have expressed the antioxidant capacities as equivalents of ascorbic acid in μmol/g of sample. A similar trend is also observed in our findings for the antioxidant capacity of these three leaves. The total antioxidant capacity of leaf extracts may be attributed to their chemical composition and phenolic content. In our study, we found that the phenolic contents of leafy vegetables extracts strongly correlate with total antioxidant capacity assay ($r^2 = 0.890$). A good correlation between phenolics and antioxidant properties has also been previously reported by Zheng and Wang (2001) and Rudnicki et al. (2007). However, a comparatively poor correlation was found between chlorophyll and carotene content of studied leafy vegetables and antioxidant assays used (data not shown).

In conclusion, the data from this screening study indicated that these leafy vegetables have shown remarkable variations in antioxidant activities. Among the leafy vegetables studied, *Sesbania grandiflora*, *Cassia auriculata*, *Murraya koenigii* Spreng, *Passiflora edulis*, *Gymnema lactiferum* and *Olax zeylanica* showed comparatively higher antioxidant properties. A good correlation between the results from total polyphenolics and the four antioxidative assays of leafy vegetables was observed in this study.

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