Antioxidative Properties of 34 Green Leafy Vegetables

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Abstract

Green leafy vegetables available in Sri Lanka have not been fully exploited though 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 analyzed 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 *Olex 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.

Keywords: Green leafy vegetables, polyphenolics, antioxidant activities, reducing power, carotene

1.0 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 et al., 2006; Madamanchi et al., 2005). Though an organism is naturally equipped with antioxidant protection systems to cope with the harmful effects of ROS, the endogenous antioxidant defense 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 antioxidant vitamins (Subhasree et al., 2009). Various research findings have suggested that leafy vegetables have medicinal properties such as anticarcinogenic (Rajeshkumar et al., 2002), antibacterial (Kubo et al., 2004) and antidiabetic (Kesari et al., 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 (Subhasree et al., 2009; Andarwulan et al., 2012; Khanam et al., 2012; Deng et al., 2013). Lutein is one of the major carotenoids in green leafy vegetables which show a marked antioxidant activity (Chandrika et al., 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.

2.0 Materials and method

2.1. Materials

Thirty four types of fresh green leafy vegetable samples were collected from various places in Colombo, Negambo 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 792g. 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 et al., (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 (0.5N) were mixed and incubated at room temperature for 15 min in the dark. Then 2.5 mL 7.5% sodium carbonate were added to the mixture and further incubated for 2 hours in the dark at room temperature and then the absorbance were 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 1000-15000 μg GAE/L.

2.5. Total chlorophyll and carotene contents

The chlorophyll and carotene contents were analyzed according to the method described by Türlerinde et al. (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 245g. The supernatant was separated and the absorbance was read at 470, 653, 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 = $11.75 (A_{662}) - 2.350(A_{645})$.

Chlorophyll b = $18.61 (A_{645}) - 3.960 (A_{662})$.

Carotene = $1000 (A_{470}) - 2.270 (C_a) - 81.4 (C_b)/227$.

where C_a , Chlorophyll a and C_b , 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 et al. (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 antioxidant activity of leaf extracts was calculated using following formula.

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

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 352g. 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 Ohkawa et al. (1979). Briefly 0.5 mL egg homogenate (10%, v/v) was mixed with 0.1 mL leaf extract, 0.4 mL distilled water and 0.05mL 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) thiobarbuturic 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 were analyzed according to the method described by Prieto et al. (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 $\pm$ 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 analyzed 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 were performed using MINITAB 17.

3.0 Results

Total phenolics, chlorophyll and carotene contents of 34 leafy vegetables were analyzed and the results are summarized in Table 1. Total phenolic content of the methanolic extracts of leafy vegetables were 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).

144 However, among the leaves studied, *Asteracantha longifolia* (0.92 mg GAE/g dry weight) and
145 *Alternanthera sessilis* (0.98 mg GAE/g dry weight) contained a significantly lower phenolic
146 content.

147 Chlorophyll content in leafy vegetables ranged from 9.20 to 37.10 µg/g dry weight. *Boerhavia*
148 *diffusa* (9.20 µg/g), *Alternanthera sessilis* (9.55 µg/g), *Allium cepa* (9.45 µg/g) and *Amaranthus*
149 *caudatus* (11.37 µg/g) showed significantly lower chlorophyll content, whereas *Asteracantha*
150 *longifolia* (37.10 µg/g) had the highest chlorophyll content. Carotene content of studied leafy
151 vegetables was within the range of 0.47-4.17 mg/g dry weight. Among the leafy vegetables
152 analyzed, *Passiflora edulis* had the highest carotene content (4.17 mg/g) followed by *Cassia*
153 *auriculata* (3.45 mg/g), *Solanum nigrum* (3.18 mg/g) and *Syngonium angustatum* (3.13 mg/g).
154 The lowest carotene content was observed in *Alternanthera sessilis* (0.47 mg/g), *Allium cepa* (0.53
155 mg/g), *Boerhavia diffusa* (0.67 mg/g), *Osbeckia octandra* (0.79 mg/g) and *Bacopa monnieri*
156 (0.89 mg/g).

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

167 Reducing power of the leaf extracts was within the range of 0.55-10.90 mg ascorbic acid
168 equivalents (AAE/g dry weight of leaf) and *Passiflora edulis* (10.90 mg AAE/g dry weight)
169 showed significantly higher ($p<0.05$) reducing power compared with other leaves. Leaves of
170 *Olax zeylanica* and *Allium cepa* also have shown higher reducing properties. *Boerhavia diffusa*
171 (0.55 mg AAE/g dry weight), *Triathema monogyna* (0.59 mg AAE/g dry weight), *Syngonium*
172 *angustatum* (0.64 mg AAE/g dry weight), and *Asteracantha longifolia* (0.61 mg AAE/g dry
173 weight), showed lower reducing power comparatively.

174 Percentage inhibition of lipid peroxidation by leaf extracts were in the range from 34.3%
175 (*Boerhavia diffusa*) to 89.8% (*Asparagus racemosus*). *Polyscias scutellaria*, *Manihot esculenta*,
176 *Cucurbita maxima*, *Centella asiatica*, *Costus speciosus*, *Cardiospermum halicacabum*, *Murraya*
177 *Koenigii* Spreng, *Amaranthus spp.*, *Leucas zeylanica* and *Solanum nigrum* also showed higher
178 lipid peroxidation inhibition ability. Total antioxidant capacity of the leaf extracts were within
179 the range of 4.42-25.70 mg AAE/g dry weight. *Murraya Koenigii* Spreng showed the highest
180 antioxidant capacity (25.70 mg AAE/g dry weight) followed by the *Hemidesmus indicus* (19.43
181 mg AAE/g dry weight) and *Cassia auriculata* (18.16 mg AAE/g dry weight). Other leafy
182 vegetables with relatively high antioxidant capacity were *Passiflora edulis*, *Olax zeylanica* and
183 *Costus speciosus*.

184 Figure 1 shows the regression analysis to correlate the four antioxidant assays with relevant to
185 total phenolic content of different leaf extracts. The highest correlation coefficient ($r^2=0.903$)
186 was exhibited between the DPPH assay and the total pheolic content of leafy vegetable extracts
187 (Figure 1b), followed by antioxidant capacity assay and the total phenolic content ($r^2=0.890$)
188 (Figure 1a); reducing power assay and total phenolic ($r^2=0.846$) (Figure 1c) and between lipid
189 peroxidation and total phenolic content ($r^2=0.769$) (Figure 1d). As lower correlation values were

observed in four antioxidant assays with reference to total carotene and chlorophyll contents, they are not summarized here.

4.0 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 method because of their complex nature of phytochemicals (Chu et al., 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 analyzed 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, also use in medicinal formulations to control various chronic diseases. However, there is not much published research available on antioxidant properties of leaves of *Gymnema lactiferum* though it has higher phenolics compared with other leafy vegetables tested. *Gymnema* leaf possesses hypoglycemic properties (Bandara et al., 2009) and the antidiabetic components of this plant have been identified as gymnemic acid, a phenolic triterpene (Kanetkar et al., 2006; Surveswaran et al., 2007). *Sesbania grandiflora*, *Passiflora*

214 *edulis* and *Murraya koenigii* Spreng are well known green leafy vegetables in Asia. Leaves of
215 *Passiflora edulis*, is a rich source of bioactive polyphenols and in a previous rat study, it was
216 mentioned that leaf extract could be an option to enhance the supply of antioxidants to safeguard
217 against oxidative stress (da Silva et al., 2013). Further, flavonoids present in *Passiflora edulis*
218 leaves were identified by a high-performance liquid chromatography–diode array detection–
219 tandem mass spectrometry method by Ferreres et al. (2007) and they have characterized sixteen
220 apigenin or luteolin derivatives such as vitexin, isovitexin, orientin and isoorientin. Major
221 antioxidant constituents of *Cassia auriculata* leaves are kaempferol-3-*O*-rutinoside together with
222 kaempferol, quercetin and luteolin (Juan-Badaturuge et al. (2011). *Hemidesmus indicus* is a
223 twining shrub and known to have wide pharmacological actions. 2-Hydroxy-4-methoxybenzoic
224 acid (HMBA) and a number of pregnane glycosides are believed to be responsible for its various
225 bioactivities and cinnamic acid, coumaric acid, gentisic acid and gallic acid are main phenolics
226 reported in this plant (Jayaram and Dharmesh, 2011). Major phenolics in *Murraya koenigii*
227 Spreng leaves are myricetin-3-galactoside, quercetin-*O*-pentohehexoside, quercetin-3-diglucoside,
228 quercetin-3-*O*-rutinoside, quercetin-3-glucoside, quercetin-3-acetylhexoside, quercetin-*O*-
229 xylopentoside, kaempferol-*O*-glucoside, and kaempferol-aglucoside (Singh et al., 2011).
230 Carotenoids are pigments which play a role in the protection of plants against photo-oxidative
231 processes and they are much effective antioxidant scavenging singlet molecular oxygen and
232 peroxy radicals (Stahl & Sies, 2003). Lutein is a major carotenoid in leafy vegetables which
233 showed marked antioxidant activities (Nicolle et al., 2004). Chandrika et al. (2010) have
234 quantified the carotenoid content in selected leafy vegetables. They have reported that leaves of
235 *Ipomoea batatas* showed the highest carotene content and *Synгонium angustatum* leaves
236 contained the highest amount of lutein as major carotenoid. In another study, carotenoid

composition of green leafy vegetables was analyzed by HPLC and reported that *Solanum nigrum* were 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 et al., 2007). In our study also we found that, leaves of *Passiflora edulis*, *Cassia auriculata*, *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 et al., 1993). The radical scavenging 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 et al., 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 colorless product. Hence, 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 though 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 correlate with radical scavenging activities of *Ipomoea batatas* leaves (Islam et al., 2002).

Leaves of *Murraya koengii*, *Cassia auriculata* and *Sesbania grandiflora* also have shown higher free radical scavenging activity and this may be due to 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 et al., 2007) and alcohol (Ningappa et al., 2008) have 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 et al., 2003). *Sesbania grandiflora* contains high levels of kaempferol and this may be the main contributor to the antioxidant activity (Andarwulan et al., 2012; Mustafa et al., 2010). Further, scavenging ability toward DPPH is increased by the length of the effective conjugated double bonds of a compound (Jiménez-Escrig et al., 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 lividis*, *Amaranthus caudatus* and *Syngonium angustatum* contained lowest amount polyphenolics compared with other leaves. Therefore, they exhibited lower radical scavenging activities. However, *Alternanthera sessilis* plant extracts was studied for its antioxidant activity by Borah et al. (2011) and reported that methonolic extract have shown highest radical scavenging activity.

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

298 Oxidation damages to the tissues through lipid peroxidation could result in changes in cellular
299 biomembranes (Skrzydowska et al., 2002). We used three types of Amaranthus in the present
300 study: *Amaranthus caudatus*, *Amaranthus lividis* and *Amaranthus viridis* and they have shown
301 higher lipid peroxidation inhibition activity. Ozsoy and co-authors (2009) have reported that
302 Amaranth leaves contained naturally occurring antioxidant components and possessed
303 antioxidant activity which may be attributed to its lipid peroxidation inhibitory activities.
304 Isoquercetin and rutin were the most abundant flavonoids and salicylic acid, syringic acid, gallic
305 acid, vanilic acid, ferulic acid, p-coumaric acid and sinapic acid are the most common phenolic

306 acids in amaranth cultivars (Khanam et al., 2013). Though *Boerhaavia diffusa* exhibited lower
307 antioxidant properties compared with other leafy vegetables in this study, in a previous *in vivo*
308 study, ethanolic extracts of *Boerhaavia diffusa* leaves have shown antioxidant and
309 hepatoprotective properties such as inhibition of acetaminophen induced lipid peroxidation
310 (Olaleye et al., 2010). Further, in another *in vitro* study, the antioxidant potency of methanolic
311 extract of *Boerhaavia diffusa* and showed significant scavenging activity against hydroxyl and
312 superoxide radicals and also it inhibited the lipid peroxidation in linoleic acid emulsion system
313 (Prathapan et al., 2011). Antioxidant properties of leaves of *Hemidesmus indicus* (L.) was
314 evaluated by Murali et al. (2011) using an *in vivo* models (wister rats) and found that leaf of *H.*
315 *indicus* var. *pubescens* possesses equipotent antioxidant effects with the standard antioxidant
316 drug, silymarin. Lipid peroxidation is the net result of any free radical attack on membrane and
317 other lipid constituent present in the system and this can be enzymatic or non-enzymatic. Since
318 we have used egg-yolk as a substrate for this assay, it could be suggested that leaf extracts are
319 active against non-enzymatic oxidation and the inhibition of lipid peroxidation may be either due
320 to chelation of Fe or by free radical trapping (Pandey et al, 2007). Lipid peroxidation inhibition
321 activity may be due to presence of polyphenolics and showed a good correlation with phenolic
322 contents of leaf ($r^2 = 0.769$).

323 A spectrophotometric method described by Prieto et al. (1999) was used for the quantitative
324 determination of antioxidant capacity. This assay is based on the reduction of MO(VI) to MO(V)
325 by the sample analyte and subsequent formation of a green phosphate/MO(V) complex at acidic
326 medium. Measuring a total antioxidant capacity of a sample might be more useful than
327 determining that of specific antioxidant species in a sample. Total antioxidant capacity of leafy
328 vegetables such as *Murraya koenigii* Spreng, *Hemidesmus indicus*, *Cassia auriculata*, *Passiflora*

329 *edulis* and *Oxalys zeylanica* and have shown higher antioxidant activities including higher reducing
330 potentials. In another study, Gupta and Prakash (2009) reported that antioxidant capacities were
331 in the order of *Murraya koenigii* > *Amaranthus* sp. > *Centella asiatica*, and they have expressed
332 the antioxidant capacities as equivalents of ascorbic acid in $\mu\text{mol/g}$ of sample. A similar trend is
333 also observed in our findings for the antioxidant capacity of these three leaves. The total
334 antioxidant capacity of leaf extracts may be attributed to their chemical composition and
335 phenolic content. In our study, we found that the phenolic contents of leafy vegetables extracts,
336 strongly correlate with total antioxidant capacity assay ($r^2 = 0.890$). A good correlation between
337 phenolics and antioxidant properties has also been previously reported by Zheng and Wang
338 (2001) and Rudnicki et al. (2007). However, a comparatively poor correlation was found between
339 chlorophyll and carotene content of studied leafy vegetables and antioxidant assays used (data
340 not shown).

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

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

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

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

Values are presented as mean $\pm$ SD, n = 3.

* mg gallic acid equivalent per g dry weight (dw) leaves

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

Values are presented as mean ± SD, n = 3.

* mg Ascorbic acid equivalent per g dry weight leaves

Table 3: major bioactive content in selected leafy vegetables

Bioactives	Leafy vegetables	Content in mg/g dry weight	References
Gymnemic acid	<i>Gymnema lactiferum</i>	10-54	Kadiragamanathar et al., 2009
2-Hydroxy-4-methoxybenzoic acid	<i>Hemidesmus indicus</i>	0.10	Jayaram and Dharmesh, 2011
Gentisic acid		1.35	
Ferulic acid	<i>Hemidesmus indicus</i> <i>Amaranthus tricolor</i>	0.25 0.003*	Jayaram and Dharmesh, 2011 Khanam et al., 2013
Caffeic acid	<i>Hemidesmus indicus</i> <i>Ipomoea batatas</i>	0.12 2.17	Jayaram and Dharmesh, 2011 Islam et al., 2002
Kaempferol-3- <i>O</i> -rutinoside	<i>Cassia auriculata</i>	Not given	Juan-Badaturuge et al., 2011
myricetin-3-galactoside	<i>Murraya koenigii</i> Spreng	0.33	Singh et al., 2011
quercetin- <i>O</i> -pentohexoside		0.71	
quercetin-3-diglucoside		0.06	
quercetin- <i>O</i> -xylopentoside		0.70	
kaempferol- <i>O</i> -glucoside		2.41	
quercetin-3-glucoside	<i>Murraya koenigii</i> Spreng	1.44	Singh et al., 2011
	<i>Cassia auriculata</i>	0.31*	Rao et al., 2000
Lutein	<i>Polyscias scutellaria</i> <i>Gymnema lactiferum</i> <i>Syngonium angustatum</i> <i>Manihot esculenta</i> <i>Triathema monogyna</i> <i>Spinaces oleracea</i> <i>Amaranthus caudatus</i> <i>Cucurbita maxima</i> <i>Ipomoea batatas</i> <i>Sesbania grandiflora</i> <i>Alternanthera sessilis</i> <i>Centella asiatica</i>	0.89 0.93 1.73 1.66 0.76 0.64 0.49 1.53 1.69 1.08 0.29 0.98	Chandrika et al., 2010
	<i>Murraya koenigii</i> Spreng	0.33	Zhang et al., 2011
β-carotene	<i>Polyscias scutellaria</i> <i>Gymnema lactiferum</i> <i>Syngonium angustatum</i> <i>Manihot esculenta</i> <i>Triathema monogyna</i> <i>Spinaces oleracea</i> <i>Amaranthus caudatus</i> <i>Cucurbita maxima</i> <i>Ipomoea batatas</i> <i>Sesbania grandiflora</i> <i>Alternanthera sessilis</i> <i>Centella asiatica</i>	0.17 1.63 0.26 0.52 0.56 0.30 0.40 0.66 0.74 0.40 0.28 0.26	Chandrika et al., 2010
	<i>Murraya koenigii</i> Spreng	0.11	Zhang et al., 2011
acylated cyanidins and peonidins	<i>Ipomoea batatas</i>	Not given	Islam et al., 2002
3- <i>O</i> -caffeoylquinic acid 3,4-di- <i>O</i> -caffeoylquinic acid 3,5-di- <i>O</i> -caffeoylquinic acid 4,5-di- <i>O</i> -caffeoylquinic acid	<i>Ipomoea batatas</i>	Not given	Islam et al., 2002

3,4,5-tri- <i>O</i> -caffeoylquinic acid			
Carbazole alkaloids	<i>Murraya koenigii</i> Spreng		Tachibana et al., 2003
Quercetin	<i>Sesbania grandiflora</i> <i>Cassia auriculata</i>	0.03* Not given	Andarwulan et al., 2012 Juan-Badaturuge et al., 2011
Kampferol	<i>Sesbania grandiflora</i> <i>Cassia auriculata</i>	0.18* Not given	Andarwulan et al., 2012 Juan-Badaturuge et al., 2011
anthraquinone glycosides terpenoid glycosides protoanthocyanidin phenolic acids	<i>Cassia auriculata</i>	Not given	Surveswaran et al., 2007
salicylic acid vanilic acid	<i>Amaranth tricolor</i>	0.02* 0.01*	Khanam et al., 2013
vitexin isovitexin isoorientin	<i>Passiflora edulis</i>	0.40 0.50 1.05	da Silva et al., 2013; Ferreres et al., 2007
Rutin (quercetin-3-O-rutinoside)	<i>Murraya koenigii</i> Spreng	2.36	Zhang et al., 2011
	<i>Centella asiatica</i>	1.92	
	<i>Amaranth tricolor</i>	0.003*	Khanam et al., 2013
	<i>Cassia auriculata</i>	0.77*	Rao et al., 2000

*mg/g fresh weight