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Assessment of free radical scavenging activities of leaves and stem fractions of green leafy vegetables

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Green leafy vegetables (GLVs) symbolize a class of low cost, neglected plants that are known to be wealthy spring of natural antioxidants. In the present study, the phytochemical and radical scavenging activities of both stem and leaf fractions of 20 commonly consumed GLVs of tropical India were evaluated using different systems of assays like total antioxidant assay, ferric reducing antioxidant potential (FRAP), 2,2-diphenylpicrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), hydrogen peroxide, hydroxyl and superoxide radical-scavenging activity. The results showed that all the vegetable extracts were found to have variable levels of antioxidant properties in different assay systems. Furthermore most of the GLVs are good sources of phytochemicals like flavonoids and phenolics that are excellent free radical scavengers. The results of this study will be very useful for health conscious consumers and also for current generation especially those neglecting the leafy vegetables. This study will be a prelude for future exploration of these green leafy vegetables as potential functional foods. However, still, detailed studies are needed to reveal the role of individual bioactive components that influence the antioxidant properties of these vegetables.

Key words: Antioxidants, flavonoids, leafy vegetables, phenolics, radical scavenging activity.

INTRODUCTION

India, being a tropical country has abundant and diversified plant species that grow naturally, and in earlier days many of them are part of the conventional diet (Murugan et al., 2013). In recent days, the attractiveness of fast food is becoming an obligate to the busy lifestyles of current generation that eventually lead to obesity and other adverse effects (Gacche et al., 2010; Murugan et al., 2013). In this mechanical world, people find it expedient to grab some low nutritional food as a fashion rather than to spend a penny for highly nutritious diet. This mentality of modern generation towards the adoption of globalised diet lead to losening the texture of our traditional nutritional diet that are rich in fruits and vegetables. One such neglected vegetable is leafy greens, commonly called green leafy vegetables (GLV).

Green leafy vegetables (GLVs) comprise an imperative part of a balanced diet and shown to be rich in bioactive compounds like polyphenols, carotenoids, flavonoids, flavones, isoflavones, catechins, vitamins and minerals (Gazzani et al., 1998; Vinson et al., 1998). Intake of leafy

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vegetables helps in the normal functioning of the body, as they serve as good source of antioxidants, hence GLVs are considered as “Nature’s anti-aging wonders” due to its curative properties apart from basic nutrition (Gupta and Prakash, 2009). The routine consumption of these vegetables may probably reduce the risk of oxidative induced damage and other degenerative diseases (Van Duyn and Pivonka, 2000; Oboh et al., 2008; Sreeramulu and Ragunath, 2010). In traditional system of medicine, GLVs are used to treat various illnesses. For example, the leaves of *Daucus carota* have been used as purgative. *Portulaca oleracea* can be used as an anti-scorbutic and its paste has been applied for wound healing (Ambastha, 1986).

Reactive oxygen species (ROS) is a group of highly knee-jerk molecules generated as a result of oxidative metabolism that causes major damage to cell membranes and other macromolecules leading to membrane lipid peroxidation, decreased membrane fluidity and other DNA mutations which appears to be the major factor for the pathogenesis of many diseases including cancer, aging and other degenerative diseases (Finkel and Holbrook, 2000). Antioxidants are agents which scavenge the free radicals, thereby preventing the oxidizing chain reactions and cell damage caused by reactive oxygen species (Namiki, 1990). Even though our body has its own antioxidant components to overcome this free radical induced damage, their production in animal cells are limited (Halvorsen et al., 2002), hence antioxidants have to be included in diet to maintain the balance between the antioxidants and free radicals. Leafy vegetables contain different antioxidant compounds whose activities have been well-known in modern existence (Khanam et al., 2012). Even though leafy vegetables are consumed all over the world, not much is known about its antioxidant properties. Hence, in the present investigation, an attempt has been made to demonstrate the phyto-chemical and free radical-scavenging activities of some of the neglected green leafy vegetables.

MATERIALS AND METHODS

Samples

In the present study, 20 different commonly consumed green leafy vegetables (*Allium ampeloprasum, Allium wakegi, Apium graveolens, Amaranthus gangeticus, Anethum graveolens, Basella rubra, Beta vulgaris, Brassica oleracea var italica, Brassica chinensis, Corchorus aestuans, Daucus carota, Erythrina variegata, Lactuca sativa, Mukia leiosperma, Oxalis corniculata, Petroselinum crispum, Portulaca oleracea, Premna corymbosa, Rumex vesicarius, Tamarindus indica*) of tropical India were selected. The plants were authenticated by Prof. S. Ramachandran, Taxonomist, Department of Botany, Bharathiar University, Tamil Nadu, India.

Chemicals

The compounds 2,2'-azino-di-[3-ethylbenzthiazoline sulfonate (ABTS)], 2,2-diphenyl-1-picrylhydrazyl (DPPH), aluminium chloride, ammonium molybdate, ammonium persulfate, ethylene diamine tetraacetic acid (EDTA), Folin-Ciocalteu reagent, ferrous sulfate, 2,4,6-tris (2-pyridyl)-s-triazine (TPTZ) gallic acid, sodium carbonate, sodium salicylate and quercetin were obtained from Sigma Chemical Co. (St. Louis, MO). All the organic solvents were of high performance liquid chromatography (HPLC) grade and were purchased from Fisher Scientific (Nepean, Canada).

Preparation of sample extract

The vegetables were cleaned, air dried and cut into small pieces prior to extraction. The stem and leaf (0.5 g) fractions were weighed and homogenized in 5 ml of methanol until it becomes a fine paste. The samples were then centrifuged at 4000 rpm for 10 min. The procedure was repeated twice and the supernatants were collected and stored for the further analysis.

Phytochemical and radical scavenging analysis

The total flavonoid content in the extract was estimated by aluminium chloride ($AlCl_3$) method (Chang et al., 2002). Briefly, the methanolic extract of vegetables was mixed with $AlCl_3$ and potassium acetate; the mixture was incubated in room temperature for 30 min and then the absorbance was read at 415 nm. The total phenolic content of the vegetable extract was quantified by Folin-Ciocalteu method (Singleton and Rossi, 1965). The stem and leaf extracts were mixed with 10% of Folin-Ciocalteu’s reagent and 5% of sodium carbonate. Then the mixture was incubated for 30 min at room temperature and absorption was measured at 765 nm.

The total antioxidant activity of the vegetable extracts was analyzed using phosphomolybdenum method and the results were expressed as milligrams of ascorbic acid equivalents per gram fresh weight of the sample (Prieto et al., 1999). The ability of the plant extracts to reduce the ferrous ions was measured using the method followed by Benzie and Strain (1996). The Ferric reducing/antioxidant power assay was determined using the FRAP reagent and then the result was expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1 mM $FeSO_4$. DPPH radical quenching ability of the vegetable extracts was analyzed following the method of Gyamfi et al. (1999). The vegetable extract was mixed with 1 ml of 1 mM DPPH methanolic solution and incubated for 30 min. The percentage of DPPH radical scavenging activity was calculated using the formula.

$$\text{DPPH radical scavenging activity (\%)} = [(A_0 - A_1) / A0] \times 100$$

Where A_0 is the absorbance of the control (without extract) and A_1 is the absorbance of the sample. The ability of the extract to scavenge hydrogen peroxide was determined according to the method employed by Ruch et al. (1989). The percentage of H_2O_2 scavenging by plant extracts was calculated as follows:

$$\text{Hydrogen peroxide scavenging activity (\%)} = [(A0 - A1 / A0) \times 100]$$

Where, A0 = absorbance of control; A1 = absorbance in presence of sample of extracts and standard. Hydroxyl radical scavenging activity was assayed by the method of Wang et al. (2008) and the absorbance of the hydroxylated salicylate complex was read at 562 nm. The ABTS assay was carried out according to the method of Arnao et al. (2001). ABTS radical scavenging activity of the extract was calculated by:

$$\text{ABTS scavenging activity (\%)} = [(A0 - A1 / A0) \times 100]$$

Where, A0 = absorbance of control; A1 = absorbance of sample/

Table 1. Total Flavonoids and phenolic content of green leafy vegetables[a]

Scientific name	Total flavonoids[b] (mg/g)		Total phenolics[c] (mg/g)	
	Leaf	Stem	Leaf	Stem
Allium ampeloprasum	9.6±0.04	3.2±0.04	11.8±0.06	21.4±0.03
Allium wakegi	5.4±0.03	9.0±0.12	13.8±0.02	20.6±0.06
Apium graveolens	7.8±0.03	4.6±0.05	16.0±0.17	22.2±0.04
Amaranthus gangeticus	8.0±0.03	3.8±0.02	13.8±0.09	20.0±0.06
Anethum graveolens	12±0.06	7.6±0.06	13.8±0.02	20.6±0.02
Basella rubra	8.6±0.08	3.8±0.06	16.0±0.06	20.0±0.05
Beta vulgaris	8.6±0.03	4.2±0.10	11.8±0.12	16.0±0.02
Brassica oleracea var italica	9.0±0.04	4.2±0.03	17.8±0.03	13.8±0.02
Brassica chinensis	2.6±0.05	2.0±0.03	13.8±0.07	11.8±0.13
Corchorus aestuans	11.6±0.02	5.6±0.03	21.8±0.07	17.8±0.14
Daucus carota	5.8±0.01	6.0±0.09	30.4±0.10	11.8±0.11
Erythrina variegata	9.2±0.04	4.2±0.02	26.2±0.07	20.0±0.31
Lactuca sativa	3.0±0.02	3.0±0.02	11.8±0.10	12.2±0.03
Mukia leiosperma	6.4±0.13	7.2±0.04	20.6±0.21	17.8±0.07
Oxalis corniculata	11.2±0.02	5.6±0.02	25.2±0.03	17.8±0.02
Petroselinum crispum	22.4±0.08	6.4±0.02	23.2±0.02	20.0±0.02
Portulaca oleracea	13.2±0.11	3.8±0.08	21.0±0.02	16.0±0.03
Premna corymbosa	6.4±0.14	4.2±0.04	20.2±0.07	11.9±0.04
Rumex vesicarius	19.6±0.06	5.4±0.02	23.6±0.03	17.8±0.04
Tamarindus indica	13.4±0.03	6.4±0.09	20.6±0.02	22.6±0.20

[a]Data represented are the means of three replicates±SD. [b]Data expressed as milligrams of Quercetin equivalents per gram fresh weight. [c]Data expressed as milligrams of Gallic acid equivalents (GAE) per gram fresh weight .

standard. The capacity of the vegetable extract to inhibit the formation of superoxide radical generated in the riboflavin light NBT system was determined by the method of Dasgupta and De (2004) and the percentage inhibition was calculated.

RESULTS

In the present study, the flavonoid content of 20 commonly consumed leafy vegetables was evaluated and the results were expressed as quercetin equivalents (Table 1). Among the 20 GLVs analyzed, the total flavonoid content in the leaf and stem tissues varied from 2.6 to 22.4 mg quercetin equivalents/g and 2.0 to 9 mg quercetin equivalents/g fresh weight (FW), respectively. High flavonoid content was recorded in the leaves and stem fractions of *P. crispum* (22.4 mg/g) and *A. wakegi* (9 mg/g), respectively, whereas least flavonoid content was observed in the stem (2 mg/g) and leaves (2.6 mg/g) of *B. chinensis*. The polyphenolic content of 20 GLVs were assessed and summarized in Table 1. All the GLVs were found to have varying levels of polyphenols in leaves ranging from 11.8 mg of gallic acid equivalents (GAE)/g FW to 30.4 mg GAE/g FW. Stem fraction of *T. indica* (22.6 mg/g) showed the highest phenolic content and *D. carota, B. chinensis* (11.8 mg/g) showed the lowest among the vegetables tested.

The total antioxidant activity of the vegetables was estimated by using phosphomolybdenum method and the results are expressed as ascorbic acid equivalents (Table 2). Among the 20 GLVs, total antioxidant capacity was found to be higher in the leaves of *P. crispum* and stem of *T. indica* (6.4 and 4.4 mg/g) and lower in the leaves and stem of *B. Chinesis* (2.0 mg/g) and *A. graveolens* (1.0 mg/g), respectively.

The reducing power of the vegetable extract was assessed by using FRAP assay. The results indicate significant differences in the reducing power of leafy vegetables evaluated. As shown in Table 2, FRAP activity ranged from 1.4 to 8 µM ferrous equivalent Fe(II)/g FW of stem and leaves; *D. carota* and *E. variegata* exhibited higher reducing power in the leaves and stem, respectively suggesting the electron donating ability of the extract (Gordon, 1990). In the present study, the free radical scavenging activity of the antioxidants present in the vegetable extracts were evaluated by using synthetic radical DPPH, which is a stable free radical, capable of accepting an electron or hydrogen radical to become a stable diamagnetic molecule (Baumann et al., 1979). The results showed that the highest radical

Table 2. Total antioxidant and FRAP activities of green leafy vegetables[a]

Scientific name	Total Antioxidant[d](mg/g)		FRAP[e] (µM)	
	Leaf	Stem	Leaf	Stem
Allium ampeloprasum	2.2±0.02	1.6±0.02	2.2±0.02	2.0±0.02
Allium wakegi	5.8±0.07	1.4±0.02	2.6±0.02	2.0±0.02
Apium graveolens	3.2±0.03	1.0±0.05	2.6±0.04	2.2±0.02
Amaranthus gangeticus	2.4±0.02	1.6±0.02	1.8±0.09	1.6±0.03
Anethum graveolens	4.0±0.04	2.2±0.07	4.2±0.04	2.2±0.02
Basella rubra	3.6±0.06	1.4±0.07	4.2±0.02	1.6±0.09
Beta vulgaris	4.6±0.05	1.6±0.03	2.6±0.12	1.8±0.03
Brassica oleracea var italica	3.6±0.02	1.2±0.03	2.0±0.11	2.2±0.08
Brassica chinensis	2.0±0.02	1.4±0.01	1.8±0.06	1.4±0.03
Corchorus aestuans	6.2±0.05	2.6±0.10	4.4±0.12	2.2±0.02
Daucus carota	4.2±0.08	2.6±0.02	8.0±0.14	2.6±0.07
Erythrina variegata	6.2±0.02	3.4±0.31	6.0±0.04	8.0±0.05
Lactuca sativa	4.0±0.03	2.4±0.11	1.4±0.02	1.4±0.04
Mukia leiosperma	5.2±0.02	2.6±0.09	2.2±0.02	1.8±0.09
Oxalis corniculata	4.6±0.11	1.1±0.02	5.4±0.31	1.8±0.04
Petroselinum crispum	6.4±0.03	2.2±0.04	2.6±0.02	1.6±0.02
Portulaca oleracea	4.0±0.09	1.8±0.12	4.8±0.05	1.6±0.02
Premna corymbosa	4.8±0.03	2.2±0.07	2.8±0.03	1.8±0.02
Rumex vesicarius	4.0±0.02	2.4±0.09	3.6±0.02	1.8±0.03
Tamarindus indica	3.4±0.04	4.4±0.02	3.8±0.05	4.4±0.08

[a]Data represented are the means of three replicates±SD. [d]Data expressed as milligrams of Ascorbic acid equivalents per gram fresh weight..[e]Data expressed as µM of ferrous equivalent Fe (II) per gram fresh weight.

scavenging activity was observed in the leaves of *A. graveolens* (85.78%) and stem of *D. carota* (87.86%) (Table 3).

Hydrogen peroxide scavenging activities of the leafy vegetable extracts were measured at 230 nm and the results were presented in Table 3. In the present investigation, H_2O_2 radical scavenging activity was found to be higher in the stem and leaves of *R. vesicarius* (21.3%) and *L. sativa* (20.22%), respectively. The hydroxyl radical scavenging ability of vegetable extracts was shown in Table 4. The leaves of *T. indica* (69.7%) and the stem of *A. ampeloprasum* (75%) was found to possess the highest hydroxyl radical scavenging activity while the other vegetables showed a significant amount of scavenging activities ranging from 8.8 to 69.7% and 31.9 to 75% in the leaves and stem, respectively. Among the vegetables tested, the ABTS radical scavenging activity was found to be higher in the leaves and stem of *P. oleracea* (94.19%) and *T. indica* (93.76%) whereas leaves of *A. gangeticus* (55.94%) and stem of *B. chinensis* (51.27%) (Table 4) showed the least. The superoxide radical scavenging activities of the selected green leafy vegetables were determined using riboflavin-NBT-light system. The relative scavenging effect of all the vegetable extracts on superoxide radical are listed in Table 5. Superoxide radical scavenging activity of the vegetable extracts varied from 71.39 to 7.08% and 4.81 to 23.54% in case of leaves and stem, respectively.

DISCUSSION

Flavonoids occur ubiquitously in plant kingdom as glycosides and contain several phenolic hydroxyl groups on their ring structure (Larson, 1988). The presence of phenolic hydroxyl groups make the flavonoids as potential antioxidants that are capable of effectively scavenging the reactive oxygen species formed inside the cell (Cao et al., 1997). In the present study, all the vegetables contain considerable amount of flavonoids both in their stem and leaf fractions. Earlier studies have shown an optimistic relationship between improved consumption of flavonoids and abridged risk of cardiovascular diseases and cancer (Yang et al., 2001). Polyphenols are aromatic secondary metabolites that are associated with imparting color, sensory and nutritional qualities in plants; comprising a broad range of compounds such as flavonoids (anthocyanins, flavanols, flavones, flavonols, and others) and proanthocyanidins (Zujko and Wittowska, 2001). In general, the polyphenolic compounds present in plants can act as reducing agents, hydrogen donors, heavy metal chelators, hydroxyl radical quenchers and lipid peroxidation stabilizers. Hence, phenolic compounds are known to possess high antioxidant

Table 3. DPPH and hydrogen peroxide scavenging activities of green leafy vegetables[a]

Scientific name	DPPH[f](%)		Hydrogen peroxide scavenging activity[g](%)	
	Leaf	Stem	Leaf	Stem
Allium ampeloprasum	67.59±0.05	63.30±0.08	19.38±0.07	20.22±0.07
Allium wakegi	69.49±0.02	86.13±0.02	20.08±0.03	19.80±0.07
Apium graveolens	84.22±0.02	66.37±0.04	19.80±0.02	20.01±0.07
Amaranthus gangeticus	64.47±0.05	55.28±0.03	18.86±0.05	19.80±0.07
Anethum graveolens	85.78±0.02	85.96±0.08	18.66±0.06	19.80±0.08
Basella rubra	83.36±0.11	52.85±0.06	18.24±0.05	19.80±0.14
Beta vulgaris	80.76±0.04	61.00±0.07	18.03±0.10	20.01±0.07
Brassica oleracea var italica	80.93±0.03	85.44±0.05	19.49±0.20	19.80±0.07
Brassica chinensis	54.59±0.07	44.19±0.03	20.01±0.07	20.01±0.32
Corchorus aestuans	81.80±0.31	46.44±0.03	17.93±0.07	19.70±0.01
Daucus carota	85.61±0.02	87.86±0.10	17.82±0.10	19.80±0.07
Erythrina variegata	78.68±0.04	80.06±0.21	17.51±0.07	19.90±0.11
Lactuca sativa	56.67±0.13	47.14±0.02	20.22±0.07	20.22±0.02
Mukia leiosperma	81.10±0.11	63.95±0.04	19.07±0.05	19.70±0.10
Oxalis corniculata	85.44±0.05	43.67±0.22	17.93±0.07	20.01±0.05
Petroselinum crispum	50.25±0.09	37.78±0.08	16.99±0.08	19.70±0.03
Portulaca oleracea	81.10±0.05	44.54±0.03	20.11±0.05	19.90±0.02
Premna corymbosa	72.79±0.05	48.52±0.07	20.01±0.07	19.80±0.07
Rumex vesicarius	76.94±0.07	29.28±0.06	20.11±0.05	21.30±0.04
Tamarindus indica	79.20±0.21	82.66±0.04	20.11±0.03	20.11±0.10

[a]Data represented are the means of three replicates±SD. [f]Data expressed as percentage of DPPH radical scavenging activity. [g]Data expressed as percentage of hydrogen peroxide radical scavenging activity.

Table 4. Hydroxyl radical scavenging activity and ABTS scavenging activities of green leafy vegetables[a]

Scientific name	Hydroxyl radical scavenging activity[h] (%)		ABTS[i](%)	
	Leaf	Stem	Leaf	Stem
Allium ampeloprasum	45.2±0.02	75.0±0.05	69.12±0.05	62.46±0.02
Allium wakegi	47.0±0.08	57.3±0.18	65.58±0.03	75.21±0.06
Apium graveolens	20.4±0.05	31.9±0.04	78.89±0.07	66.71±0.03
Amaranthus gangeticus	37.9±0.02	56.1±0.06	55.94±0.02	59.63±0.04
Anethum graveolens	23.4±0.01	41.0±0.10	89.23±0.05	63.88±0.05
Basella rubra	15.5±0.03	41.6±0.05	91.64±0.07	56.76±0.02
Beta vulgaris	23.1±0.04	62.8±0.03	89.09±0.02	60.76±0.03
Brassica oleracea var italica	34.6±0.05	51.3±0.08	72.80±0.07	65.86±0.11
Brassica chinensis	48.8±0.03	53.7±0.09	56.65±0.03	51.27±0.03
Corchorus aestuans	8.8±0.003	35.5±0.07	92.49±0.02	66.85±0.02
Daucus carota	13.7±0.07	43.2±0.04	87.96±0.02	69.26±0.03
Erythrina variegata	17.6±0.02	48.2±0.10	93.90±0.10	73.65±0.03
Lactuca sativa	44.9±0.06	62.2±0.02	62.03±0.07	61.33±0.21
Mukia leiosperma	28.5±0.09	37.0±0.02	75.07±0.05	70.82±0.04
Oxalis corniculata	39.4±0.02	44.3±0.03	89.80±0.06	59.63±0.02
Petroselinum crispum	24.3±0.02	48.8±0.04	81.44±0.04	63.03±0.03
Portulaca oleracea	26.1±0.05	51.6±0.02	94.19±0.02	91.64±0.02
Premna corymbosa	9.4±0.04	37.9±0.11	83.42±0.11	74.36±0.04
Rumex vesicarius	14.6±0.02	38.8±0.04	86.54±0.02	68.27±0.04
Tamarindus indica	69.7±0.02	57.3±0.02	83.14±0.02	93.76±0.04

[a]Data represented are the means of three replicates±SD. [h]Data expressed as percentage of hydroxyl radical scavenging activity. [i]Data expressed as percentage of ABTS radical scavenging activity.

Table 5. Superoxide radical scavenging activity green leafy vegetables[a]

Scientific name	Superoxide Radical Scavenging activity[j]	
	Leaf (%)	Stem (%)
Allium ampeloprasum	22.27±0.09	17.46±0.08
Allium wakegi	21.77±0.08	20.50±0.09
Apium graveolens	21.77±0.07	17.72±0.04
Amaranthus gangeticus	20.50±0.09	13.67±0.03
Anethum graveolens	22.02±0.08	10.37±0.03
Basella rubra	13.16±0.04	16.96±0.04
Beta vulgaris	20.50±0.09	14.68±0.09
Brassica oleracea var italica	14.43±0.02	10.88±0.02
Brassica chinensis	21.01±0.05	14.17±0.20
Corchorus aestuans	23.29±0.04	23.54±0.03
Daucus carota	31.39±0.02	20.25±0.11
Erythrina variegata	25.06±0.04	11.13±0.02
Lactuca sativa	20.25±0.04	18.48±0.02
Mukia leiosperma	17.72±0.02	21.77±0.01
Oxalis corniculata	21.26±0.02	18.22±0.03
Petroselinum crispum	13.67±0.04	12.91±0.10
Portulaca oleracea	7.08±0.05	4.81±0.03
Premna corymbosa	16.70±0.08	18.48±0.09
Rumex vesicarius	56.45±0.02	5.56±0.08
Tamarindus indica	71.39±0.02	14.43±0.02

[a]Data represented are the means of three replicates±SD. [j]Data expressed as percentage of superoxide radical scavenging activity.

antioxidant properties than carotenoids and vitamins (Gardener et al., 2000; Kaur and Kapoor, 2002). The phenolic content of vegetables varies among the vegetables tested. Similar study by Shyamala et al. (2005) showed that the total polyphenol content of some common Indian leafy vegetables such as cabbage, coriander leaves and spinach was reported to be in the range of 5 to 69.5 mg of tannic acid/g of extract. However, the phenolic contents of vegetables may vary depending on the assay system, standards used, environmental conditions, cultivars and stages of maturity, hence the comparison is always challenging (Gupta and Prakash, 2009; Hue et al., 2012).

The total antioxidant activity of the vegetable extracts showed that all the extracts have considerable amount of antioxidant activity. Reports showed that polyphenol content might play a major role in determining the antioxidant activity of the vegetables (Wong et al., 2006). However, the ability of vegetable extracts to inhibit the production of free radicals also depends on the age of the plant and the temperature where it grows (Uusiku et al., 2010). The reducing capability of antioxidants present in the GLV extracts was measured by using FRAP assay. This method is based on the reaction of antioxidants present in the extracts with the ferric tripyridyltriazine complex (Fe^{3+}-TPTZ) to form the colored ferrous tripyridyltriazine complex (Fe2+-TPTZ), which corresponds to the antioxidants or reductants present in the sample. The result showed that *D. carota* and *E. variegata* in the stem and leaves of the GLV has higher FRAP values that corresponds to the higher reducing capacity of the extract. Devi et al. (2012) showed correlation between chelating ability and the phenolic content of red sorghum suggesting that polyphenols in the extracts may act as reductones, thereby preventing the oxidative chain reactions.

The ability of antioxidants to inhibit lipid peroxidation inside the cell depends on its free radical scavenging activity (Blokhina et al., 2003). The results of DPPH radical scavenging analysis showed that the percent inhibition of the DPPH radical among the GLVs varied significantly. In a similar study by Gupta and Prakash (2009) on the antioxidant activity of the Indian green leafy vegetables, *M. koenigii* showed the strongest radical scavenging activity (83.44%) and *Amaranthus* sp. showed the lowest (38.18%) as compared to the other GLVs used. The higher content of total phenolic compounds, tannin and flavonoid in the vegetable extracts might have contributed towards the scavenging of free radicals (Murugan et al., 2013). Earlier reports have shown the radical scavenging activity of methanolic extracts of several leafy vegetables including *Allium,* Brocolli celery

and spinach (Chu et al., 2000; Marinova et al., 2005; Gacche et al., 2010).

The presence of hydrogen peroxide inside the cell is non-reactive, but the conversion of hydrogen peroxide to hydroxyl radical is toxic, since hydroxyl radicals are the major mediators of most free radical induced damage since it reacts with most of the macromolecules found in living cells causing oxidative damage to DNA, lipids and proteins (Gutteridge, 1984; Spencer et al., 1994; Lloyd et al., 1997). In this study, the hydrogen donating ability of the vegetable extracts was analysed since it prevents the toxic effects of H_2O_2 by converting them into water (Khan et al., 2012; Magama et al., 2013). The radical scavenging activity of the extract varied widely in the vegetables tested. The proton radical scavenging activity of the leafy vegetable extract may contribute to its antioxidant and free radical scavenging activity (Shyamala et al., 2005). These results also suggest that the antioxidant property of the vegetable extracts is related to its ability to scavenge hydroxyl radicals.

Superoxide radicals are formed in the cell as a result of various cellular reactions and it is also shown to be involved in the formations of hydroxyl radical precursors and other free radicals including singlet oxygen that ultimately leads to lipid peroxidation (Meyer and Isaksen, 1995; Blokhina et al., 2003). The results of the present study showed that the radical scavenging activities of all the vegetables are directly proportional to the antioxidants present in the extracts.

In summary, phytochemical and radical scavenging activities of both the leaves and stem fractions of 20 leafy vegetables commonly consumed in India were evaluated and the results proved that all the vegetables possess high antioxidant properties. As these vegetables shown to have good antioxidant potential, we can recommend that these vegetables should be part of our daily diet to circumvent the free radical induced damage/disorders. The present finding validates the traditional knowledge of the neglected/underexploited green leafy vegetables that will be helpful for the current generation. This study also paves the way for the identification of natural antioxidants and also further studies are essential to assess the antioxidant activity of vegetables after cooking and their effects on neurodegenerative disorders.

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Conflict of interest

Authors declare that there are no conflicts of interest

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