TOTAL PHENOLIC CONTENT AND TOTAL ANTIOXIDANT ACTIVITY OF SIXTEEN COMMONLY CONSUMED GREEN LEAFY VEGETABLES STORED UNDER DIFFERENT CONDITIONS

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Abstract

The health benefits of Green leafy vegetables (GLVs) have been related to their phytochemical components. In Ayurvedic system of medicine they have been used to cure various lifestyle-related disorders. An attempt has been made to quantitate the Total Antioxidant activity (TAA) and to correlate it with the Total Phenolic content (TPC) of leafy vegetables consumed in the Eastern part of Sri Lanka.

TAA was estimated by ferric reducing ability method while TPC was measured using Folin–Ciocalteau reagent.

TAA of fresh GLVs ranged from 4.12±0.16 µmol g^{-1} Wet Weight (WW) for Moringa oleifera to 38.59±1.05 µmol g^{-1}WW for Murraya koenigii. TPC of leafy vegetables when fresh ranged from 21.82±15 mg Tannic Acid Equivalent (TAE)/ 100 g WW for Mollugo oppositifolia to 560.1±51 mg TAE/ 100 g WW for Delonix elata. Most of the GLVs showed an increase in TPC and TAA content during storage but the increase was observed more at room temperature than at 4ºC. The increase in TPC and TAA may be due to breakdown to free phenolics during storage. A positive correlation existed between TPC and TAA in fresh GLVs as well as GLVs stored at room temperature and 4ºC.

Introduction

Consumption of green leafy vegetables may play a role in preventing human disease in which free radicals are involved, such as cancer, cardiovascular diseases and aging. (Guptha and Prakash, 2009). Fruits and vegetables contain a wide variety of bioactive, non-nutritive compounds known as phytochemicals. These phytochemicals impart health benefits beyond basic nutrition (Oomah and Mazza, 2000). Some of the

bioactive compounds neutralize free radical species generated during biochemical reactions in our body (Halliwell 1994). The ability of various parts of plants to cure diseases has been recognized since ancient times by physicians practicing traditional health system. The scientific basis of traditional health practices are being currently investigated especially to identify the bioactive compounds in plants that are regularly used. Some of the GLVs are very popular among rural communities in many parts of the world, including Sri Lanka, and well accepted in their traditional diets and medicines.

Most of the phytochemicals from plant extracts, fruits and green vegetables have been identified to exhibit antioxidant activities. They have different antioxidants such as Vitamin C, Vitamin E, Carotenes, Lycopenes, Polyphenols and other phytochemicals (Prior and Cao, 2000).

GLVs are living tissues subject to continuous changes after harvest. Enzymatic browning, microbial spoilage, dehydration, rapid wilting and senescence are the factors that shorten shelf life of GLVs (Reyes, 1996). Packaging is often used to increase the shelf life of food as well as to improve the quality (Cutter, 2002). Refrigerated storage has been reported to enhance the shelf life quality of green herbs (Sankat and Maharaj, 1996)

Gupta et al (2005) reported that several GLVs are rich sources of antioxidant vitamins. The antioxidant activity of GLVs grown in Batticaloa district, which is located in the eastern part of Sri Lanka with extremes of temperature, where a number of medicinal plants are available, has not been reported. Therefore a study was initiated to determine the of total phenolic content (TPC) and total antioxidant activity (TAA) of selected GLVs under common storage conditions (fresh- soon after harvest, stored under refrigerated condition, stored at room temperature) and to find out the relationship between TPC and TAA of selected GLVs.

Materials and methods

Chemicals

All chemicals used in the study were of analytical grade and distilled water was used for the preparation of reagents.

Sources of green leafy vegetables

Ten popular market places in Batticaloa district namely Arayampathy, Batticaloa, Chenkalady, Eravur, Kaluwanchikudy, Kallar, Kattankudy, Kiran, Oddamavadi and Valaichenai were selected for the study. During the preliminary study it was found that roughly 20,000 people/ month regularly used these markets. Interviews were conducted for a period of 10 months and 5 subjects were interviewed during each visit to the market selected on a random basis. A total of one thousand subjects were interviewed from these markets, at the end of the survey (5 people x 2 times per month x 10 places x 10 months). The interview mainly focused on the types and purpose of consuming green leafy vegetables. Percentage consumption of each GLV for food or medicine based on the interview was calculated. GLVs consumed frequently were selected for the study and they were purchased from the markets. Identification of the GLVs was done with the help of herbarium specimens from the Department of Botany, Eastern University and other published and unpublished documents. The identified species were re-confirmed with the help of National Herbarium, Peradeniya

Preparation of GLVs, storage and packing

The GLVs collected from field were cleaned and shoots separated. The shoots were washed thoroughly under running tap water for 5 minutes to remove soil particles and dirt. Then the washed shoots were placed in a plastic container with paper towel and allowed to drain for 5 minutes. The cleaned, washed, air-dried shoots of GLVs were wrapped initially with paper and then in polyethylene bags for each GLVs. They were stored both at room temperature (30± 2ºC) and 4ºC (refrigerator) for 4 days.

Estimation of total phenolic content (TPC)

Preparation of extracts for estimation of TPC

Samples of GLVs (1 g of each), in 30 ml of methanol were ground using a mortar and pestle. After recovery of the finely ground mixture, 15 ml methanol was used to wash the mortar and pestle and then pooled with the first mixture. It was made up to 50 ml with methanol, mixed thoroughly and centrifuged to obtain a clear supernatant. The extracts were prepared using 3 samples collected at different time for each GLVs and all analysis were carried out in triplicates.

TPC was determined by adopting the procedures of Gupta and Prakash (2009). A sample of methanolic extract (0.2 ml) was mixed with 1 ml of Folin–Ciocalteau reagent (tenfold diluted) followed by the addition of 0.8 ml of 2% Na_2CO_3. The volume was made to 10 ml with 4:6 water/ methanol. This was allowed to stand for 30 minutes and absorbance read at 740 nm in a specrotphotometer (Hach DR 2500). Concentration was calculated using tannic acid as standard and the results were expressed as mg tannic acid equivalent /100 g wet weight (WW).

Tannic acid, in varying concentrations (0, 50, 100, 150, 250 and 500 mg/l), were used to prepare a standard curve. This curve is used to relate the absorbance of the unknown samples to Tannic acid equivalents (TAE).

Determination of total antioxidant activity (TAA)

Preparation of extracts for estimation of TAA

Samples of GLVs (1 g of each) were cut into small pieces and mashed using a mortar and pestle with 9 ml cool 0.1 M phosphate buffer (pH 7.6). It was then made up to 10 ml with the phosphate buffer and centrifuged to obtain a clear supernatant for measurement of antioxidant activity. The extracts were prepared using 3 different samples of each GLVs and all analysis were carried out in triplicates.

Antioxidant activity was measured using Ferric reducing antioxidant power (FRAP) assay (Benzie and Strain 1996). One ml of freshly prepared FRAP reagent (Acetate buffer: TPTZ solution: $FeCl_3.6H_2O$ solution in the ratio of 10:1:1) was mixed with the extract and absorbance measured exactly after 4 minutes at 593 nm. TAA was calculated using 1 mm $FeSO_4$ standard and expressed as μmol/g WW.

Data analysis

The data were analyzed using MINITAB 14 statistical software. The relationship between TPC and TAA were determined by Pearson's correlation.

Results and Discussion

Total phenolic content (TPC)

Selected GLVs showed differences in phenolic contents when fresh and under two storage conditions for 4 days. TPC of fresh GLVs ranged from 21.8 ± 15 mg TAE/100 g WW for *Mollugo oppositifolia* to 560.1 ± 51 mg TAE/100 g WW for *Delonix elata* (Table 1). TPC of some common Indian leafy vegetables was found to be in the range of 5 – 69.5 mg of tannic acid equivalents per gram extract (Shyamala *et al*., 2005).

TPC of fresh *Centella asiatica* and *Murraya koenigii* were 168.2 ± 57.3 mg TAE/100 g WW and 430.6 ± 44.2 mg TAE/100 g WW respectively. These values are slightly higher than the values reported by Gupta and Prakash (2009) for *C. asiatica* (150 mg tannic acid/100 g) and *M. koenigii* (387 mg of tannic acid/100 g fresh weight). TPC of leaf, root and petiole extracts of four *C. asiatica* varieties were found to vary from 3.23 g gallic acid equivalent/ 100 g to 11.7g gallic acid equivalent/ 100g of dry sample (Zainol *et al*., 2003). A comparison of these values could not be made with the present study because of the difference in the standards used as well as the nature of the samples.

During storage at room temperature (30±2ºC) and 4ºC, TPC of most of the GLVs studied increased. It ranged from 87.9 mg TAE /100 g WW of *M. oppositifolia* to 651.2 mg TAE/ 100 g of WW *D. elata* when stored at room temperature (Table 1). This increase in total phenol agrees with the observation of Oboh (2005) and Rivera, *et al*. (2006). Generally, GLVs stored at room temperature showed higher TPC than that stored at 4ºC. Akindahunsi and Oboh (1999) stated that the basis for the increase could not be categorically stated, however, it could be attributed to the possible breakdown of the tannins present in the vegetables to simple phenol. The breakdown could be slowed down when stored at 4ºC due to slowing down of the metabolic process at low temperature (Ragaert *et al*., 2007). However, Amanatido *et al*., (2000) pointed out phenol build up as a physiological response to infection and damage. Therefore, it can be said that the TPC of GLVs varies widely depending on the variety and the environmental conditions.

Table 1. Total phenolic content of selected GLVs when fresh and during storage for 4 days

Green leafy vegetables	TPC (mg/100g WW)		
	Fresh	Room temperature 30±2ºC (for 4 days)	Refrigeration 4^0C (for 4 days)
Aerva lanata	127.9±39.4[a]	152.3±39.3[a]	144.1±45.9[a]
Alternanthera sessilis	181.1±42.4[a]	236.1±51.5[b]	176.9±43.2[a]
Amaranthus caudatus	164.3±26.8[a]	261.7±25.5[b]	188.6±16.5[c]
Amaranthus viridis	147.4±40.4[a]	289.6±41.8[b]	207±44.3[c]
Argyreia pomacea	74.7±31.1[a]	93.3±35.9[a]	16.71±13.0[b]
Cardiospermum helicacabum	206.4±29.4[a]	292.3±34.9[b]	218.4±35.3[a]
Centella asiatica	168.2±57.3[a]	241.5±39.4[b]	185.3±36.9[a]
Delonix elata	560.1±51.8[a]	651.2±65.8[b]	598±59.0[a,b]
Drega volubilis	187.7±55.8[a]	128.8±41.4[b]	73.5±34.6[c]
Mollugo oppositifolia	21.8±15.3[a]	87.9±5.8[b]	52.5±28.9[a]
Moringa oleifera	208.5±42.5[a]	345.2±86.7[b]	449.5±30.5[c]

Murraya koenigii	430.6±44.2[a]	545.7±124.1[b]	555.6±25.19[b,c]
Pisonia grandis	125.2±76.5[a]	90.0±18.9[a]	239.1±13.0[b]
Sauropus androgynus	160.1±37.3[a]	105.1±31.1[b]	144.7±37.7[a]
Sesbania grandiflora	102.7±41.9[a]	76.82±17.1[a]	100.9±26.2[a]
Solanam trilobatum	170.9±36.6[a]	23.62±16.3[b]	128.2±29.8[c]

Values are means of 3 replicates ± Standard deviation. Different superscripts a to c in rows indicate significantly different at $p<0.05$

Six species of the GLVs showed significantly higher TPC when stored at room temperature than when fresh or stored at 4ºC, such as *A. sessilis, A. caudatus, A. viridis, C. helicacabum, C. asiatica* and *M. oppositifolia* (Table 1). *M. oleifera* and *P. grandis* showed significantly higher TPC when stored at 4ºC. In two species TPC was higher in fresh GLVs than the other two stored conditions, such as D. *volubilis* and *S. trilobatum* (Table 1). TPC of *M. oppositifolia* increased nearly 4 fold when stored at room temperature, while in *M. oleifera* and *P. grandis* two fold increase was observed when stored at 4^0C. TPC of *A. caudatus* and *A. viridis* increased 1.5 and 2 fold respectively when stored at room temperature (Table 2). Latif and ABD El-Aal, (2007) observed in six different species of vegetables such as *Allium kurrat* (Egyptian leek) *Anethum graveolans* (Dill), *Coriandrum sativum* (Coriander), *Eruca sativa* (Eruca), *Petroselinum sativum* (Parsley) and *Raphanus sativus* (Raddish) 2 to 4 fold increase in TPC at 4ºC.

TPC of *A lanata, D. elata*, *M. koenigii* and *S. grandiflora* stored at 4^0C was not significantly different when stored at room temperature.

Eight fresh GLVs showed significant differences in TPC when stored at 4ºC, such as *A. caudatus*, *A. viridis*, *A. pomacea*, *D. volubilis*, *M. oleifera, M. koenigii*, *P. grandis* and *S. trilobatum*. Generally TPC increased during storage conditions. Only in 4 species TPC declined in both room temperature and at 4ºC such as *D. volubilis, S. androgynus, S. grandiflora* and *S. trilobatum* (Table 2). TPC increased in *P. grandis* when stored at 4ºC but not at room temperature. But in *A. pomacea,* TPC declined when stored in refrigerator. Latif, and ABD El-Aal, (2007) reported that TPC of fresh GLVs showed an initial decrease, followed by an increase back to original level and subsequent repeat of this trend, when stored at 4^oC for 8 days. *A. lanata* and *M. koenigii* did not show notable changes in TPC during both storage conditions (Table 2).

Table 2. Variation of TPC in GLVs stored under room temperature and refrigerator for 4 days

Green leafy vegetables	Percentage of gain during storage	
	Room temperature 30±2ºC	Refrigerator 4ºC
Aerva lanata	19.0[a]	12.7[a]
Alterenthera sessilis	30.4[a]	-2.3[b]
Amaranthus caudatus	59.3[a]	14.8[b]
Amaranthus viridis	96.4[a]	40.4[b]
Argyreia pomacea	24.9[a]	-77.6[b]
Cardiospermum helicacabum	41.7[a]	5.8[b]
Centella asiatica	43.6[a]	10.2[b]
Delonix elata	16.3[a]	6.8[b]
Drega volubilis	-31.4[a]	-60.8[b]
Moringa oleifera	65.6[a]	115.6[b]
Mollugo oppositifolia	303.1[a]	140.5[b]
Murraya koenigii	26.73[a]	29.04[a]
Pisonia grandis	-28.1[a]	91.0[b]
Sauropus androgynus	-34.4[a]	-9.6[b]
Sesbania grandiflora	-25.2[a]	-1.8[b]
Solanam trilobatum	-86.2a	-25.0[b]

Values are means of 3 replicates ± Standard deviation. Different superscripts a and b in rows indicate significantly different at $p<0.05$

Phenolic antioxidants appear to be more responsive to environmental factors such as water availability, light quality and temperature. Phenolics also seem to be more affected by storage factors such as temperature, atmosphere and light, than vitamin C or carotenoids (Latif and ABD El-Aal, 2007; Ragaert *et al*., 2007). Among the GLVs studied *D. volubilis* had the highest TPC. We have also observed that *D. volubilis* had the highest ascorbic acid content among the common GLVs studied (Unpublished observation). However, the ascorbic acid content declined in *D. elata* during storage while TPC increased when stored at either room temperature or at 4ºC. Even though *M. koenigii* was a poor source of ascorbic acid (Unpublished observation), it is a good source of TPC and storing it at either room temperature or 4ºC further increases the TPC similar to *D. volubilis*. The stability of TPC of GLVs during storage showed wide and noticeable variations. Therefore studies on individual phenolic substances are required to understand this phenomenon.

Total antioxidant activity (TAA)

Fresh GLVs had different TAA. Highest TAA of 38.6 ± 1.50 µmol g^{-1} WW was obtained for *M. koenigii* followed by *D. volubilis* and *S. androgynus* (31.6± 0.19 and 30.8±0.83 µmol g^{-1} WW respectively). *M. koenigii* was observed to be a good source of TPC (Table 1) and *D. volubilis* is rich in vitamin C (Unpublished observation) as such the high TAA is as expected. The lowest value of 4.12±0.16 µmol g^{-1} WW was observed in *M. oleifera* (Table 3). Wong *et al*., (2006) reported TAA values of *C. asiatica, S. androgynus, S. grandiflora and M. koenigii* as 92.85 ±18.55, 187.30 ± 19.29, 45.47 ± 6.97 and 99.25 ± 15.6 µmol Trolox Equivalents per g dry plant material respectively. However a comparison of the TAA could not be made between the 2 studies because of the different standards used and the nature of the samples (wet *vs*

dry) used. *M. oleifera* was found to be good 2, 2-diphenyl 1-2-picryllhydrazyl (DPPH) radical scavenger with a percent inhibition of 63.05± 2.135 (Vimal *et al*., 2009).

Ten species of GLVs stored at room temperature showed significantly higher TAA than when fresh and when stored at 4ºC, such as *A. lanata, A. sessilis, A. caudatus, A. viridis, D. elata, D. volubilis, M. oppositifolia, M. koenigii, S. androgynus* and *S. trilobatum* (Table 3). This may be due to breakdown of tannins, which is high at room temperature than at 4ºC (Akindahunsi and Oboh, 1999). Low temperature slows down plant metabolic processes (Ragaert *et al*., 2007).

Six GLVs such as *A. lanata*, *A. sessilis*, *C. asiatica*, *D. volubilis*, *M. oppositifolia* and *M. oleifera* showed significantly higher TAA under both storage conditions than fresh (Table 3). Six GLVs such as *A. caudatus*, *A. viridis*, *C. helicacabum*, *D. elata*, *M. koenigii* and *S. grandiflora* showed no significant differences in TAA between fresh and that of stored at 4ºC.

Table 3. Total antioxidant activity of GLVs at fresh when fresh and during storage for 4 days

Green leafy vegetables	TAA (µmol g^{-1} WW)		
	Fresh	Room temperature 30±2ºC (for 4 days)	Refrigeration 4^0C (for 4 days)
Aerva lanata	25.19±0.91[a]	34.96±0.57[b]	27.55±0.92[c]
Alternanthera sessilis	22.43±0.58[a]	28.04±1.70[b]	25.78±0.61[c]
Amaranthus caudatus	26.39±0.11[a]	30.88±1.57[b]	26.86±1.01[a]
Amaranthus viridis	26.57±0.74[a]	32.71±1.24[b]	25.71±0.78[a]
Argyreia pomacea	6.85±0.20[a]	6.14±0.21[b]	7.03±0.28[c]
Cardiospermum helicacabum	4.99±0.34[a]	3.55±0.30[b]	5.03±0.40[a]
Centella asiatica	6.21±0.29[a]	7.58±0.35[b]	8.57±0.26[c]
Delonix elata	23.81±0.74[a]	25.71±0.61[b]	23.97±0.38[a]
Drega volubilis	31.59±0.19[a]	40.08±0.82[b]	38.40±0.73[c]
Mollugo oppositifolia	5.18±0.32[a]	6.59±0.17[b]	5.72±0.17[c]
Moringa oleifera	4.12±0.16[a]	4.77±0.19[b]	7.26±0.24[c]
Murraya koenigii	38.59±1.05[a]	41.40±0.66[b]	39.41±0.94[a]
Pisonia grandis	9.47±0.28[a]	7.74±0.26[b]	8.78±0.24[c]
Sauropus androgynus	30.75±0.83[a]	36.92±0.86[b]	26.37±0.53[c]
Sesbania grandiflora	6.84±0.36[a]	3.26±0.21[b]	6.84±0.26[a]
Solanam trilobatum	6.94±0.14[a]	9.96±0.26[b]	6.47±0.27[c]

Values are means of 3 replicates ± Standard deviation. Different superscripts a to c in rows indicate significantly different at $p<0.05$

TAA of GLVs was higher when stored at room temperature than at 4ºC (Table 4). Six species showed increased TAA when stored at both room temperature and 4ºC, such as *A. lanata*, *A. sessilis*, *C. asiatica, D. volubilis, M. oppositifolia and M. oleifera*. TAA of *P. grandis* declined under both storage conditions. In *A. pomacea*, *C. helicacabum*, *P. grandis* and *S. grandiflora* TAA was lowest when stored at room temperature.

The variation of TAA in GLVs stored at room temperature and refrigerator for 4 days is given in Table 4.

Table 4. Variation of TAA in GLVs stored at room temperature and refrigerator for 4 days

Selected GLVs	Percentage of gain during storage	
	Room temperature (30±2ºC)	Refrigerator (4ºC)
Amaranthus caudatus	17.01[a]	1.74[b]
Aerva lanata	38.73[a]	9.37[b]
Alternanthera sessilis	25.01[a]	14.94[b]
Amaranthus viridis	23.11[a]	-3.24[b]
Argyreia pomacea	-10.32[a]	6.62[b]
Cardiospermum helicacabum	-29.00[a]	0.60[b]
Centella asiatica	22.06[a]	38.00[b]
Delonix elata	7.98[a]	0.67[b]
Drega volubilis	26.88[a]	21.56[b]
Mollugo oppositifolia	27.12[a]	10.39[b]
Moringa oleifera	16.06[a]	76.64[b]
Murraya koenigii	7.28[a]	2.12[b]
Pisonia grandis	-18.29[a]	-7.25[b]
Sauropus androgynus	20.08[a]	-14.25[b]
Sesbania grandiflora	-52.36[a]	0.05[b]
Solanam trilobatum	43.88[a]	-6.80[b]

Values are means of 3 replicates ± Standard deviation. Different superscripts a and b in rows indicate significantly different at $p<0.05$

Relationship between TPC and TAA

The correlation between TPC and TAA when fresh and under different storage conditions showed positive correlation but was not significant (Table 5). However this indicates the close relationship between TPC and antioxidant activity as reported by Prasad *et al*., 2005 and Lei *et al*., 2009. Phenolic compounds are the major antioxidant constituents in selected herbs, vegetables and fruits and there are direct relationships between their antioxidant activity and TPC (Dorman *et al*., 2004; Velioglu *et al*., 1998).

Table 5: Correlation between TPC and TAA

Conditions	Relationship	Pearson correlation
Fresh	TAA / TPC	0.439 [ns](p =0.089)
Room temperature (30±2ºC)	TAA / TPC	0.315 [ns](p= 0.288)
Refrigeration (4ºC)	TAA / TPC	0.288 [ns](p =0.279)

ns- not significant ($p>0.05$)

Conclusion and Recommendation

TPC of fresh GLVs ranged from 21.8±15 TAE mg/100 g WW for *Mollugo oppositifolia* to 560.1±51 TAE mg/100 g WW for *Delonix elata*. TAA of fresh GLVs ranged from 4.12±0.16 µmol $Fe^{2+}g^{-1}$ WW for *Moringa oleifera* to 38.6±1.05 µmol g^{-1} WW for *Murraya koenigii.* Most GLVs showed increased TPC and

TAA during storage but the increase was observed more at room temperature than at 4ºC. The increase of TPC and TAA may be due to breakdown of tannins to free phenolics during storage. A positive correlation was observed between TAA and TPC even though it was not significant.

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