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Iron and vitamin C content in green leafy vegetables

DN Dahanayake[1,#], S Ekanayake[1]

[1] *Department of Biochemistry, Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka*
[#] Corresponding author; < dndahanayake@kdu.ac.lk >

Abstract—Commonly available green leafy vegetables (GLV) are rich sources of minerals and vitamins. Nutrient analysis of green leafy vegetables contributes to determine the health benefits that can be achieved by consumption. The present study determined the iron and vitamin C (ascorbic acid) content in some locally available *GLV (Sesbania grandiflora* (*kathurumurunga*), *Alternanthera sessilis* (*mukunuwenna*), *Trianthema portulacastrum* (*sarana*), *Amaranthus viridis* (*thampala* (green) and *Gymnema lactiferum* (*kurinnan*)).

Leaves for the study were obtained from a vendor in Kottawa, Sri Lanka. Moisture content of cleaned, washed and dried leaves were determined using Association of Official Analytical Chemistry (AOAC) approved oven dry method. Ascorbic acid of fresh leaves sample was determined by AOAC standard 2,6-dichlorophenolindophenol titrimetric methods. Iron content was determined in dried leaves using standard spectrophotometric method.

Moisture contents of leaves ranged from 94-79 mg/100g on fresh weight basis with sarana and kathurumurunga having the highest and lowest moisture contents. On wet weight iron content ranged from 6-25.2 mg/100g. Tampala had the highest iron content with lowest in sarana. Vitamin C ranged from 45.8 – 163.4 mg/100g on fresh weight basis with kathurumurunga having the highest and mukunuwenna having the lowest vitamin C contents.

Accordingly, an edible portion of 50 g of *Sesbania grandiflora* provides 64 mg of the RDA requirement (75-90mg/day) of ascorbic acid and a 50 g of *Amaranthus viridis* provides 10.5 mg of RDA requirement (8-18mg/day) of iron. Thus, incorporation of these leaves in the meal will provide essential micronutrients necessary for metabolic functions.

Key words*:* green leafy vegetables, ascorbic acid, iron content

Introduction

Different varieties of green leafy vegetables, which are available in Sri Lanka, are inexpensive and nutritious. Leafy vegetables provide low dietary energy making them valuable components in energy limiting diets (Nwanekezie and Obiakor, 2014) and at the same time they provide considerable amounts of vitamins (beta carotene, ascorbic acid, vitamin E, and vitamin K), fiber and minerals (calcium (Ca), mgnesium (Mg), potassium (K), sodium (Na) and iron (Fe)) (Borah *et al.,* 2009). Consumption of at least 50g of green leafy vegetables is a necessity to satisfy the daily requirement of minerals and vitamins (Wikramanayake, 1996) and supplements the rice diet.

Methodology & Experimental Design

A. Collection and preparation of plant materials

Samples of green leafy vegetable were obtained from a vendor who supplies green leafy vegetables to the consumers collected from a field situated at Kottawa in the western province. Foreign material and deteriorated plant parts were removed from the plants by hand sorting. Soil and sand adhered to the plant material were washed off. Excessive washings were avoided to prevent leaching of nutrients. Dried samples were finely grounded and were stored at room temperature in tightly capped, labeled bottles.

B. Determination of moisture content in GLVs

Moisture content of plant material was determined using the (Association of Official Analytical Chemistry) AOAC (930.15) method. Edible parts of fresh plant samples were cut into small pieces and 0.5000 g of fresh plant samples was added to oven dried crucibles (105 ºC for 30 min). Samples and crucibles were dried repeatedly at 105 ºC for 3 to 4 hours till a constant weight is gained. Crucibles were transferred to a desiccator each time after drying, cooled and weighed. Loss of weight on drying (LOD) was calculated.

C. Determination of vitamin C content in GLVs

Vitamin C concentration was determined on fresh samples by the 2, 6-dichlorophenolindophenol (DCPIP) titrimetic method (AOAC official method 967.21). The dye remains colourless till all ascorbic acid is oxidized. At the equivalence point addition of dye causes the solution to be rose pink.

Ascorbic acid in fresh samples of GLVs (10.0 g of each) were extracted in to 50 mL of metaphosphoric acid-acetic acid solution. The supernatant layer was decanted through a filter funnel and volume made to 100mL with distilled water. Metaphosphoric acid-acetic acid solution 5 mL and 2 mL of plant juice samples was pipette into each of three 50 mL Erlenmeyer flasks. Each sample was titrated with DCPIP dye solution until a light, but distinct pink colour produced and persisted above 5 seconds. The results were expressed as mg ascorbic acid/100 g wet weight (WW).

D. Determination of Iron content in GLVs

Iron in GLVs determined in dried plant samples using spectroscopy method. Iron (111) reacts with thiocyanate ions and forms a blood red complex $[Fe\ (SCN)\ (H_2O)_5]^{2+}$]. Absorbance of this colored complex measured at 480 nm corresponds to ferric concentration.

Dried plant sample (3.000g) was ignited in a porcelain crucible at 550ºC until residue is white. The residue was moistened with conc. HCl, evaporated to dryness and moistened with 5mL of conc. HCl and boiled for 2 minutes. Distilled water (50 mL) was added to the preparation and heated on a water bath for few minutes. The solution was filtered in to a 100 mL volumetric flask through few filter papers stacked together. Conc. H_2SO_4 [5 mL] was added and volume made up to 100mL with distilled water.

A series of ferric ammonium sulphate [Fe (NH_4) SO_4] solution [1 mL, 2 mL, 3 mL, 4 mL and 5 mL] was prepared using standard ammonium sulphate solution. Unknown ferric solution (7 mL) was prepared from plant samples. The solutions were made up to 7 mL by adding distilled water, Nitric acid (4N HNO_3) and ammonium thiocyante (2M NH_4CNS) 1 mL each. Optical density was measured at 480 nm wavelength. Standard curve was plotted against known ferric ammonium sulphate [Fe $(NH_4)SO_4$]concentrations and absorbance corresponding to unknown ferric concentration was found using the standard curve. All tests were replicated 6 times. Using the concentration mg of iron in 100mg of dried plant sample was calculated. Using the moisture value of each plant sample iron mg per 100g fresh weights was calculated.

E. *Statistical analysis*

The results obtained were presented as mean +/- standard deviation (SD). Student's t-test was used to determine the significant variations of the determined parameters. Probability value (P value) < 0.05 was considered as statistically significant.

Results

The moisture and iron content of studied GLV are stated in Table 1. According to the results in fresh samples, Iron content ranged from 6 to 25.2 mg/100g in GLVS. *Amaranthus viridis* had the highest ($p < 0.05$) iron content among the vegetables studied. *Sesbania grandiflora* and *Gymnema lactiferum* had similar iron contents and high iron contents after *Amaranthus viridis*.
Significantly low ($p < 0.05$) iron content was found in *Trianthema portulacastrum* among the vegetables studied (Table 1).

Previous studies also have found high iron contents in *Amaranthus viridis* (20.7 mg/100g) (Borah *et al.,* 2009, Nadeeshani *et al.*, 2018) and *Gymenema lactiferum* (14.64 mg/100g) (Maheshika & Jagath, 2018) and *Sesbania grandiflora (6.49 mg/100g)* (Nadeeshanietal, 2018), *Alternanthera sessilis* (6.72 mg/100g) (Nadeeshanietal, 2018), to have low iron contents compared to *Amaranthus viridis*.

Table 1. Moisture, iron and vitamin C content in GLVs

Sample Type	Moisture content(g/100g)	Iron in mg/100g of fresh sample #	Vitamin c content in mg/100g of fresh sample #
Kathurumurunga (*Sesbania grandiflora*)	79 ±1.0	20.3 ± 1.5^{a}	163.4 ± 6.7^{b}
Mukunuwenna (*Alternanthera sessilis)*	86±1.4	14.6 ± 1.2^{b}	45.8 ± 8.8^{c}
Thampala (*Amaranthus viridis)*	84±3.5	25.2 ± 0.8^{c}	122.5 ± 13.0^{a}
Sarana (*Trianthema portulacastrum)*	95±6.5	6.0 ± 0.3^{d}	65.0 ± 16.5^{d}
Kurinnan (*Gymnema lactiferum)*	82±2.1	20.27 ± 2.6^{a}	125.7 ± 16.2^{a}

Results are average of 6 replicates; different superscripts in the same column indicate significantly different values for the same mineral at 95% confidence interval ($p < 0.05$).

Moisture content ranged from 79.15 to 94.64 mg/100g in the GLV in the study. Highest moisture content was noted in *Trianthema portulacastrum* and lowest was observed in *Sesbania grandiflora. Alternanthera sessilis,* *Amaranthus viridis* and *Gymenema lactiferum* had similar moisture contents (Table 1). Moisture content in GLV allows them to serve as reserve for trace elements and vitamin C.

According to the results the vitamin C ranged from 45.8 – 163.4 mg/100g in GLVs. Significantly high ($p<0.05$) vitamin C content was in *Sesbania grandiflora* leaves (Table 1). Significantly low vitamin C content was in *Alternanthera sessilis* leaves. *Amaranthus viridis* and *Gymnema lactiferum* had similar vitamin C levels, but values were significantly lower than *Sesbania grandiflora* leaves (Table 1). Previous studies also have found the highest vitamin C content in *Sesbania grandiflora* (169 mg/100g) (Duke, 1983), (134.75 mg/100g) (Umaramani & Sivakanesan, 2015). *Alternanthera sessilis* (36.17 mg/100mg) (Umaramani & Sivakanesan, 2015) *and Trianthema portulacastrum* (22 mg/100mg) (Umaramani & Sivakanesan, 2015) leaves reported to have low vitamin C contents in previous studies as well.

Disscussion and Conclusion

Amaranthus viridis had the highest iron content among the vegetables studied. *Recommended dietary allowance (RDA) values of iron (age of 19-50) in both males and females respectively are 8 mg/day and 18 mg/day. The RDA requirement of iron and vitamin C provided by the GLVs in the study is shown in Table 2.* A normal edible portion of 50 g of *Amaranthus viridis* (moisture content 84 %) provides 10.5 mg of RDA requirement of iron. *Sesbania grandiflora and Gymnema lactiferum* provide 8 and 8.3 mg of RDA requirement of iron. *Sesbania grandiflora and Gymnema lactiferum* are good sources of iron that can be used to satisfy iron requirement in vegetarians after *Amaranthus viridis*. *Trianthema portulacastrum* is the poorest source of iron compared to the other GLVs in the study.

Table 2: RDA value of iron and vitamin C provided by GLVs.

Sample Type	Vitamin C content in edible potion of GLVs (mg/50g)	Iron content in edible potion of GLVs (mg/50g)
Kathurumurunga (*Sesbania grandiflora*)	64.54	8.02
Mukunuwenna (*Alternanthera sessilis)*	19.69	6.28
Thampala (*Amaranthus viridis)*	51.45	10.58
Sarana (*Trianthema portulacastrum)*	30.875	2.85
Kurinnan (*Gymnema lactiferum)*	51.53	8.31

RDA value of ascorbic acid in males and females above age of 19 is 90 mg/day and 75 mg/day respectively. A portion of 50 g of *Sesbania grandiflora* (moisture content 79 %) provides 64 mg of the RDA requirement.

In conclusion, *Amaranthus viridis* can be considered as a good potential source of vitamin C as a portion of 50 g of fresh *Amaranthus viridis* provides 51.45 mg of RDA requirement of vitamin C. A portion of 50 go fresh *Sesbania grandiflora* provides 64 mg of RDA requirement of vitamin C and 8.02 mg of RDA requirement of iron and considered as a good source of both. Kurinnan is also good potential sources of vitamin C as it provides 51.53 mg of vitamin C from the RDA requirement and provides a 8.13 mg of RDA requirement of iron. *Amaranthus viridis* and *Gymnema lactiferum* provides similar RDA requirement of vitamin C. *Alternanthera sessilis* is a poor source of iron and vitamin C comparing to the other leaves in the study (figure 1).

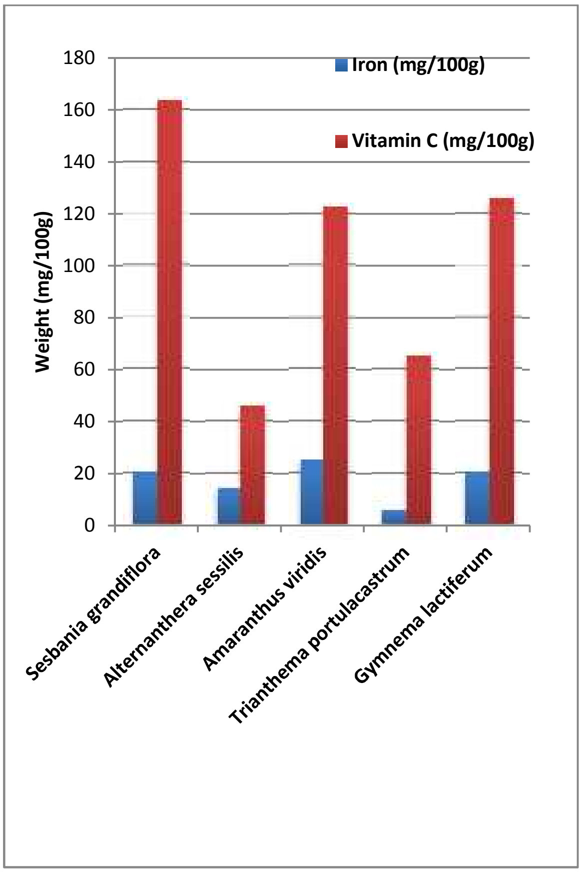

Figure 1. Iron and vitamin C content in GLVs

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Author Biographies

Dilhara Nayomini Dahanayake

Ms. D.N dahanayake is a lecture in Physiology at General Sir John Kotelawala Defence University. She received a BSc Human Biology degree specilaizing in Biochemistry from the University of Sri Jayewardenapura in 2012, and the M.Sc degree in Medical Physics from University of Colombo in 2018. Her primary research interest is in Human physiology due to her teaching interests. Although her concurrent research is in nutrient analysis in vegetables and dosimetry in radiotherapy. The current study on Nutrient analysis of green leafy vegetables is conducted for partial fulfilment of B.Sc. Human biology degree. She has presented oral and poster presentations at local and international conferences. And has published abstracts in international conferences and symposiums proceedings.

Prof. Sagarika Ekanayake

Prof. Sagarika is currently the president of Institute of Chemistry Ceylon. Prior to her current appointment she was Professor in Biochemistry, Faculty of Medicine, University of Sri Jayewardenapura.

She received her BSc Chemistry (honours) from University of Peradeniya, Sri Lanka in 1992 and her MPhil in Biochemistry, from University of Sri Jayewardenepura, in 1999. She obtained her PhD in Applied Nutrition & Food Chemistry, in 2005, from University of Lund, Sweden. Her primary research interest is in the field of biochemistry and food science. She has more then 200 abstracts and 65 papers published in the field of food science and biochemistry in peer review journals and international and local conference proceedings. She was the supervisor of the present study.