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RESEARCH ARTICLE

Determination of selenium content in selected edible green leaves

D.S.M. De Silva[1,*] and A.G.S. Dayarathna[2]

[1]*Department of Chemistry, Faculty of Science, University of Kelaniya, Kelaniya, Sri Lanka*
[2]*Postgraduate Institute of Science, University of Peradeniya, Peradeniya, Sri Lanka*

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Abstract: The selenium (Se) contents in seven conventional Edible Green Leaves (EGL) that consumed by Sri Lankans were determined using Hydride Generation Atomic Absorption Spectrometric method (HGAAS). The EGLs that were grown in five districts under different climatic conditions in Sri Lanka were collected from Gampaha, Kandy, Kurunegala, Anuradhapura and Puttalam areas. The EGL that were subjected to current study are *Centella asiatica (Sin. Gotukola), Alternanthra sessilis (Sin. Mukunuwenna), Basella alba (Sin. Nivithi/ Spinach), Boerhavia diffusa (Sin. Sarana), Ipomoea aquatica (Sin. Kankun), Amaranthus spinosus (Sin. Thampala)* and *Hygrophila schulli (Sin. Neeramulliya)*. Soil samples corresponding to each EGL sample were also collected from Gampaha, Kandy and Anuradhapura districts in order identify a relationship between the Se content in plants and soils. Prior to the analysis EGL samples and corresponding soil samples were subjected to acid digestion with nitric acid. Se contents in the EGL were in the range of 31.2 – 103.2 µg kg^{-1}on dry weight basis. According to the results, *Centella asiatica* and *Hygrophila schulli* varieties showed relatively higher Se content, while *Hygrophila schulli* shown the highest value and the lowest was reported in *Boerhavia diffusa*. The Se content in corresponding soil samples were ranged from 96.4 to 133.9 µg kg^{-1}on dry weight basis. The Se content in soil was higher than that in plants, but there was no significant correlation between the Se content in soil and EGLs.

Keywords: Selenium, edible green leaves, soil, Sri Lanka.

INTRODUCTION

Selenium is an essential micro-nutrient for both human and animals that specially incorporated as amino acids. It is an important trace element in human for good thyroid function and promotes immunity system (Hatfield, 2012). Its anti-oxidant activity reduces the risk of cancer and coronary heart diseases (Patric, 2004; Briggs, 1999). Selenium is also involved in the regulation of variety of cellular functions in living organisms (Rayman, 2000). Tolerable intake level of Se is quite narrow and its deficiencies and toxicities may cause considerable impact on human and animals (Food and Nutrition Board, 2000) and that indicate the vital importance of determining Se content in human diet.

There are relatively many reports on Se content in food (Sunde *et al*., 2006), but limited studies carried out on the Se content in foods consumed by Sri Lankans; i.e.: studies done on rice (Mahagama, 2013, Prasanna 2014), vegetables and cereals (Bandara, 2012; Buwaneka, 2014; Prasanna, 2014). The Se content in meals consumed for lunch by Sri Lankans has also been reported (Kiridena, 2017).

Majority of South Asians including Sri Lankans are consumed plant-derived foods. Particularly, EGLs are dominant choice in their daily meals while the staple food is rice. The contribution of EGLs to dietary intakes of Se has not been reported in Sri Lanka. EGL that were grown on selenium enriched soil and their selenium content was determined by spectrophotometry elsewhere (Petro *et al.,* 2015)

The aim of the present study was to evaluate the Se content in selected edible green leaves consumed by Sri Lankans. The Analysis was done by extracting Se by acid digestion followed by determination using Hydride Generation Atomic Absorption Spectrometry (HGAAS) technique.

MATERIALS AND METHODS

Reagents

Nitric acid (AR, 69%, Sigma Aldrich), hydrochloric acid (AR, 37%, Fluka), hydrogen peroxide (60%, BHD), sodium borohydride (98%, Sd fine-CHEM), sodium hydroxide (99%, Sigma Aldrich), Selenium (AAS standard, Reagecon).

Instrument

Hydride generation atomic absorption spectrophotometer (Analytikjena - nov AA 400P)

Sampling

Seven varieties of edible green leaf samples were selected for the analysis from five districts including Gampaha, Kandy, Kurunegala, Anuradhapura and Chilaw. The EGL samples that were selected for the current study are *Centella asiatica (Sin. Gotukola), Alternanthra sessilis (Sin. Mukunuwenna), Basella alba (Sin. Nivithi/ Spinach), Boerhavia diffusa (Sin. Sarana), Ipomoea aquatica (Sin. Kankun), Amaranthus spinosus (Sin. Thampala)* and *Hygrophila schulli (Sin. Neeramulliya)*. Corresponding soil samples were also collected from three districts including

**Corresponding Author's Email:* sujeewa@kln.ac.lk
http://orcid.org/0000-0001-9407-8455

Gampaha, Kandy, and Anuradhapura. The plant and soil samples were collected randomly from farms during the period of November 2014 to April 2015.

Preparation of samples

Collected samples were prepared for analysis by removing damaged leaves and foreign matter. Cleaned samples were cut into small pieces. Each cleaned green leaves samples (25.00g) and corresponding soil samples (10.00 g) were dried in an oven at (90 ± 5) ˚C until constant weight is obtained (about 6 hours); and the dry weight of each sample and thus the moisture content was measured. The dried soil samples were crushed into powder using mortar and pestle, and sieved through a wire mesh to remove large particles. Finally the working sample of soil was obtained as dried, powdered soil while the dried pieces of green leaves were directly conveyed for analysis.

Digestion of samples

Prepared working sample of green leaves (2.000 g) was digested with concentrated nitric acid (15.00 mL) at (95 ± 5)˚C for 2 hours, by heating in a water bath (until brown fume, NO_2 emission ceased) and the sample was evaporated until the extract was approximately 10 mL. The sample was cooled to room temperature. De-ionized water (4.00 mL) and 60% hydrogen peroxide (6.00 mL) were added and heated to 60 ˚C for 20 minutes (to occur peroxide reaction), and heating was continued until effervescence subsides. The addition of 60% hydrogen peroxide, and heating was continued until effervescence ceased. The sample was concentrated at (90 ± 5)˚C until the volume is approximately reduced to 10 mL. The sample was allowed to cool to room temperature and filtered through a Whatmann No.1 filter paper. Concentrated hydrochloric acid (4.10 mL) was added to filtrate (to obtain 3% HCl solution ultimately) and heated at 70˚C for 30 minutes. Finally, the solution was allowed to cool to room temperature and diluted up to 50.00 mL with de-ionized water. Prepared soil samples (2.000 g) were digested using similar procedure. A control sample was also digested using the above procedure. To analyze the recovery percentage of Se, a standard solution of Se (100 µg/L) was subjected to the same digesting process.

Analysis of samples

The selenium content of various species in dry weight basis, were analyzed for different locations separately. The Se concentrations of different plant and soil samples were analyzed using one way ANOVA followed by Tukey's pairwise comparison test. This was used to analyze the significant difference of each sample together at 95% confidence interval. The normality test (Anderson-Darling test) was performed for each data set to check whether the set of data is in normal distribution. A log conversion was performed, if the set of data is not normally distributed.

RESULTS AND DISCUSSION

There was no significant difference between moisture content in edible green leaf samples collected from various districts. The average moisture content in edible green leaf varieties analyzed ranged from 87.32 – 92.49% (w/w). Hence, the weight of sample highly diminished in the drying process. The higher moisture content leads to lower Se contents in wet weight basis of these plants (Table 1).

Selenium content in different edible green leave varieties

The variation of selenium concentrations on dry weight base in different varieties of conventional edible green leave samples from the five districts are summarized in Table 2.

Centella asiatica (Sin. Gotukola) and *Hygrophila schulli* (Sin. Neeramulli) were reported significantly higher selenium concentrations than other conventional edible green leaves analyzed in the present study. *Alternanthra sessilis* (Sin. Mukunuwenna), *Basella alba* (Sin. Spinach) and *Ipomoea aquatica* (Sin. Kankun) have relatively similar selenium contents, while the selenium contents in *Boerhavia diffusa* (Sin. Sarana) and *Amaranthus spinosus* (Sin. Thampala) were significantly lower.

The selenium contents of EGLs were reduced significantly than their respective contents measured on the dry weight basis due to higher moisture contents (≈ 90%w/w) in EGLs (Table 3). The decrease of selenium contents is more or less proportional to the values of dry weight basis. Among the green leaves analyzed, *Hygrophila schulli* and *Centella asiatica* contains significantly higher selenium contents while *Boerhavia diffusa* having lower amounts.

In a previous study on selenium in rice grown in 12 districs in Sri Lanka, it was observed that the amount of selenium present in Bg 350 rice variety was in the range, 7.5 – 56.9 µg/kg while that in corresponding soil ranged

Table 1: The average moisture content in edible green leave samples.

Variety of EGL	Moisture content (as a %)
Centella asiatica (Gotukola)	87.32 (±1.87)
Alternanthra sessilis (Mukunuwenna)	87.55 (±1.20)
Basella alba (Spinch)	92.49 (±0.76)
Boerhavia diffusa (Sarana)	91.97 (±1.04)
Ipomoea aquatica (Kankun)	89.21 (±1.74)
Amaranthus spinosus (Thampala)	89.15 (±1.10)
Hygrophila schulli (Neeramulli)	88.17 (±0.58)

Table 2: Selenium content (dry weight based) in different edible green leave varieties in different sampling sites.

Species	Se concentration (µg/kg)				
	Sampling sites				
	Gampaha	**Kandy**	**Kurunegala**	**Anuradhapura**	**Chilaw**
Centella asiatica (Gotukola)	73.9 (±2.6)^{a}	71.7 (±3.0)^{a}	60.8 (±3.5)^{a}	84.1 (±4.9)^{a}	66.7 (±2.0)^{a}
Alternanthra sessilis (Mukunuwenna)	51.4 (±1.2)^{b}	46.7 (±2.4)^{b}	44.8 (±2.9)^{b}	45.9 (±1.7)^{b}	42.7 (±1.8)^{b}
Basella alba (Spinach)	52.0 (±3.5)^{b}	51.3 (±3.0)^{b}	46.9 (±5.2)^{b}	55.4 (±2.2)^{c}	46.5 (±2.5)^{b,c}
Boerhavia diffusa (Sarana)	35.6 (±1.7)^{c}	39.6 (±2.2)^{c}	36.7 (±2.2)^{c}	42.7 (±2.0)^{b}	33.0 (±2.0)^{d}
Ipomoea aquatica (Kankun)	50.4 (±1.3)^{b}	51.1 (±0.9)^{b}	48.8 (±2.5)^{b}	53.0 (±2.4)^{c}	50.9 (±2.3)^{c}
Amaranthus spinosus (Thampala)	47.9 (±2.9)^{b}	31.2 (±2.0)^{d}	33.5 (±1.3)^{c}	45.7 (±1.9)^{b}	41.5 (±2.4)^{b}
Hygrophila schulli (Neeramulliya)	102.6 (±1.1)^{d}	84.0 (±2.5)^{e}	93.3 (±3.5)^{d}	103.2 (±1.7)^{d}	88.7 (±2.7)^{e}

*Average selenium content ±standard deviation carried out in triplicates; Different superscript letters in a column show significant differences.

Table 3: Selenium content (wet weight based) in different EGL varieties in different sampling sites.

Species	Se concentration (µg/kg)				
	Sampling sites				
	Gampaha	**Kandy**	**Kurunegala**	**Anuradhapura**	**Chilaw**
Centella asiatica (Gotukola)	9.4 (±0.3)^{a}	9.1 (±0.4)^{a}	7.7 (±0.4)^{a}	10.7 (±0.6)^{a}	8.5 (±0.3)^{a}
Alternanthra sessilis (Mukunuwenna)	6.4 (±0.1)^{b}	5.8 (±0.3)^{b}	5.6 (±0.4)^{b}	5.7 (±0.2)^{b}	5.3 (±0.2)^{b}
Basella alba (Spinach)	3.9 (±0.3)^{c}	3.9 (±0.2)^{c}	3.5 (±0.4)^{c}	4.2 (±0.2)^{c}	3.5 (±0.2)^{c}
Boerhavia diffusa (Sarana)	2.9 (±0.1)^{d}	3.2 (±0.2)^{d}	2.9 (±0.2)^{c}	3.4 (±0.2)^{d}	2.7 (±0.2)^{d}
Ipomoea aquatica (Kankun)	5.4 (±0.1)^{e}	5.5 (±0.1)^{b}	5.3 (±0.3)^{b}	5.7 (±0.3)^{b}	5.5 (±0.2)^{b}
Amaranthus spinosus (Thampala)	5.2 (±0.3)^{e}	3.4 (±0.2)^{c,d}	3.6 (±0.1)^{c}	5.0 (±0.2)^{e}	4.5 (±0.3)^{e}
Hygrophila schulli (Neeramulliya)	12.1 (±0.1)^{f}	9.9 (±0.3)^{e}	11.0 (±0.4)^{d}	12.2 (±0.2)^{f}	10.5 (±0.3)^{f}

*Average selenium content ±standard deviation carried out in triplicates; Different superscript letters in a column show significant differences.

Table 4: Selenium content in soil samples in three sampling sites.

Species	Selenium content in soil (µg/kg)		
	Sampling sites		
	Gampaha	**Kandy**	**Anuradhapura**
Centella asiatica (Gotukola)	108.3 (±3.4)^{a,b,c}	108.6 (±6.0)^{a,b}	110.5 (±4.1)^{a}
Alternanthra sessilis (Mukunuwenna)	109.1 (±4.1)^{a,c,d}	100.2 (±4.4)^{b,c}	133.9 (±7.5)^{b}
Basella alba (Spinach)	114.8 (±3.7)^{a,d}	101.1 (±4.3)^{a,b,c}	122.1 (±5.6)^{c}
Boerhavia diffusa (Sarana)	101.1(±3.5)^{b,c}	111.6 (±4.3)^{a}	105.2 (±5.0)^{a}
Ipomoea aquatica (Kankun)	116.4 (±3.4)^{a,d}	99.8 (±5.1)^{b,c}	129.7 (±3.8)^{b,c}
Amaranthus spinosus (Thampala)	99.8 (±5.1)^{b}	96.4 (±4.7)^{c}	125.3 (±2.4)^{b,c}
Hygrophila schulli (Neeramulliya)	117.9 (±4.6)^{d}	105.2 (±3.6)^{a,b,c}	122.3 (±3.2)^{c}

*Average selenium content ±standard deviation carried out in triplicates; Different superscript letters in a column show significant differences.

from 9.5– 69.8 µg/kg (Mahagama, 2013). Also another analysis of selenium concentration in rice and soil in selected 15 villages in Sri Lanka where goiter is prevalant revealed that the total Se contents in soil and rice were in the range of 0.113 - 5.238 µg/g and 0.1 to 776 µg/g respectivily (Ferdyce et al., 2000). Selenium content in meals consumed for lunch by Sri Lankans has also been reported and found to be in the range of 48-70 µg/kg and 53-60 µg/kg respectively (Kiridena, 2017). Recently, Se deficiency has been identified as a responsible factor in the occurrence of Chronic Kidney Disease of Unknown etiology (CKDu) (Jayathilake *et al*., 2013).

Selenium content in corresponding soil samples

Soil selenium contents in the three districts (Table 4) were ranged from 96.4 to 133.9 µg/L. Any positive correlation among selenium contents of edible green leaves and respective soils could be hardly found.

Table 5: Selenium concentration variation in edible green leave samples and corresponding soil samples in three sampling sites.

Species	Selenium content (µg/kg)					
	Gampaha		Kandy		Anuradhapura	
	plant	soil	plant	soil	Plant	soil
Centella asiatica (Gotukola)	73.9 (±2.6)[a]	108.3 (±3.4)[b]	71.7 (±3.0)[a]	108.6 (±6.0)[b]	84.1 (±4.9)[a]	110.5 (±4.1)[b]
Alternanthra sessilis (Mukunuwenna)	51.4 (±1.2)[a]	109.1 (±4.1)[b]	46.7 (±2.4)[a]	100.2 (±4.4)[b]	45.9 (±1.7)[a]	133.9 (±7.5)[b]
Basella alba (Spinach)	52.0 (±3.5)[a]	114.8 (±3.7)[b]	51.3 (±3.0)[a]	101.1 (±4.3)[b]	55.4 (±2.2)[a]	122.1 (±5.6)[b]
Boerhavia diffusa (Sarana)	35.6 (±1.7)[a]	101.1 (±3.5)[b]	39.6 (±2.2)[a]	111.6 (±4.3)[b]	42.7 (±2.0)[a]	105.2 (±5.0)[b]
Ipomoea aquatica (Kankun)	50.4 (±1.3)[a]	116.4 (±3.4)[b]	51.1 (±0.9)[a]	99.8 (±5.1)[b]	53.0 (±2.4)[a]	129.7 (±3.8)[b]
Amaranthus spinosus (Thampala)	48.0 (±2.8)[a]	99.8 (±5.1)[b]	31.2 (±2.0)[a]	96.4 (±4.7)[b]	45.7 (±1.9)[a]	125.3 (±2.4)[b]
Hygrophila schulli (Neeramulliya)	102.6 (±1.1)[a]	117.9 (±4.6)[b]	84.0 (±2.5)[a]	105.2 (±3.6)[b]	103.2 (±1.7)[a]	122.3 (±3.2)[b]

*Average selenium content ±standard deviation carried out in triplicates; Different superscript letters in a row in each sampling site, show significant differences.

Although no positive correlation between Se contents of plants and corresponding soils was recorded, the results revealed that soil Se contents were always significantly higher than that in plant samples. The amount of absorption of selenium from soil may differ between plants perhaps due to many reasons including different modes of accumulation, diverse metabolism mechanisms and bioavailability of selenium in corresponding soil. The selenium content in soil samples represents the total selenium content while in plants the Se contents represent only the biologically available fraction, which is lower than the total selenium content. However, selenium contents in those EGLs were found to be in an acceptable range; 55-400 µg/kg. The tolerance level of selenium for human is 400 µg/kg (Food and Nutrition Board, 2000). However, none of the EGL samples that were analyzed have exceeded the tolerance level of selenium.

CONCLUSIONS

The present study reveals that the conventional edible green leaves consumed by Sri Lankans contain significant amount of selenium. Selenium contents in selected leafy vegetables were in the range of 31.2 – 103.2 µg/kg. *Hygrophila schulli* showed the highest Se content (101.3 µg/kg), while *Boerhavia diffusa* (Sin. Sarana) and *Amaranthus spinosus* (Sin. Thampala) contained comparatively lower Se contents. The results conclude that the EGLs tested contained adequate amounts of selenium. *Centella asiatica* and *Hygrophila schulli* can be introduced as selenium rich edible green leave sources for human diet.

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