Proceedings of 15th Agricultural Research Symposium (2016) 326-330

Antioxidant Properties and Mineral Contents of Selected Plant Species used in Green Leafy Porridge *Kola Kenda*

W.M.D. RUWANMALI[1], J.P. EESWARA[2] and R.H.M.K. RATNAYAKE[1]

[1]*Department of Horticulture and Landscape Gardening, Faculty of Agriculture and Plantation Management, Wayamba University of Sri Lanka, Gonawila (NWP), 60170, Sri Lanka*

[2]*Department of Crop Science, Faculty of Agriculture, University of Peradeniya, Peradeniya, 20400, Sri Lanka*

ABSTRACT

Green leafy porridge or *Kola kenda* is a unique food in Sri Lanka. Plants used to make green leafy porridge have medicinal properties and health benefits. This study was conducted to scientifically validate the nutritional compounds and mineral contents in five plant species used for making *Kola kenda, viz., heen bovitiya* (*Osbeckia octandra*), *welpenela* (*Cardiospermum halicacabum*), *hathawariya* (*Asparagus racemosus*), *neeramulliya* (*Hygrophila schulli*) and *gotukola* (*Centella asiatica*). Highest antioxidant scavenging capacity was observed in the leaf extracts of *O. octandra* (67.80 mg g^{-1} Gallic acid equivalence) while *A. racemosus* and *O. octandra* had the highest ascorbic acid contents (126.89 and 124.43 mg 100 g^{-1},respectively). Highest fiber content (41.85%) was recorded in *A. racemosus. C. halicacabum* contained the highest amount of nitrogen (53.41 mg N g^{-1}) and potassium (13.8 g K kg^{-1}) while the level phosphorus (4.81mg P g^{-1}) was highest in *H. schulli.*

KEYWORDS: Green leafy porridge, *Kola kenda*, Nutrient components, Plant species

INTRODUCTION

Green leafy porridge, which is commonly known as *Kola kenda* is a food unique to Sri Lanka. *Kola kenda* is more palatable and fulfilling compared to a water extract of herbs as it is a mixture of leaf extracts, coconut milk and rice, and is ingested as a meal. Furthermore, *Kola kenda* provides many health benefits due to the presence of nutraceuticals (Senadheera *et al.*, 2014).

Herbal dietary remedies consumed from ancient times are still widely used to treat hyperglycemic conditions all over the world. The presence of antioxidants could be the main reason for the health benefits of *Kola kenda* because antioxidants protect cells from oxidation by scavenging harmful free radicals which are involved in dangerous ailments, including cancer (Kaur and Kapoor, 2001).

With the introduction of herbal products in the global market, their safety has become a major concern in public health care (Atanassova *et al.*, 2011). Therefore, it is essential to investigate the nutritional value of herbs used for the preparation of *Kola kenda* to scientifically validate their health benefits. In the current study, the antioxidant properties and mineral contents of five plant species *viz., heen bovitiya* (*Osbeckia octandra*), *welpenela* (*Cardiospermum halicacabum*), *hathawariya* (*Asparagus racemosus*), *neeramulliya* (*Hygrophila schulli*) and *gotukola* (*Centella asiatica*), which are commonly used as ingredients of *Kola kenda* , were investigated.

Osbeckia is a genus of family Melastomataceae which is used in traditional medicine to treat jaundice and other liver disorders. *Osbeckia octandra* leaf decoction is used in the treatment of diabetes mellitus in Ayurvedic medicine.

Cardiospermum halicacabum L. (Sapindaceae) is a herb with slim twigs that climb by tendrillar hooks.

In *Hygrophila schulli* (Acanthaceae), the whole plant, roots, seeds and ashes of the plant are extensively used in traditional system of medicine for the treatment of pnemeham (Diabetes) and athisaram (Dysentery). The plant is known to possess antitumor, hypoglycemic, antibacterial and hepatoprotective activities.

Centella asiatica (Umbelliferaceae) contains several active constituents, of which the most important are the triterpenoid saponins, including asiaticoside, centelloside, madecassoside, and Asiatic acid.

The roots of *Asparagus racemosus* (Liliaceae) are used in various medicinal preparations. The stem is used in preparation of *Kola Kenda.*

MATERIALS AND METHODS

Experimental Site and Materials

The experiments were conducted at the Department of Crop Science, Faculty of Agriculture, University of Peradeniya. Semi-hard wood portion of plants were collected. Leaves, shoots and stem parts were separated and extracted as required for specific chemical analyses. All chemicals used were of Analytical Reagent grade (Sigma). Preliminary trials were

conducted to standardize the procedures for plant species.

Ascorbic Acid Content (AA)

Five grams of plant parts were blended with 3% HPO_3 acid (30 g of HPO_3 pellets diluted in 1000 mL of distilled water). Then, the extract was filtered and volume was made up with 3% HPO_3 acid (Ranganna, 1986). Ascorbic acid standard (100 mg in 100 mL) was prepared using 3% HPO_3. Then, 10 mL from that solution was again diluted with 100 mL by using 3% HPO_3. Dye solution was prepared by dissolving 50 mg of the Sodium-salt of 2, 6–dichlorophenol-Indophenol in 150 mL of hot glass distilled water containing 42 mg of Sodium bicarbonate. Then, it was cooled and diluted with distilled water to 100 mL and was kept in the refrigerator. Five milliliters of ascorbic acid solution was mixed with 5 mL of HPO_3 solution and was titrated with dye solution on a magnetic stirrer for the blank titration. Thereafter, 5 mL of leaf extract was mixed with 5 mL of HPO_3 solution and it was titrated with the dye solution on a magnetic stirrer. Ascorbic acid content was calculated by following equation:

$$\text{Dye factor} = \frac{0.5}{titre}$$

$$\text{mg ascorbic acid per 100 g} = \frac{Titre \times Dye\ factor \times volume\ made\ up \times 100}{Aliqout\ of\ extract\ taken\ for\ estimation \times Weight\ or\ volume\ of\ sample\ taken\ for\ estimation}$$

Antioxidant Scavenging Capacity (AC)

1,1–Diphenyl-2-picryllhydrazyl radical (DPPH) assay was used to measure the antioxidant scavenging capacity of plants. Plant parts were air dried for 2-3 days and 0.5 g of air dried samples were thoroughly crushed into a powder. Then, the sample was mixed with 100 mL of 80% methanol and shaked under 170 rpm for 24 h. The extract was filtered and used for the analysis. Antioxidant scavenging capacity was analyzed by Gallic acid equivalent method (Wojdyol *et al.*, 2007). Gallic acid (0.1 g) was dissolved in 1 L of distilled water and 4 mg of DPPH was dissolved in 100 mL of ethanol. Leaf extract (100-1,000 µL) was mixed with 3 mL of DPPH and the total volume was adjusted to 6 mL with 80% methanol. Then, the sample was shaken vigorously and allowed to stand at room temperature in the dark for 20 min. The decrease in absorbance of the resulting solution was measured at 517 nm at 20 min. The data were collected and the results were expressed in µg g^{-1} weight (DW) in Gallic acid equivalence (GAE). Additional dilution was done if the absorbance value measured was over the liner range of the standard curve (Placio *et al.*, 2014).

Chlorophyll Content

Plant samples of 1 g were cut in to small pieces and finely ground with 80% acetone. The extract was volumerized up to 100 mL with 80% acetone (Arnon, 1949). Five milliliters of the extract was diluted up to 50 mL with 80% acetone and the absorbance was measured at 645 and 663 nm. Calculations were done as:

Total Chlorophyll (µg mL^{-1}) = 20.2(A_{645}) +8.02 (A_{663})

Chlorophyll a (µg mL^{-1}) =12.7 (A_{663}) - 2.69 (A_{645})

Chlorophyll b (µg mL^{-1}) = 22.9 (A_{645}) - 4.68 (A_{663})

Fiber Content

Oven dried 5 g leaf sample was finely ground. Then, 50 mL of 5% H_2SO_4 was added to the beaker and volume was made up to 200 mL with distilled water. It was placed in a water bath at 85 °C while being stirred using a glass rod for 30 min. Afterwards, the hot solution was filtered under suction through Buchner funnel (Haldenw-anger)covered with a piece of muslin cloth. The residue was washed on the cloth with hot water until the filtrate was free from acid. The residue was transferred into same beaker by scrapping off with a spatula and hot water. Then, 50 mL of 0.5 M NaOH was added. It was boiled while stirring for 30 min and the liquid level was maintained at 200 mL by adding distilled water. Thereafter, the sample was filtered using a cloth and the residue was washed for a few minutes with hot water followed by washing with 1% HCl acid. The sample was then re-washed with hot water and 50 mL of isopropyl alcohol (95% ethanol) was added before washing with hot water again. Then, the residue was allowed to dry under suction followed by drying in an oven at 105 °C until the weight of the sample was constant. Oven dried sample was weighed, transferred into a previously weighed crucible and was kept in muffle furnace until only the amount of gray ash remained (Ranganna, 1986).

Nitrogen (N) Content

Dried 0.5 g of plant parts were analyzed by Kjeldhal method (Ranst, 1999). Dried samples were transferred into Kjeldhal tubes. Seven milliliters of Sulphosalicylic acid was added to each tube and was kept for 30 min. Thereafter, 0.5 g of $Na_2S_2O_3$ was added to each sample and kept for 15 min. Then, 0.2 g of catalyst mixture, 3 mL of conc. H_2SO_4 and H_2O_2 were added to the tubes. The samples were kept in the digester for 1.5 h and later allowed to cool. Then, 30 mL of distilled water was added to each sample. In the distillation unit, 40% of

NaOH was added to the samples (when NaOH is added, if N is present, boric acid becomes green). The green solution was titrated against 0.1 N HCl (at the end point, green turns into pinkish red). Total N content was analyzed by;

$$\text{mg of N g}^{-1} = \frac{14\,(V1-V0)t}{W}$$

where, V1- Sample titer reading, V0 – blank reading, t- normality of HCl, W –weight of the sample

Protein content = Nitrogen content x 6.25%

Potassium (K) and Phosphorus (P) Contents

Dried plant parts were analyzed by muffle furnace method (Ranst, 1999). Plant samples (0.5 g) were transferred into porcelain crucibles. Pre-calcination was done on a hot plate or muffle furnace at 200 °C. Calcination was done at 450 °C until complete mineralization of the sample. Crucibles were removed from muffle furnace, placed directly on a hot plate and boiled with 5 mL of 6 M HNO_3. Then, 5 mL of 3 M HNO_3 was added and reheated for a few minutes. Thereafter, the warm solution was filtered into a 50 mL volumetric flask (using a glass rod to recover the residue on the filter). The filtrate was allowed to cool and was diluted to 50 mL with distilled water. Two milliliters of ash extract, 6 mL of distilled water and 2 mL of nitro vanado molybdate were homogenized and allowed to react for 1 h. The same procedure was followed for the standard. Absorbance was measured at 430 nm by UV-Vis spectrophotometer. Phosphorous and K contents were calculated as follows.

Phosphorus:

$\mu g\ P\ g^{-1}\ DM = (Ce\ x\ 1)/Dx50$

$mg\ P\ g^{-1}\ DM = (Ce\ x1)/Dx0.050$

Potassium:

$$mg\ K\ kg^{-1} = \left(\frac{Cde\ x\frac{1}{D}x1}{ER}\right) \text{ or (Ce x 0.5 g)}$$

Where, DM-Dry mattered, Ce- Concentration of the extract, D- dilution, Cde- Concentration of diluted extract, ER-Extraction ratio.

Sugar Content

Leaves (2 g) were ground with distilled water and the extract was filtered. Anthrone (2 g) was dissolved in 1 L of conc. H_2SO_4 and stored in the refrigerator. Fresh solutions of Perchloric acid (0.46 N), and glucose stock solution (100 mg L^{-1}) were prepared daily. Each of the standards was prepared at a concentration of 100 mg L^{-1}. Then, 0.6 mL of 0.46 N $HClO_4$ was added to each tube and the volume was made up to 5 mL with distilled water. Additional dilution with distilled water was done if the measured absorbance value was out of the liner range of the standard curve (Ranganna, 1986).

Statistical Analysis

Data were analyzed by Microsoft Excel 2013 and Minitab Version 15.

RESULTS AND DISCUSSION

Ascorbic Acid Content

Highest ascorbic acid levels were found in *hathawariya* (*A. racemosus*; 126.89 mg 100 g^{-1}) and *heen bovitiya* (*O. octandra*) shoots (124.43 mg 100 g^{-1}) and those were significantly different ($P<0.05$) from other three species (Table 1).

Antioxidant Scavenging Capacity (AC)

Highest AC was recorded by *heen bovitiya* leaves (*O. octandra*; 67.80 mg g^{-1}) while *welpenela* (*C. halicacabum*) recorded the lowest level of AC (3.60 mg g^{-1}) among the five plants (Table 1).

Chlorophyll Content (Chl)

Chlorophyll content of five plants were highly variable. Highest chlorophyll level was recorded in *welpenela* (*C. halicacabum L*; 12.18 $\mu g\ g^{-1}$; Table 1).

Fiber Content (F)

The highest crude fiber percentage was recorded in *hathawariya* (*A. racemosus*, 41.85%) which was significantly different ($P<0.05$) from all other species (Table1).

N, P and K Contents

Welpenela (*C. halicacabum*) recorded the highest significant total N content (49.22 mg N g^{-1}) followed by *gotukola* leaves (*C. asiatica;* 43.73 mg N g^{-1}) and *neeramulliya* (*H. schulli*; 37.94 mg N g^{-1}). *Heen bovitiya* (*O. octandra*) shoots showed a significantly lower ($P< 0.05$) N content (8.8 mg N g^{-1}) compared to its own leaves and other species (Table 2).

Highest P content was recorded by *neeramulliya* (4.81 mg P g^{-1}) while the highest K content was recorded by *welpenela, hathawariya, gotukola* stalk and *neeramulliya* (Table 2).

Sugar Content

The highest sugar level was recorded by *hathawariya* (*A. racemosus;* 0.92 $\mu g\ mg^{-1}$) and it was significantly different from all other species, except from *gotukola* (Table 2).

Nutritional components contained in green leafy plants help prevent diseases and maintain human health. Consumption of green leafy porridge would develop a strong immune system in the human body. Antioxidants neutralize free radicals before the radicals get a

Table 1. Mean Ascorbic acid content (AA), antioxidant scavenging capacity (AC), chlorophyll (Ch) and fiber (F) contents of five plant species

Plant type	Mean quantity of compounds			
	AA (mg 100g^{-1})	AC (mg g^{-1})	Ch (µg g^{-1})	F (%)
GS	10.50^{d}	-	-	26.78^{c}
GL	32.15^{c}	30.39^{b}	4.20^{b}	-
W	24.32^{c}	3.60^{d}	12.18^{a}	17.52^{d}
N	66.60^{b}	12.67^{c}	3.06^{b}	30.31^{b}
HBS	124.43^{a}	-	2.5°C	20.19^{d}
HBL	37.0°C	67.80^{a}	2.8°C	-
H	126.89^{a}	6.21^{d}	1.23^{d}	41.85^{a}

Within a column, values with different superscript letters are significantly different at P=0.05 level. Plant type: GS- Gotukola stalk, GL-Gotukola leaf, W- Welpenela, N- Neeramulliya, HBS- Heen bovitiya shoots, HBS - Heen bovitiya leaves AA- Ascorbic acid, AC- Antioxidant Scavenging Capacity, Ch- Chlorophyll, F- Fiber

Table 2. Mean quantities of nitrogen (N), phosphorus (P), potassium (K) and sugar (Su) in the five plant species

Plant type	Mean quantity of compounds			
	Nitrogen (mg N g^{-1})	Phosphorus (mg P g^{-1})	Potassium(g K kg^{-1})	Sugar (µg g^{-1})
GS	23.31^{c}	2.20^{b}	12.60^{a}	-
GL	43.73^{b}	2.50^{b}	8.40^{b}	0.76^{a}
W	49.22^{a}	4.22^{a}	13.80^{a}	0.14^{c}
N	37.94^{b}	4.81^{a}	11.50^{a}	0.07^{c}
HBS	8.80^{d}	2.11^{b}	7.50^{b}	0.55^{b}
HBL	26.0°C	2.40^{b}	6.90^{b}	-
H	25.77^{c}	1.38^{c}	12.66^{b}	0.92^{a}

Within a column, values with different superscript letters are significantly different at P=0.05 level.GS- Gotukola stalk, GL- Gotukola leaf, W- Welpenela, N- Neeramulliya, HBS- Heen bovitiya shoot, HBL- Heen bovitiya leaf, H- Hathawariya

chance to create havoc, by donating one of their own electrons ending the electrons stealing chain reaction, and play the housekeeper's role in the human body (Kaur and Kapoor, 2001).

Antioxidants even offer protection against critical illnesses like cardiovascular diseases, chronic diseases and certain types of cancer. Fiber, polyphenols, conjugated isomers of linoleic acid, D-limolene, epigallocatechin, gallate, soya protein, isoflavanones, vitamins A, B, C and E, tocopherols, calcium, selenium, chlorophyllin, alipharin, sulphides, catechin, tettahydrocurcumn, seasaminol, glutathione, uric acid, indoles, thiocyanates and protease inhibitors are the most thoroughly investigated dietary components in fruits and vegetables acting as antioxidants (Kaur and Kapoor, 2001).

In the present study, the total antioxidant activity, ascorbic acid and mineral contents of five plant species used in making green leafy porridge were investigated. Results indicated that the antioxidant activity and ascorbic acid content were significantly high in *heen bovitiya* leaves which is used in Ayurvedic medicine in Sri Lanka as a remedy for hepatitis. Thus, it may be possible that antioxidants present in Heen bovitiya play a hepatoprotective role in the human body.

Study of inter relationships between nutrient components is important because they do not act individually inside the plant cells. In the present study, a positive relationship between N content of the plant and chlorophyll content was observed (Figure 1). The nitrogen is essential for chlorophyll synthesis and therefore, a higher N supply is associated with higher chlorophyll content.

However, in the present study, a negative relationship between chlorophyll content and sugar was also found (Figure 2). Theoretically, plants containing high amounts of chlorophyll should produce more starch. It is possible that the external structures of plant (eg. thick cuitical layer, hairy structures, stomatal density of leave surface, *etc.*) and external environmental conditions may affect the synthesis of starch in addition to the presence of chlorophyll in the plant.

CONCLUSIONS

Based on the results of the present study, *heen bovitiya* (*Osbeckia octandra*) leaves can be considered as the best source for making green leafy porridge considering their high antioxidant activity as well as ascorbic acid content. Nevertheless, the method of preparation may affect the antioxidant activity and ascorbic acid content ingested by the consumer. Therefore, further studies are required to identify the changes occurred in nutritional value of porridge during preparation.

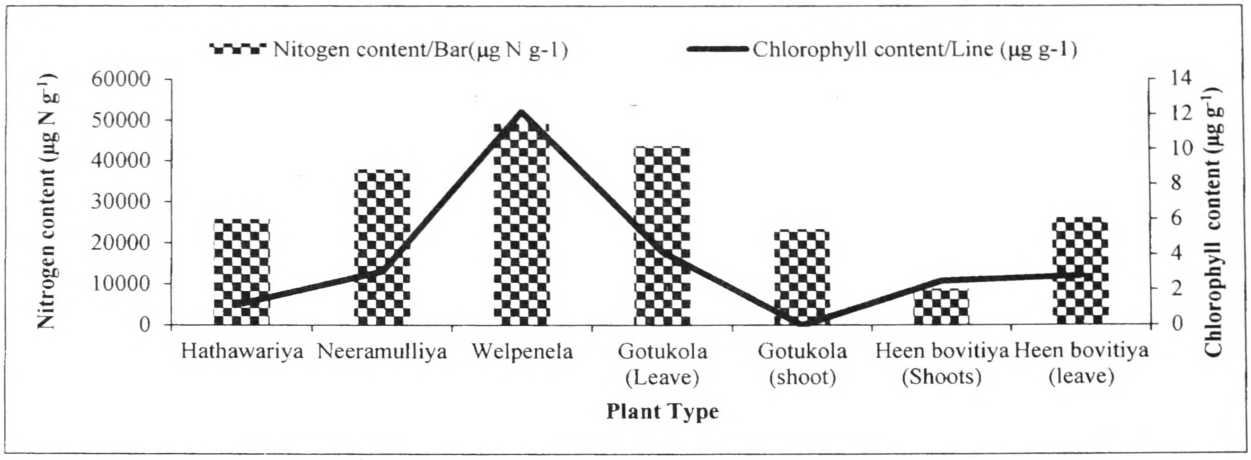

Figure 1. Relationship between nitrogen content and chlorophyll content in five plant species

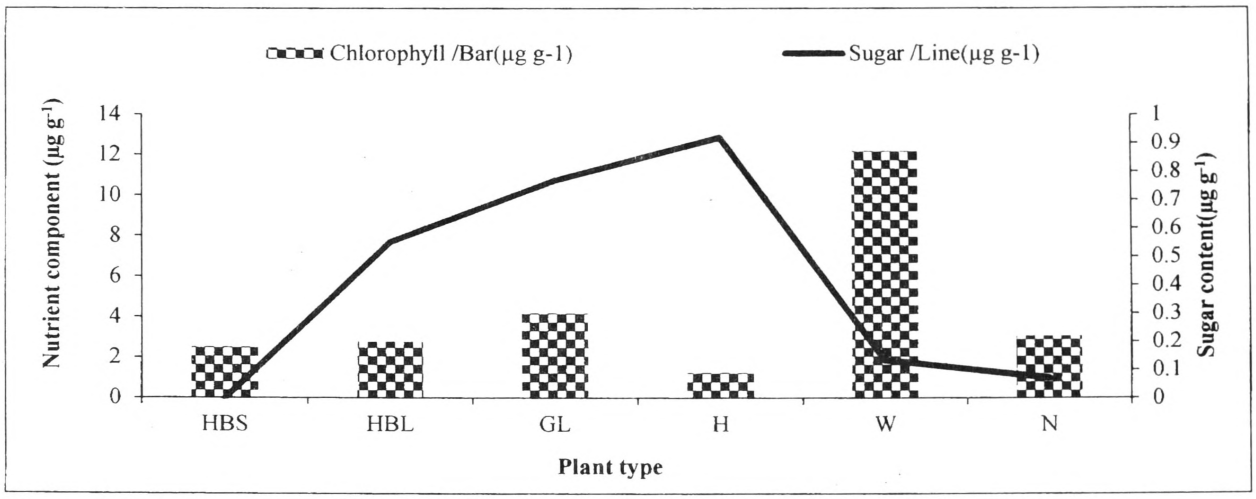

Figure 2. Relationship between sugar content and chlorophyll content in five plant species

ACKNOWLEDGEMENTS

The authors acknowledge the support given by Mr. Gemunu Wejesooriya, Technical officer, Department of Crop Science, Faculty of Agriculture, University of Peradeniya. Authors thank Dr. Keminda Herath, Department of Agribusiness Management and Mr. Indika Karunarathne, ICT Center, Wayamba University of Sri Lanka, for their assistance and statistical analysis.

REFERENCE

Anon. (2012). Available from: https:// www. google.com (Asian cuttings Lanka (pvt) limited.

Arnon. D. (1949). Measuring chlorophyll and Anthocyanin Concentration. *Plant Physiology,* **24,** 1-15.

Atanassova, M., Georgieva, S. and Ivancheva, K. (2011). Total phenolic and total flavonoid, antioxidant capacity and biological contaminants in medicinal herbs. *Journal of the University of Chemical Technology and Metallurgy*, **46** (1), 81-88.

Kaur, C. and Kapoor, H.C. (2001). Antioxidants in fruits and vegetables the millennium's health. *International Journal of Food Science and Technology*, **36,** 703 -725.

Placio, E.F., Alvarez, L.A., Camacho, N.A., Farias, G.A., Cruz, O. and Rodriguez, J.A. (2014). Antioxidant capacity, radical scavenging kinetics and phenolic profile of methanol extracts of wild plants of Southern Sonora, Mexico. *Tropical Journal of Pharmaceutical Research,* **13** (9), 1487 -1493.

Ranst, E. (1999). *Manual for the Soil Chemistry and Fertility Laboratory. Analytical Methods for Soils and Plants Equipment, and Management of Consumables.* Ghent University Academic Bibliography.

Ranganna, S. (1986). Hand Book of Analysis and Quality Control for Fruits and Vegetable products. Tata McGraw-Hill company limited, New Delhi, India.

Senadheera, S.P.A.S., Ekanayake, S. and Wanigatunga, C. (2014). Antioxidant potential of green leafy porridges. *Ceylon Medical Journal,* **59,** 4-8.

Wojdylo, A., Oszmianski, J. and Czemerys, R. (2007). Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chemistry*, **105,** 940-949.