

Protein and Micronutrient Contents of *Moringa oleifera* (Murunga) Leaves Collected from Different Localities in Sri Lanka

R. Liyanage¹, C. Jayathilaka², O.S. Perera³, S.A.Kulasooriya⁴, B.C.Jayawardana⁵, S.Wimalasiri⁶

¹Research Fellow, Institute of Fundamental Studies, Kandy, Sri Lanka
Corresponding author Email: ruvini@ifs.ac.lk

²Research Assistant, Institute of Fundamental Studies, Kandy, Sri Lanka

³Research Assistant, Institute of Fundamental Studies, Kandy, Sri Lanka

⁴Research Fellow, Institute of Fundamental Studies, Kandy, Sri Lanka

⁵Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Sri Lanka

⁶Department of Food Science, Faculty of Agriculture, University of Peradeniya, Sri Lanka

ABSTRACT— *Deficiency in protein and micronutrients especially iron, zinc and calcium among children and pregnant mothers has become a major health issue in Sri Lanka today. Moringa oleifera is an important multipurpose tropical tree under-utilized for its nutritional and medicinal properties. In the present study, M. oleifera leaves collected from eight districts falling within different agro-climatic localities in Sri Lanka were analyzed for their protein and some micronutrient. The collected samples were dried, milled to particle sizes of less than 125 µm and protein as well as mineral contents were analyzed by Kjeldhal method and microwave assisted acid digestion followed by the Flame Atomic Absorption Spectrophotometer, respectively. Results revealed wide variations in the ranges of protein and micronutrients in Moringa leaves collected from the different localities, and among all the districts studied, leaves collected from Polonnaruwa contained significantly higher iron, zinc and protein contents compared to those from the other districts and calcium and magnesium were also present in considerable amounts. Therefore, Polonnaruwa district appears to offer the best environmental conditions for the intensive cultivation of moringa to obtain leaves of high nutritive value. However, moringa leaves collected from all the locations contain appreciable amounts of nutrients and could be used as a food supplement to combat protein and micronutrient malnutrition among the affected people of Sri Lanka.*

Keywords— *Moringa oleifera*, proteins, micronutrients, malnutrition, Sri Lanka

1. INTRODUCTION

Food is the primary source of essential and non essential nutrients for human wellbeing and health [1, 2]. It is important to maintain good supply of quality nutrients throughout life for better functioning of the human body [3-5]. Over one billion people around the world are known to be undernourished due to lack of balanced diet [6-8]. At present malnutrition has also become a multifaceted problem in Sri Lanka [9, 10]. According to a Demographic and Health Survey in Sri Lanka, 22% of married women in the reproductive age group are malnourished, while children under five years have been born as low birth-weight babies [11]. Obviously mother's nutritional status affects the unborn child, and a low birth weight child would show a higher vulnerability to ill health, as well as physical growth in the formative years of life. [12]. Recently released surveys by Sri Lanka's health ministry, revealed that child malnutrition is an emerging nutritional problem in Sri Lanka [13]. In particular, children of school age do not consume nutritious and healthy meals on a daily basis [12, 14].

Poor purchasing power has been the main reason for insufficient access to a healthy diet. Hence it is important to introduce a low cost and sustainable source of nutrients that people can consume directly or can be incorporated into other food products. Moringa is a natural, whole-food source for vitamins, minerals, protein, antioxidants, and other important compounds that body needs to stay healthy.[15-19]. As a source of good nutrition, its leaves are considered the best of tropical legumes with its high quantities of vitamin A and significant quantities of vitamin C, calcium, iron, protein, potassium, magnesium, selenium, zinc and a good balance of all the essential amino acids. One 100 g portion of

leaves could provide a woman with over one-third of her daily need of calcium and give her important quantities of iron, protein, copper, sulfur and B-vitamins. Some clinical studies have shown that supplementation of moringa leaf powder is effective in improving the nutritional status of children and mothers [20,21]. Importantly, Moringa leaves have not been found to be and safety evaluation studies showed that ethanolic and aqueous extract of both fruit and leaf of moringa was well tolerated by experimental animals[22].

Moringa oleifera L. belongs to one of the 14 species in the family *Moringaceae*, native to India, Africa, South Asia, South America and the Pacific and Caribbean Islands[23]. Several parts of this plant are consumed by humans from the ancient times. The plant is recognized by many names such as drumstick tree, horseradish tree, miracle tree etc[24]. In Sinhala it is called “Murunga” and in Tamil it is called “Murungakai”.

Different parts of the Moringa plant has been exploited for its nutritional, medicinal, and flavor enhancing activities. The leaves of Moringa can be eaten fresh, cooked or stored as a dried powder and used in stews and sauces [25, 26]. *Moringa oleifera* leaves are especially selected as a suitable source for fighting against protein and micronutrient malnutrition in the developing countries because it has some specific favorable characters [27].

Leaves form *Moringa oleifera* Lam. (Murunga) is considered a cheap and sustainable source protein and micronutrients for human consumption which is available year around in most parts of Sri Lanka [28]. Thus it can be used as a suitable source for combating protein and micronutrient deficiency existing in the developing countries. Moringa leaf supplemented products such as sauces, juices, spices, cereal mixtures, milk, instant noodles, instant tea etc have been developed recently.

Even though various studies have been carried out on Moringa species, there are few or no experimental studies regarding the potential nutritional value of this plant growing in different parts of Sri Lanka. The objective of this study was to determine the protein and some micronutrient contents of *Moringa oleifera* leaves as a function of agro-climatic locations in Sri Lanka.

2. MATERIALS AND METHODS

2.1. Sample Collection

Fresh green *Moringa oleifera* leaf samples were obtained from Anuradhapura, Galle, Hambantota, Jaffna, Kandy, Kurunegala, Matara and Polonnaruwa Districts of Sri Lanka. From each district 2kg of leaf samples were collected from four different locations.

2.2. Sample preparation

The leaves were removed from the stem and damaged, yellow coloured ones were excluded. They were washed with double de-ionized water, spread on trays and initially air dried at room temperature. Then they were dried in a dry air oven at 65°C for 48 hours[29]. From each dried leaf sample, 100g were ground using a stainless steel grinder and sieved into particle size less than 125 µm and stored in air tight high density polyethylene bags at room temperature in a dark place for further analysis. The leaves of each four locations from the eight districts were assayed and analyzed individually in triplicate.

2.3. Mineral analysis

Mineral contents were determined according to the methods described by the Association of Official Analytical Chemists [30] using flame atomic absorption spectrophotometry (AAS), (GBC 933 AA). From the each dried and powdered sample 0.25 g was acid digested using the Microwave digestion unit (Milestone start D, MLS47100). Conc. 69% HNO₃ (9 ml) and 30% H₂O₂ (1 ml) were used as the digestive solutions. Then the digestion unit was programmed (warming time: 0-5 minutes, warming temperature: 0-180 °C/ Digestion time: 5-15 minutes, Digestion temperature: 180 °C/ Cooling time: 15-25 minutes) and samples were digested. After the digestion was completed the digestion vessels were allowed to cool and then the digested solutions were filtered using Whatman 42 filter paper and diluted to 50 ml with double-deionized water and the absorbance of the samples was read directly on the Atomic Absorption

Spectrophotometer (AAS). For each element a calibration curve was obtained for concentration vs absorbance for the suitable concentration series. Working standard solutions of calcium (Ca), potassium (K), iron (Fe), zinc (Zn) and magnesium (Mg) were prepared from stock standard solution (1000 ppm), in 2N HNO₃ and absorbance was read for standard solution of each element and samples using atomic absorption spectrophotometer (AAS). The samples were diluted as necessary and the concentrations were taken for all the samples. A blank reading was also taken and necessary corrections were made during the calculation of concentration of various elements.

2.4. Crude protein content analysis

The crude protein content of the samples was estimated by macro-Kjeldhal method (Kjeldhal system- Tecator 2006 Digester and Tecator 1002 distillation unit). 0.2 g of the dried leaves was digested with 10 mL of Conc. Sulfuric acid

and the catalyst mixture for 30 min. at 420 °C. The digested material was distilled after addition of alkali. The release of ammonia was collected in 4% boric acid. The resultant boric acid which now contained the ammonia released was then titrated against 0.1 N HCl. The percentages of nitrogen were converted to protein by multiplying by 6.25.

3. STATISTICAL ANALYSIS

Nested classification was used as the experimental model for analysis of all the experimental data of microelements and protein. Protein and micronutrient comparison were carried out based on districts and locations using SAS system for windows V9.1 (SAS Institute Inc. NC. USA). Statistical analyses of data on micronutrients and total protein for location based district comparison (Anuradhapura, Galle, Hambantota, Jaffna, Kandy, Kurunegala, Matara and Polonnaruwa) were analyzed using General Linear Model (GLM) procedure. Probability value was taken as 0.05. Turkey's Honest Significant Different Test (THSD) was used to carry out the mean comparison.

4. RESULTS & DISCUSSION

According to data for mineral analysis, highest Fe content of 26.99±12.0 mg/100 g was observed in leaves from Polonnaruwa district which was significantly higher ($p<0.05$) compared to other districts. According to the research done in Punjab, Pakistan by Aslam *et al*, 2005, [31] Fe contents in *Moringa* leaves varied as 20.5±1.52 mg/100 g to 57.3±5.64 mg/100 g and values were rather higher than the data obtained in current study.

Leaves from Kurunegala district had the second highest Fe content (13.09±6.93 mg/100 g) which was significantly higher ($p<0.05$) than the other six districts (Anuradhapura, Galle, Hambantota, Jaffna, Kandy and Matara). When the Zn content was considered, (Table.1) Polonnaruwa, Kurunegala and Galle districts had shown significantly ($p<0.05$) higher values (4.21±0.97 mg/100 g, 4.97±2.68 mg/100 g and 4.41±0.54 mg/100 g). Study done by Aslam *et al*, 2005 [31] again showed higher Zn content (10.9± 0.2 mg/100 g) than this study. K content was significantly high ($p<0.05$) in Polonnaruwa and Jaffna districts compared to other districts. The values were 2428.00±476.12 mg/100 g and 2215.87±186.96 mg/100 g, respectively (Table.1). When the present data was compared with study done by Yameogo *et al*, 2011[32] the values for potassium were almost same. (1973.2±6.83 mg/100 g to 2493.7±8.52 mg/100 g). There was no significant difference in calcium content (Table. 2) among studied districts except, Kandy and Galle which had significantly lower ($p<0.05$) content compared to other districts (1300.00±318.26 mg/100 g and 1157.55±218.38 mg/100 g). When consider the Ca content in *Moringa* leaves in the study done in Punjab by Aslam *et al*, 2005 [31] the Ca content values were in the range of 1895.0±6.52 mg/100 g to 3512.6±335.7 mg/100 g). There was a significantly ($p<0.05$) high Mg content in leaves (Table.2) from Kurunegala (818.28±204.95 mg/100 g) compared to other districts, while the leaves from Polonnaruwa had the second highest content (569.71±127.07 mg/100 g) which was significantly ($p<0.05$) different compared to other districts. In study done by Jongrungruangchok *et al*, 2010 [29] Mg content was reported as 500.0 ± 23.5 mg/100 g. When the protein content in leaves (Table.3) was considered, leaves collected from Polonnaruwa had significantly ($p<0.05$) higher values (40.84±0.62 %) compared to Matara, Kurunegala and Kandy districts (30.43±1.24% , 31.68±6.19 and 29.45±1.48%) . However, there was no significant difference in protein content among Polonnaruwa, Anuradhapura, Galle, Hambantota and Jaffna (Table.3).

Table 1: Iron (Fe), Zinc (Zn) and Potassium (K) concentrations of *Moringa oleifera* leaves (Average district value)

District	Mineral Concentration (mg per 100 g)		
	Fe	Zn	K
Anuradhapura	9.11±2.58 ^c	2.99±1.41 ^b	1747.75±254.57 ^d
Galle	5.77±3.37 ^c	4.41±0.54 ^a	1936.87±342.98 ^b
Hambantota	5.95±5.03 ^c	2.68±1.09 ^b	1613.75±198.60 ^d
Jaffna	8.85±2.44 ^c	3.42±2.65 ^b	2215.87±186.96 ^a
Kandy	8.02±4.05 ^c	2.73±2.25 ^c	1607.00±254.57 ^d
Kurunegala	13.09±6.93 ^b	4.97±2.68 ^a	1747.62±204.18 ^c
Matara	6.50±3.12 ^c	0.92±1.09 ^d	1851.62±422.32 ^{bc}
Polonnaruwa	26.99±12.0 ^a	4.21±0.97 ^a	2428.00±476.12 ^a
Mean±SD	10.54±4.05	3.29±0.85	1839.56±291.82

Means followed by the same letter(s) within each column are not significantly different at $p\leq 0.05$ according to least significant difference test.

Table 2: Calcium (Ca) and Magnesium (Mg) concentrations of *Moringa oleifera* leaves (Average district value)

District	Mineral Concentration (mg per 100 g)	
	Ca	Mg
Anuradhapura	1755.04±290.68 ^a	412.13±134.52 ^{cd}
Galle	1157.55±218.38 ^d	471.15±141.57 ^c
Hambantota	1555.76±400.84 ^b	375.21±222.81 ^d
Jaffna	1645.64±289.34 ^{ab}	290.89±133.99 ^f
Kandy	1300.00±318.26 ^c	451.92±112.51 ^c
Kurunegala	1589.83±253.52 ^b	818.28±204.95 ^a
Matara	1579.12±314.44 ^b	331.74±135.24 ^{ef}
Polonnaruwa	1673.37±514.56 ^{ab}	569.71±127.07 ^b
Mean±SD	1532.04±201.00	465.13±66.86

Means followed by the same letter(s) within each column are not significantly different at $p \leq 0.05$ according to least significant difference test.

Table 3: Protein content values of *Moringa oleifera* (average district value)

District	Protein content (%)
Anuradhapura	33.87±2.85 ^{ab}
Galle	35.00±0.49 ^{ab}
Hambantota	34.66±0.24 ^{ab}
Jaffna	33.13±1.80 ^{ab}
Kandy	29.45±1.48 ^b
Kurunrgala	31.68±6.19 ^b
Matara	30.43±1.24 ^b
Polonnaruwa	40.84±0.62 ^a
Mean±SD	33.63±3.52

Means followed by the same letter(s) within each column are not significantly different at $p \leq 0.05$ according to least significant difference test.

These results revealed that the *Moringa* leaves collected from different districts of Sri Lanka contain appreciable amount of micro nutrients and proteins. Proteins and micronutrients in *Moringa* leaves from different districts exhibited wide ranges of variation and variability. These results are in agreement with previous studies done in other countries which showed that the nutrient content of *Moringa* leaves vary according to the area where plants are grown [31-34]. In this study, similar results were observed for other investigated minerals as well proving that the climatic condition and soil condition may affect the nutritional properties of plants [34-37]. Protein content in the leaf samples collected in Sri Lanka (Table.3) was generally high and in the range of 29.45±1.48 % to 40.84±0.62 %, which was higher than the protein content (30.03±0.45%) reported by Moyo *et al.*, 2011 in South Africa [35]. Due to higher protein content *Moringa* leaves could be considered as a good source for protein energy malnutrition in developing countries as they don't have purchasing power to consume animal proteins. Dried *moringa* leaves contain 17 times calcium of milk and 4 times protein of eggs and could be used as a good substitute for popular animal proteins [34].

Among all the districts studied, leaves collected from Polonnaruwa contained significantly higher iron, zinc and protein contents compared to those from the other districts and calcium and magnesium were also present in considerable amounts. Therefore, Polonnaruwa district appears to offer the best environmental conditions for the intensive cultivation of *Moringa* to obtain leaves of high nutritive value.

5. CONCLUSION

M. oleifera leaves from different areas of Sri Lanka contain considerable high range of micronutrients and proteins, and can be successfully used as a food supplement for combating protein and micronutrient malnutrition prevailing in the country at present.

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