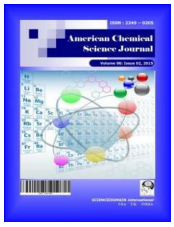

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Vitamin C (L-ascorbic Acid) Content in Different Parts of *Moringa oleifera* Grown in Bangladesh

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Authors' contributions

This work was carried out in collaboration between all authors. Author KSA performed the analysis managed the literature, wrote the first draft of the manuscript. Author RB managed the analysis and literature, wrote the manuscript. Author MHH managed the analysis and performed the statistical analysis. Author IAJ designed the study, wrote the protocol and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Vitamin C is a water soluble organic compound that participates in many biological processes. The objective of the present research is to evaluate the vitamin C contents in different parts of *Moringa oleifera* e.g. tender and matured leaves, flowers and pods grown in Bangladesh. Vitamin C content in fresh samples of six different *M. olifera* plants were determined by the HPLC method. Vitamin C content in the tender leaves of six *M. olifera* was found to be 62.66 to 143.587 mg/100 g, matured leaves contained 51.226 to 150.157 mg/100 g, flower showed 77.502 to 224.672 mg/100 g whereas four weeks aged pods were found to contain vitamin C 3.96 to 8.27 mg/100 g. In this study the vitamin C content in *M. oleifera* flowers was found to be in highest amount and the pods contained the lowest amount of vitamin C compared to the other plant parts. On the other hand

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vitamin C content in matured leaves was observed to be present in higher amount than the tender leaves.
Aims: The present study investigates the vitamin C content in different parts of *Moringa oleifera* grown in Bangladesh. The main object of this work was to see the level of vitamin C content in different parts of the study plant like leaves, flowers and pods in different Moringa tree.
Methodology: Vitamin C content was determined quantitatively by HPLC.
Results: Vitamin C content in the six *M. oleifera* plants was found to be 62.66 to 143.587 mg/100 g for tender leaves, 51.226 to 150.157 mg/100 g for matured leaves, 77.502 to 224.672 mg/100 g for flowers and 3.96 to 8.27 mg/100 g for four weeks aged pods.
Conclusion: It can be said that different parts of *M. oleifera* plants grown in Bangladesh are good sources of the vitamin C.

Keywords: Vitamin C; Moringa oleifera; leaves; flower; Pod; HPLC method.

1. INTRODUCTION

Vitamin C (L-ascorbic acid, ascorbate, AA) is a water soluble organic compound involved in many biological processes. Its major role in the organism is to help in synthesis of collagen and carnitine. AA plays crucial roles in electron transport, hydroxylation reactions and oxidative catabolism of aromatic compounds in animal metabolism [1]. In cells AA reduces hydrogen peroxide (H_2O_2) preserving cells against reactive oxygen species [2-4]. Vitamin C is also required for synthesis of dopamine, nor adrenaline and adrenaline in the nervous system or in the adrenal glands [5] and improves the assimilation of iron.

Moringa oleifera is a highly nutritive multipurpose plant grown for fresh vegetable, livestock fodder, green manure, biogas, medicine, biopesticide, seed production [6], spice and cosmetic oil [7]. It is a small or middle-sized tree, which is known as Drumstick in English and Sajna/Sajina in Bengali. It is widely distributed throughout the tropical region of the world [8]. Moringa leaf extract, being rich in K, Ca, Fe, amino acids, carotenoids, phenols, ascorbate and growth regulating hormones, is an ideal plant growth enhancer [9-11]. Leaves of Moringa are rich in zeatin, which have plant growth promoting capabilities [12], can also be used as natural source of cytokinin [6]. Antioxidants such as ascorbic acid and glutathione, which are found at high concentrations in moringa chloroplasts and other cellular compartments, are crucial for plant defense against oxidative stress [3]. *M. oleifera* has anti-inflammatory, antioxidant, antimicrobial, cardiovascular, antihyperlipidaemic, CNS depressant, antifertility, anticancer, antihepatotoxic, antiulcer, etc properties [7]. That's why it is popularly known as 'The Miracle Tree' [6]. The present research was carried out to evaluate the vitamin C content in different parts of *M. oleifera* grown in Bangladesh by HPLC method.

2. MATERIALS AND METHODS

2.1 Reagents and Chemicals

Metaphosphoric acid (MPA), L-ascorbic acid (AA), orthophosphoric acid, acetonitrile and sodium bi-phosphate were all purchased from Merck, Germany (HPLC-Grade). For chromatographic analysis, de-ionized water of 18 $MOcm^{-1}$ resistivity purified by a milli-Q system (Millipore, Bedford, USA) was used. Ascorbic acid stock standard solution was prepared in water and stored in a glass-stoppered bottle at 4℃ in the dark.

2.2 Plant Materials

Moringa oleifera samples (tender leaves, matured leaves, flowers and pods) were collected from the six different plants of BCSIR Dhaka campus, during January-May 2014. The collected *M. oleifera* samples were cleaned to remove dirt and other impurities. Six trees were designated as T-1, T-2, T-3, T-4, T-5 and T-6.

2.3 Instrumentation

A gradient HPLC (Shimadzu HPLC-20A series) with two LC-20A pumps (Shimadzu), variable wave length programmable photo diode array detector SPD-M20A (Shimadzu), column oven CTO 20A (Shimadzu), system controller CBM-20A (Shimadzu), and reverse phase column Luna 5μ C_{18} (2) Phenomenex (250 mm × 4.6 mm) was used. The HPLC system was equipped with LC-solution software (Shimadzu).

2.4 Extraction of Ascorbic Acid

This procedure is a modification of the method done by Bozan and co-workers [13]. About 10g of *M. Oleifera* fresh samples (tender leaves, matured leaves, flowers or four weeks aged pods) were separately weighed before extraction. After weighting of different samples, extraction was done with 25 mL of extracting solution, containing 5% meta-phosphoric acid (MPA), at 10℃ in the dark. Extraction process was performed using a shaker for 4 hours with continuous shaking. All extractions were carried out in triplicate and obtained solutions were then filtered and stored at 4℃ before analysis. The injection of each extract into HPLC system was performed twice.

2.5 Standard Solutions

Calibration curve for AA was obtained by plotting the AA peak area against the AA concentration at 6 levels (Fig. 1). The response of AA over a concentration range of 12.5–100 mg/L was linear (y = 1.0024x – 0.0391) with a regression coefficient (r^2) of 0.998. The limit of quantitation (LOQ) and detection (LOD) were 0.20 and 0.05 mg/L, respectively, which was taken as the amount of ascorbic acid giving a signal-to-noise ratio greater than 3. The RSD values for repeatability and intraday reproducibility (n ¼ 6) for a standard solution containing 25 mg/L of AA were 1.24% and 7.20%, respectively. For study of intra-day reproducibility the standard solution was kept at 4℃ and in the dark.

2.6 HPLC Analysis

The liquid chromatographic method used for the determination of L-ascorbic acid (AA) consisted of an isocratic elution procedure with UV-Visible detection at 245 nm. Separations were carried out on a reverse phase column Luna C_{18} (2) (Phenomenex, USA). The mobile phase employed was a mixture of 0.5% NaH_2PO_4 and acetonitrile (93:7). Flow rate of the mobile phase was 1.2 mL/min and an injection volume of 20 µL was used in quantitative analysis. The temperature of analytical column was kept constant at 25℃. The calibration curve and quantitative evaluations were accomplished at 245 nm. Standard solutions and extracts were filtered through a prefilter and then a 0.45 mm Millipore membrane before the injection.

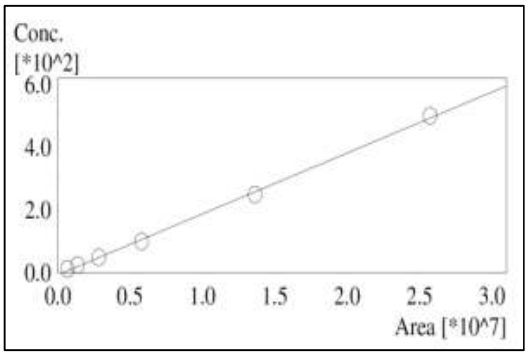

Fig. 1. Calibration curve of standard ascorbic acid

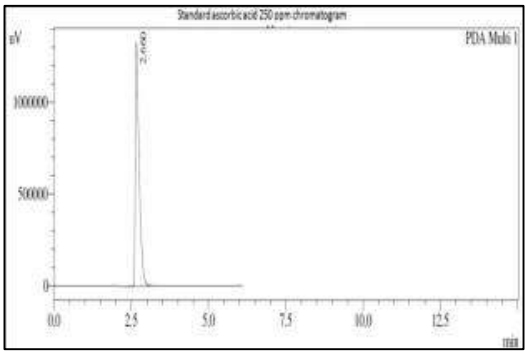

Fig. 2. HPLC Chromatogram of standard ascorbic acid (250 ppm)

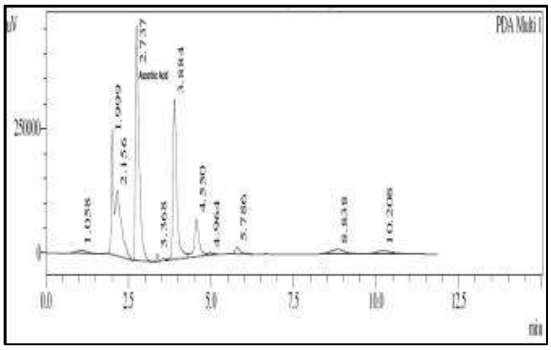

Fig. 3. HPLC chromatogram for ascorbic acid analysis of *M. oleifera* flower, tree-6

To prevent the loss of AA, standard solutions and extracted samples were protected from light using amber flasks. Quantitation was performed by comparing the chromatographic peak area with that of the external standard (Figs. 2 and 3). The calibration curve was plotted in the concentration range of 12.5–100 mg/L and based on a 6-point calibration (equation of the standard curve and r^2 value).

3. RESULTS

3.1 Quantification of AA in Samples

Table 1 shows ascorbic acid (AA) content in tender leaves, matured leaves, flowers and four week aged pods of *M. oleifera*. The range of AA content in the investigated six plants parts were found to be 62.66 to 143.587 mg/100 g for tender leaves, 51.226 to 150.157 mg/100 g for matured leaves, 77.502 to 224.672 mg/100 g for flowers and 3.96 to 8.27 mg/100g for four week aged pods by HPLC method.

4. DISCUSSION

For maintaining a good and sound health and for prevention from common cold, human body should be kept saturated with vitamin C. Numerous analysis have shown that an adequate intake of vitamin C is effective in lowering the risk of developing cancers of the breast, cervix, colon, rectum, lung, mouth, prostate and stomach [14,15,16]. A wide variety of food exists that contains vitamin C. Fruits, vegetables, and organ meats are generally the best sources of ascorbic acid; muscle, meats and most seeds do not contain significant amounts of ascorbic acid [17]. Primates and several other mammals including human are not able to biosynthesize ascorbic acid via glucuronic acid because of the mutation in pseudogene coding for enzyme (L-gulonolactone oxidase) required for this very pathway. Hence, humans and other primates have lost their ability to synthesize vitamin C [18]. Thus, vitamin C should be obtained through diet. Vitamin C is heat and light liable and generally non-toxic.

M. oleifera is quite capable of meeting the RDAs of vitamin C for all age groups (Table 2). The present study reveals that only 15-25 g of fresh leaves are sufficient to meet demand of daily vitamin C requirement for children. On other hand 50-75 g of fresh leaves will be needed for the teens and adults. Thus vitamin C can be obtained very easily form *M. oleifera.*

It is hypothesized that the leaf extract from *M. oleifera* having numerous mineral nutrients and vitamins in a naturally balanced composition, may be an attractive source of antioxidant compounds.

The differences in ascorbic acid contents might result from the variations in species, variety, ecological factors, and harvest time [20]. It is well known that ascorbic acid is a sensitive and thermo-labile compound, so its extraction with high yield and without any decomposition has been a matter of difficulty and labor-intensive for the analysts.

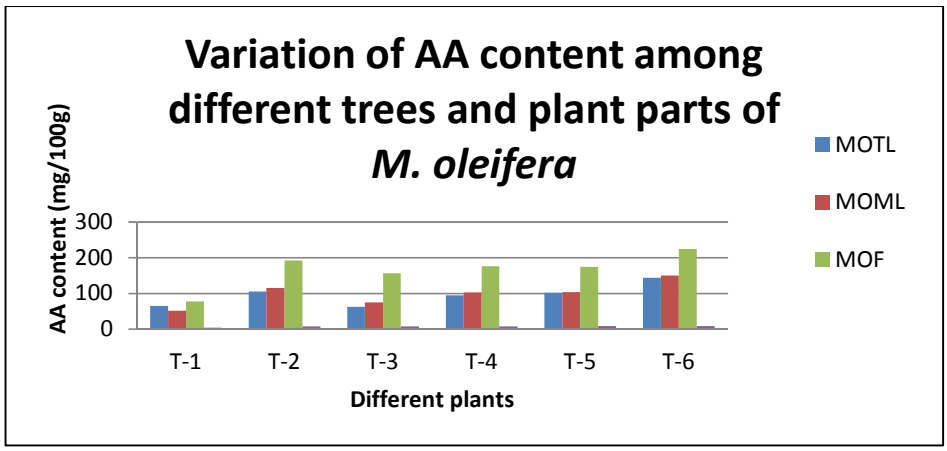

Fig. 4. Comparison of AA contents in different parts of six different *M. oleifera* plants
MOTL= M. oleifera tender leaves, MOML= M. oleifera matured leaves, MOF = M. oleifera flowers and MOP = M. oleifera Pods. T_1-T_6= Plants 1-6

Table 1. Content of ascorbic acid (AA) in different parts of *M. oleifera* (mg/100 g) samples

No.	MOTL	MOML	MOF	MOP 4 weeks
T-1	65.105	51.226	77.502	3.960
T-2	105.967	115.729	192.842	7.261
T-3	62.660	74.909	156.368	7.792
T-4	94.281	102.867	176.828	7.923
T-5	102.079	104.225	174.933	8.05
T-6	143.587	150.157	224.672	8.27

MOTL= M. oleifera tender leaves, MOML= M. oleifera matured leaves, MOF = M. oleifera flower, MOP = M. oleifera Pod

Table 2. Recommended Dietary Allowances (RDAs) for vitamin C [19]

Life Stage	Age	Male	Female	Pregnancy	Lactation
Birth	0–6 months	40 mg	40 mg	-	-
Infants	7–12 months	50 mg	50 mg	-	-
Children	1–3 years	15 mg	15 mg	-	-
	4–8 years	25 mg	25 mg	-	-
	9–13 years	45 mg	45 mg	-	-
Teens	14–18 years	75 mg	65 mg	80 mg	115 mg
Adults	19+ years	90 mg	75 mg	85 mg	120 mg

5. CONCLUSION

Vitamin C is important to human health and a necessary dietary source. However, it is very delicate and easily destroyed by many factors, particularly heat and light. Fresh *M. oleifera* samples showed vitamin C content 62.66 to 143.587 mg/100 g for tender leaves, 51.226 to 150.157 mg/100 g for matured leaves, 77.502 to 224.672 mg/100 g for flower and 3.96 to 8.27 mg/100g for four week pods. It can be concluded that vitamin C content is higher in flower compared to other plant parts. It has been observed that different Moringa plants of the same species contain different amounts of vitamin C. So standardization of different Moringa plants and its different parts is necessary.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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