COMPARATIVE ANALYSIS OF THE NUTRITIONAL COMPONENTS OF DIFFERENT PARTS OF *MORINGA OLEIFERA* FROM KARACHI, PAKISTAN

S. Khan[a*], R. Amin [a], S. Ali [a], T. F. Zeb[a], M.S. Haider[b] and O. M. Tarar[b]

[a] Dow College of Biotechnology, Dow University of Health Sciences, Ojha Campus, Karachi, Pakistan
[b] Food & Marine Resources Research Centre, PCSIR Laboratories Complex, Karachi, Pakistan
[*]Corresponding author's Email: sadaf.khan@duhs.edu.pk

ABSTRACT: *Moringa oleifera* has been reported as super food owing to its high nutritional value which has largely been explored in leaves with limited reports from other parts. Therefore, comparative study was designed to determine the nutritional components of different parts of *M. oelifera* found in Karachi, Pakistan. Samples were collected from multiple parts of *M. oleifera* plant. Proximate, minerals and Vitamin C analysis was carried out as per Association of Official Analytical Chemists (AOAC) methods. Amino acid contents were estimated using Amino Acid Analyzer. The protein content (g/100g dry weight basis) was high in the leaves with stalks and leaves samples compared to seedpods and stem. Caloric contents were in the order of leaves>leaves with stalk>seedpods>stem. The contents of different minerals (Ca, Fe and K) and Vitamin C varied from parts to parts with leaves showed comparatively high amount of Vitamin C and calcium. Total amino acid contents of different parts were in the order of leaves>flowers>seedpods. In conclusion, all the studied parts of *M. oleifera* have nutritional significance. However, leaves and seed pods due to their comparatively higher nutritional value can be more beneficial as dietary supplement.

Key words: *Moringa oleifera*, Nutrients, Amino acid composition, Leaves, Vitamin C.

INTRODUCTION

Moringa oleifera (*M. oleifera*) is one of the most widely distributed species of family Moringacea. The tree is indigenous to sub-Himalayan regions of Indo-Pakistan subcontinent and Afghanistan. However, because of its immense health benefits have spread throughout the tropical and sub-tropical regions of the world (Ma, ZF *et al*., 2020; Anwar *et al*., 2007; Fahey *et al*., 2005). *M. oleifera* has several local names, due to its healing power and lifesaving quality; it's often referred to as a "miracle tree". In Pakistan, this plant is grown in almost all areas of the country and locally known by the name of 'Sohanjna' (Anwar *et al*., 2007).

M. oleifera is a multipurpose plant providing nutritional, pharmacological and industrial significance. *M. oleifera* has been in use for thousands of years as a traditional medicine and as food for both animals and humans (Padayachee *et al*., 2020; Gopalakrishnan *et al*., 2016). The medicinal and therapeutic potential of this plant has extensively been reviewed and reported to exhibit anti-diabetic, anti-inflammatory, anti-allergy and diuretic properties (Farooq *et al*., 2012). In addition, the plant has also reported to have bactericidal, fungicidal and immunosuppressive activities (Anwar *et al*., 2007).

All parts of *M. oleifera* (leaves, seeds, seed pods, flowers and roots) are edible and have high nutritional value containing important nutrients, phytochemicals and antioxidants. The leaves and fruits (seed pods) are used as vegetables and in various cuisines indifferent South Asia & African countries (Dhakad *et al*., 2019). Recently, the potential of the plant to combat malnutrition particularly among infants and lactating mothers has been explored in different African countries and have shown promising results (Fuglie, 2005).The plant has also been used to increase the milk production in lactating mothers and to overcome the iron in anemic patients (Fahey *et al*., 2005).

M. oleifera is also considered as a 'super food', for its unique combination of macro and micro nutrients. The nutritional value of the plant can be affected from different factors such as environmental conditions, genetic background, geographical location and mode of cultivation (Moyo*et al*., 2011; Thurber*et al*., 2009). Therefore, variations have been observed in nutritional value of the plant from different parts of the world and these variations necessitate the evaluation of nutritive value of the plant from our ecotype. Moreover, dietary potentials were largely explored for Moringa leaves and information regarding other parts of the plant is lacking. Most of the studies that have been conducted in Pakistan, explored the medicinal properties of the plant. Very limited studies have evaluated the nutritional composition of this plant from Pakistan, and the evaluation is limited to the seeds flour and oil only (Anwar and Rashid, 2007; Anwar *et al*., 2005).

Therefore this study is designed to determine the nutritional profile of the different parts of the indigenous plants. This study aimed to quantitatively determine the nutritional composition (including total protein, amino acids profile, lipids, carbohydrates, fibers, vitamin C and mineral composition) of *M. oleifera* leaves, seed pods and flowers from Karachi, Pakistan. Furthermore, this study also aimed to evaluate the nutritional quality of protein from different parts of the plants based on their essential amino acids composition. This nutritional characterization scientifically provides the empirical evidence for its use and can be helpful in formulation of supplements according to nutrient requirements.

MATERIALS AND METHODS

Plant sample collection and Processing: *M. oleifera* is widely cultivated plant in Pakistan. Samples of fresh Moringa oleifera leaves, leaves with stalk, seedpods, stem, and flowers were collected from mature Moringa trees spread in different residential parts of Karachi, Pakistan. Collected samples were kept in cooled plastic bags during transportation to the lab for further processing and analysis. Samples from different parts were cleaned; grounded and chemical analysis was performed on finely ground homogenous samples.

Macronutrient Analysis: Different parts of Moringa plant including leaves, leaves with stalk, stem and seed pods was evaluated for their macronutrient composition which includes the proximate determination of total Protein, Fats, carbohydrates, dietary fiber, ash & moisture content as per the guidelines of Association of Official Analytical Chemists (AOAC) methods (AOAC, 2012).

Estimation of Moisture and Ash Content: Moisture content was determined by weight loss during drying of the samples. The ash content was estimated by incineration of the dried samples at 550-600^{o}C until all organic material is burned out (AOAC, 2012).

Total Fat Estimation: The fat contents were determined by AOAC recognized Soxhelt method (AOAC, 2012). Total fat was determined by continuous fat extraction using petroleum ether as a solvent, performed on dried samples in a Soxhlet apparatus.

Protein Determination: Protein contents were determined by Kjeldhal method (AOAC, 2012). Samples from different parts were acid digested and then distilled in a digestion and distillation unit respectively. Total nitrogen was measured by titration, and was used to calculate crude protein by multiplying the evaluated nitrogen by 6.25 [Protein (%) = N x 6.25].

Estimation of Dietary Fiber and Carbohydrate contents: Total dietary fiber content was analyzed according to the AOAC method 993.21 (AOAC, 2012). The contents of carbohydrates available in the sample were determined by the difference method by subtracting the sum of moisture, ash, protein, lipid and total dietary fiber percentages from 100 (Hart and Fisher, 1971).

Estimation of Energy Value: The caloric value of each part of Moringa was estimated in kcal/100g by multiplying the amount of protein, fat and carbohydrates with the energy conversion factors of 4, 9 and 4 respectively and summing up the products (Paul, 1988).

Micronutrient Analysis: Micronutrients (mineral contents and vitamin C) levels were determined in samples from *M. oleifera* leaves, leaves with stalk, stem and seed pods.

Calcium (Ca), potassium (K) and iron (Fe) contents of samples from *M. oleifera* were measured by atomic absorption spectrophotometry according to the AOAC official methods (AOAC, 2012).Total Vitamin C (or Ascorbic acid) levels were determined using automated LC system. Separation was carried out on C18 column, and detection was by UV-VIS detector set at 248nm (Brause *et al*., 2003).

Analysis of Amino acid composition and determination of Protein quality: Amino acid composition of the protein in *M. oleifera* leaves, seed pods and flowers was quantitatively determined as described previously (Haider *et al*., 2015). Briefly, the samples were hydrolyzed with acid under vacuum, evaporated to dryness in rotary evaporator and then amino acids were measured using Shimadzu Amino Acid Analyzer with post column derivatization system and florescence detector.

The percentage of the essential and non-essential amino acid was calculated to determine the quality of the protein.

Data Analysis: All experiments were done in duplicates and results are presented as mean value. Data analysis was performed using Microsoft Excel 2010.

RESULTS

Nutritional characterization of *M. oleifera:* Macronutrient analysis of different parts of *M. oleifera* is presented in Table-1. The mean protein content (%) was ranged from 10.08 to 26.3 in samples from various parts of *M. oleifera*, with stem contain the lowest and leaves along with stalk contain the highest protein content. Moisture content of 75.5, 72.2, 63.5 and 79.6 g/100g (wet weight basis) were found in collected fresh leaves, leaves with stalk, stem and seed pods samples respectively. The most predominant nutrient found in different parts were carbohydrates whereas fat was least abundant, these nutrients accounts for approximately 49% and 4.2 % of the total sample respectively.

The caloric and ash contents of the different parts of the plant were in the order of leaves>leaves with stalk>seedpods>stem.

Vitamin and mineral contents of different parts of *M. oleifera* is shown in Table-2. Variability was observed in micronutrient contents from different parts of *M. olifera* including leaves, leaves with stalk, stem and seed pods. Samples from leaves with stalk and seed pods contained higher calcium and potassium levels respectively as compared to other parts of *M. oleifera*.

Amino acids profile and Protein quality of *M. oleifera:* The amino acid composition and contents of *M. oleifera* leaves, seed pods and flowers is presented in Table-3. Total amino acid contents of different parts werein the order of leaves>flowers>seedpods. *M. oleifera* leaves were found to contain higher contents of total aromatic and non-polar (hydrophobic) amino acids, the total contents were 1.044 and 1.961 g/100g respectively. While seed pods contain both sulphur amino acids (Cysteine and Methionine), and their total contents were 0.267 g/100g. Comparison of the total essential and non-essential amino acid composition in different parts is shown in Figure 1. All the nine essential amino acids were found to be present in the three analyzed parts of *M. oleifera*. The total essential amino acid contents of leaves, flowers and seed pods were 2.513, 1.5 and 1.084 g/100g, accounting for 40.3, 37.5 and 35.0 % of their total amino acid contents respectively (Figure 1). Total non-essential amino acid contents were higher in seed pods, followed by flowers and leaves.Table-1: Macronutrients estimation of *Moringa Oleifera* leaves, leaves with stalk, stem and seed pods from Karachi (Pakistan), expressed as dry matter.

Nutrients	**Concentration**	**Leaves**	**Leaves with Stalk**	**Stem**	**Seed Pods**
Fat	g/100g	3.31	2.52	1.21	1.52
Protein	g/100g	17.35	26.32	10.08	13.77
Crude fiber	g/100g	12.53	16.50	51.04	29.81
Carbohydrates	g/100g	54.00	44.91	28.16	48.45
Calories	Kcal/100g	302.04	294.86	156.0	251.0
Ash	g/100g	12.61	7.80	9.51	6.44

Concentration values represent the means of duplicate measurements

Table-2: Micronutrients content of *Moringa oleifera* leaves, leaves with stalk, stem and seed pods from Karachi (Pakistan), expressed as dry matter.

Micronutrients	**Concentration**	**Leaves**	**Leaves with Stalk**	**Stem**	**Seed Pods**
Vitamin C	mg/100g	127.51	60.30	4.77	18.25
Calcium (Ca)	mg/100g	1685.71	2398.42	1123.29	295.0
Potassium (K)	mg/100g	245.71	171.88	296.16	1146.0
Iron (Fe)	mg100g	15.51	39.19	42.19	NA

Concentration values represent the means of duplicate measurements
Abbreviation: NA=Not analyzed

Table-3: Amino acid composition of *Moringa oleifera* leaves, seed pods and flowers.

Amino acids*	**Leaves (g/100g)**	**Seed Pods (g/100g)**	**Flowers (g/100g)**
Alanine (Ala)	0.411	0.146	0.227
Arginine (Arg)	0.508	0.000	0.341
Aspartic acid (Asp)	0.632	0.311	0.419
Cysteine (Cys)	0.000	0.250	0.000
Glutamic acid (Glu)	1.048	0.859	0.806
Glycine (Gly)	0.233	0.076	0.138
Histidine (His)	0.089	0.010	0.015
Isoleucine (Ile)	0.093	0.025	0.062
Leucine (Leu)	0.621	0.250	0.379
Lysine (Lys)	0.429	0.428	0.333
Metheionine (Met)	0.091	0.017	0.062
Phenyl alanine (Phe)	0.455	0.126	0.228
Proline (Pro)	0.302	0.092	0.159

Threonine (Thr)	0.249	0.110	0.158
Tryptophan (Trp)	0.276	0.037	0.135
Tyrosine (Tyr)	0.313	0.099	0.192
Serine (Ser)	0.277	0.177	0.222
Valine (Val)	0.210	0.081	0.128
Aromatic amino acids (Phe+Tyr+Trp)	1.044	0.262	0.555
Negatively charged amino acids (Asp+Glu)	1.68	1.17	1.225
Positively charged amino acids (Lys+Arg+His)	1.026	0.438	0.689
Nonpolar, aliphatic amino acids (Gly+Ala+Pro+Val+Leu+Ile+Met)	1.961	0.687	1.155
Polar uncharged amino acids (Ser+Thr+Cys)	0.526	0.537	0.38
Total Branched chain amino acids(Leu+Ile)	0.714	0.275	0.441
Total Sulphur amino acids (Cys+Met)	0.091	0.267	0.062
Total Essential Amino acids (His+Ile+Leu+Lys+Met+Phe+Thr+Trp+Val)	2.513	1.084	1.5
Total Non-Essential Amino acids (Ala+Arg+Asp+Cys+Glu+Gly+Pro+Ser+Tyr)	3.724	2.01	2.504
Total amino acids	**6.237**	**3.094**	**4.004**

Result represents g/100g of fresh sample
*Note: Asparagine (Asn) and Glutamine (Gln): Not determined

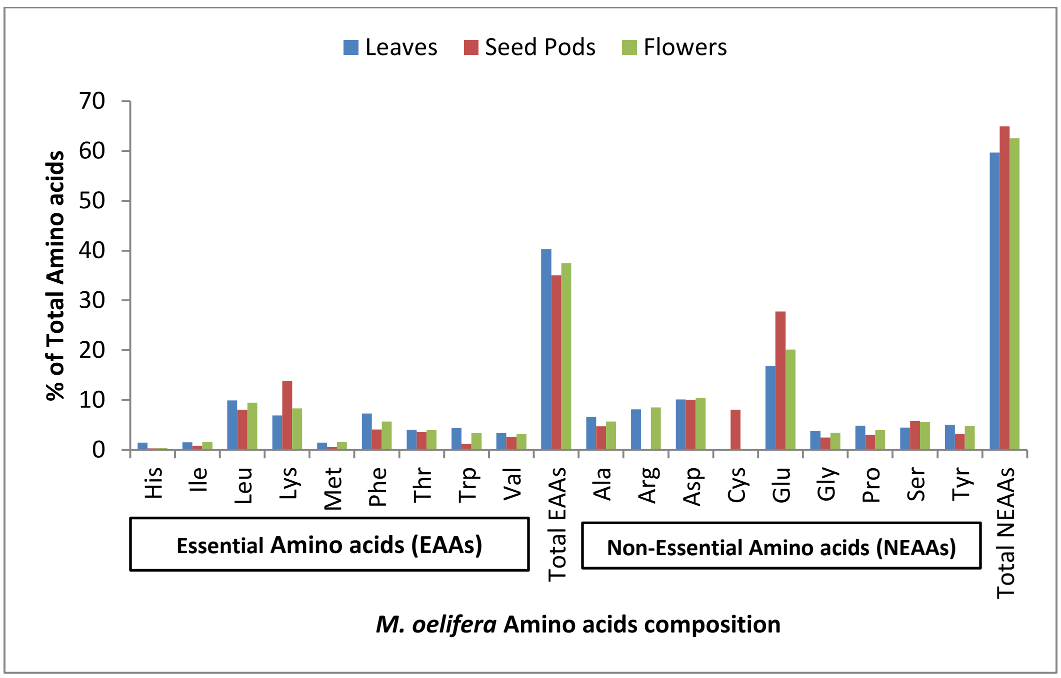

Figure-1: Percentage (%) composition of nutritionally essential and non-essential amino acids of *Moringa oleifera* leaves, seed pods and flowers.

DISCUSSION

M. oleifera is an important food source in many parts of the world. This edible plant is good source of essential nutrients including proteins, vitamins, minerals and antioxidants. Globally, nutritional evaluation of Moringa is reported from different countries. However, considerable variations have been seen among the nutritional value of this plant due to different agro-climatic factors (Anwar *et al*., 2005; Moyo *et al*., 2011). Very limited studies have evaluated the nutritional composition of this plant from Pakistan, since the utilization of *M. oleifera* as a dietary supplement in the country is increasing current study was designed to evaluate the nutritional value of different parts of this plant.

The nutritional profile (proximate, mineral and amino acid compositions) of different parts of *M. oleifera* including leaves, leaves with stalk, stem seed pods and flowers was determined. Proximate analysis carried out to assess moisture, ash, fat, protein, dietary fiber and carbohydrates contents of the samples.

Food moisture has been reported to contribute around 20-30% of the total water intake, moreover the positive relationship between hydration and health strengthen the significance of food moisture contribution in the guidelines for water intake (Guelinckx *et al*., 2016; Popkin *et al*., 2010). Samples from different parts of *Moringa oleifera* showed comparatively higher moisture contents (wet weight basis) in seeds pods and leaves than stem. Results were in accordance with previous studies that have analyzed the moisture content of fresh Moringa leaves samples (Ali *et al*., 2017; Witt, 2013). The good moisture content of fresh Moringa plant is comparable to most of the predominantly consumed Asian vegetables (Ullah *et al*., 2017; Yang *et al*., 2006). Moreover, food moisture content also determines its keeping quality. Studies determining the effect of different drying treatments on nutrient profile of Moringa leaves showed that the fresh Moringa leaves contained 4-11% more moisture than dried leaves, indicating that protein concentrates from different parts may have shorter shelf life at room temperature (Ali *et al*., 2017;Foline*et al*., 2011).

Dietary fiber has several health benefits such as improving laxation, lowering the risk of diabetes and coronary heart disease and help in maintaining the healthy body weight and composition (Lalas *et al*., 2017). The dietary fiber of all parts of Moringa is within the reported literature values (8.5-30.9%) (Melesse, 2011; Moyo *et al*., 2011; Sánchez-Machado *et al*., 2010). The carbohydrates content which accounts for the nonstructural sugar and starch was found comparatively lower in stem part. However, carbohydrate content of *M .oleifera* leaves, leaves with stalk and seed pods were higher than the reported range (27.0-38.4%) (Melesse, 2011; Sánchez-Machado *et al*., 2010) The dietary fiber and carbohydrate contents observed in *M. oleifera* leaves (100g) can provide the recommended daily requirements of these nutrients (Table 2005; Witt, 2013).

Food ash content is the index of inorganic elements and mineral contents such as calcium, sodium, and potassium in edible plant samples. The percent ash compositions in different parts of Moringa samples ranged from 6.44% in seed pods to 12.61% in leaves. Ash content were present in the order of Leaves>Stem>Leaves with stalk>SeedPods. The total ash content of the leaves was within the previously reported limits (8–15%) (Melesse, 2011; Moyo *et al*., 2011; Sánchez-Machado *et al*., 2010). Similarly, the leaf content of Ca, appeared comparable with the limits reported in the literature (1,440–3,650 mg/100g DM), while the Fe and K content was lower (Lalas *et al*., 2017, Witt, 2013). Calcium is an important multifunctional mineral required for the development and health of teeth and bones and preventing them from osteoporosis. The calcium content observed in *M. oleifera* leaves (100g) can provide more than 100% of dietary reference intakes (DRI) requirement of calcium for healthy adult individuals (Institute of Medicine, 1997). Ascorbic acid or Vitamin C act as an antioxidant in the body and are involved in proper functioning of immune system. When compared with the literature, current values were found slightly lower than the reported limits (172-203 mg/100g) (Lalas *et al*., 2017; Witt, 2013). The differences observed in micronutrient contents may be due to low soil nutrients (Anwar *et al*., 2005). However, all studied parts of Moringa contain Vitamin C with stem containing the lowest amount of this vitamin. This indicates that *M. oleifera* leaves can be a good source of vitamin C and minerals particularly calcium in human diet (Padayachee *et al*., 2020).

Protein is an essential macronutrient required not only for growth and maintenance of good health, it is also a source of energy providing around 10-15% of body dietary energy (Alain MuneMune *et al*., 2016; Lopez and Mohiuddin, 2020). The Moringa leaves with stalk were found to contained high protein content followed by leaves and seed pods, stem had comparatively lesser amount of least protein. Since most of the published literature is based on nutritional characterization of leaves and very few studies on other parts comparisons with previous were mainly made with leaves contents. Different studies have reported a range of around 16-32g/100g protein in Moringa dried leaves (Ali *et al*., 2017; Witt, 2013). Protein content found in leaves samples were comparable to the limits reported by previous studies (Ali *et al*., 2017; Moyo *et al*., 2011; Witt, 2013). Stem samples showed comparatively higher protein contents compared to previous reports (Gopalakrishnan *et al*., 2016).

Moringa has also been reported to have high quality protein, which is determined by its digestibility and amino acid composition (Ma, ZF *et al*., 2020; Alain MuneMune *et al*., 2016; Gopalakrishnan *et al*., 2016). Amino acids which are building units of proteins are classified as nutritionally essential and non-essential and perform important functions in the body (Lopez and Mohiuddin, 2020). In this study, various parts of Moringa including leaves flower and seed pods contained all the nine dietary essential amino acids (Table-3) and were comparable in amount with non-essential amino acids. Moreover, the contents and pattern of essential amino acids observed in this study were comparable to the previous reports (Alain MuneMune *et al*., 2016; Lalas *et al*., 2017; Okereke and Akaninwor 2013; Sánchez-Machado *et al*., 2010). The presence of all the essential amino acids in sufficient amounts reflects the high quality of the Moringa protein, and also highlights that Moringa can be a good source of essential amino acids.

Conclusion: In conclusion, the results of the study showed the nutritional potential of different parts of *M. oleifera* particularly the high nutritional value of leaves and seedpods. Thus, suggesting them as potential candidate for formulation of nutritional supplements. The consumption of leaves and seedpods as dietary supplement could prevent mal-nutrition and certain micronutrient deficiencies particularly in developing countries like Pakistan.

Competing Interests: The authors report that there are no conflicts of interest.

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