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Proximate and Mineral Compositions, Bioactive Compounds and Total Antioxidant Capacity of Different Parts of *Moringa oleifera* Lam

ADGL Dilruni[1], DC Abeysinghe[1], RM Dharmadasa[2]* and GA Prathapasinghe[2]

[1]Department of Plantation Management, Faculty of Agriculture and Plantation Management, Wayamba, Sri Lanka University of Sri Lanka, Makandura, Gonawila (NWP), Sri Lanka

[2]Industrial Technology Institute, Baudhaloka Mawatha, Colombo, Sri Lanka

***Corresponding Author**: RM Dharmadasa, Industrial Technology Institute, Baudhaloka Mawatha, Colombo, Sri Lanka.

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Abstract

Background and Objectives: *Moringa oleifera* Lam., popularly known as drum stick tree or horseradish tree, is a member of a family, Moringaceae. The present work is a comprehensive study that evaluates proximate and mineral compositions, phenolic compounds and antioxidant capacity of various parts (leaf, bark, root, flower, seed, immature pod and semi-mature pod) of *M. oleifera.*

Method: Proximate composition was determined according official AOAC method. Mineral composition, Total Antioxidant Capacity (TAC) and Total Phenolic Content (TPC) were determined using Atomic Absorbance Spectrophotometric method, Ferric Reducing Antioxidant Power (FRAP) assay and Folin-Ciocalteu methods respectively.

Results: Significantly higher protein content (24.66 ± 0.27%) was observed in leaves than bark and root whereas the highest fiber content was recorded in roots (46.07 ± 0.64%). High calcium content was found in all parts of *M. oleifera* when compared with other tested minerals. Our results revealed that flower extracts had the highest TAC (60.26 ± 2.69 mg TE/g DW) followed by leaf, root, immature pod, bark, seed and semi-mature pod. The highest phenolic content was also found in flower (11.44 ± 0.33 mg GAE/g DW) and a positive correlation ($R2 = 0.94$, $p < 0.001$) between phenolic compounds and antioxidant activities of *M. oleifera* extracts was observed.

Conclusion: Due to the presence of high nutrients, minerals, secondary metabolites and antioxidant activity, all parts of *M. oleifera* could be effectively used for elimination of malnutrition and production of pharmaceuticals and nutraceuticals.

Keywords: *Antioxidant Capacity; Mineral Composition; Moringa oleifera; Phenolics; Proximate Composition*

Introduction

Moringa oleifera Lam. (Moringaceae) is one of the best known and most widely distributed in tropical and subtropical climates in Sub Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan [1]. Since *Moringa oleifera* is a fast growing, well adopted plant it has been cultivated in all over the world due to its multiple utilities [2]. Moreover, almost all parts of the plant such as the leaves, roots, seeds, barks, fruits, flowers and immature pods contain rich nutritional values and therapeutic properties, it has been exhibited anti-inflammatory antimicrobial, antioxidant, anticancer, cardiovascular, hepatoprotective, anti-ulcer, diuretic, antiurolithiatic, circulatory stimulants, possess antitumor, antipyretic, antiepileptic, antispasmodic, antihypertensive, cholesterol lowering, antidiabetic, antibacterial and antifungal activities [3,4]. Based on its multiple biological activities, it has been widely used in Ayurveda and traditional systems of medicines

for the treatment of an array of ailments [5,7]. In many regions of Africa, it is widely consumed for self-medication by patients affected by diabetes, hypertension, or HIV/AIDS [8-10]. Also leaves contain trace elements that are essential to human health including magnesium, iron, selenium and zinc play an important role in metabolism and interest in these elements are increasing together with reports relating trace element status and oxidative diseases. Moreover, *M. oleifera* leaf has been used as an alternative food source to combat malnutrition, especially among children and infants [6]. Even though *M. oleifera* possess several benefits, systematic information on nutrients, phyto-chemical contents and therapeutically active molecules in different present in different maturity stages of leaf and pods are scattered Objective of the Study.

Present study was undertaken to determine the proximate and mineral compositions, Total Antioxidant Capacity (TAC), Total Phenolic Content (TPC), of different parts of *Moringa oleifera* plant.

Materials and Methods

Chemicals and reagents

Folin-Ciocalteu reagent, Gallic acid, Ferric chloride ($FeCl_3$ $6H_2O$) 2, 4, 6-trypyridyl-2-try-azine (TPTZ), 6-hydro xy-2, 5, 7, 8-tetramethyl-chroman-2 carboxylic acid (Trolox) were purchased from Sigma Aldrich Chemical Co. (St. Louis, Mo). All other chemicals used were of analytical grade.

Plant materials

Different plant parts (leaf, bark, root, flower, seed, immature pod and semi-mature pod) of *Moringa oleifera* plants were collected from Western Province in Sri Lanka.

Preparation of samples

Collected samples of *M. oleifera* were cut into small pieces and air dried for three days at room temperature (28 ± 2°C). Then samples were powdered using mortar and pestle and sieved with 0.25 mm mesh. Powdered sample (0.1g) was mixed with 5 mL of 80% methanol vortexed for 15 minutes. Then it was placed in a water bath at 60°C for 40 min and vortexed at 10 minutes intervals. After centrifugation at 4,000 rpm for 5 minutes, the supernatant was decanted into a 15 mL centrifuge tube and remaining was re-extracted with 5 mL of 80% methanol. Supernatants were pooled and stored at -20°C prior to chemical analysis.

Determination of proximate composition

The proximate analysis (Fibre, ash, fat and protein contents) of all the samples were determined. The crude fiber content was determined using fibre analyser [11]. The ash content was determined using previously published [12]. The fat content of the samples was determined using the Soxhlet extraction method [12]. The crude protein content was determined using the Kjeldahl method [12].

Determination of mineral composition

Mineral contents were determined by using an Atomic Absorbance Spectrophotometer (icetm 3000 series Thermo Scientific, U.S.A). Concentrated HNO_3 (10 mL) was added to 0.5g of the sample in a reaction tube. The dispersion was digested in a digestion block MARS 6 Microwave digestion unit (Model-MARS 6 240/50) for 1 hr. After cooling, the digested products were diluted to 50 mL with DI water. The clear solution was taken for mineral determination.

Quantification of total phenolic

The total phenolic contents of different plant parts of *M. oleifera* were determined using a modified Folin-Ciocalteu method [13]. Briefly, 0.5 mL of appropriately diluted samples or a standard solution of gallic acid was added to a 15 mL centrifuge tubes containing 4 mL of distilled water. Folin-Ciocalteu phenol reagent (0.5 mL) was added to the mixture and mixed by shaking. After 3 min, 1 mL of saturated sodium carbonate was added with mixing and incubated for 2 hr at 30°C. The absorbance relative to that of a prepared blank was

read at 760 nm using a spectrophotometer (Shimadzu, UV Mini 1240, Japan). The total phenolic content was expressed in mg of gallic acid equivalents (GAE) per g dry weight (DW) as standard.

Determination of total antioxidant capacity (TAC)

TAC was determined using the FRAP assay described by Benzie and Strain with slight modifications. Briefly, the FRAP reagent was freshly prepared by mixing 25 mL of 300 mM Sodium Acetate buffer (pH 3.6), 2.5 mL of 10 mM TPTZ solution and 2.5 mL of 20 mM Ferric Chloride solution. The absorbance at 593 nm was read 4 min after mixing of 100 µL of sample extraction with 900 µL of FRAP reagent, using spectrophotometer (Shimadzu, UV Mini 1240, Japan). The measurement was compared to a standard curve of prepared trolox solutions and expressed as mg trolox equivalents (TE)/g dry weight (DW) of samples.

Statistical analysis

To verify the statistical significance of all parameters the values of means ± SD were calculated. Statistical comparison of mean values was performed by General Linear Model (GLM) of ANOVA followed by Tukey Multiple Range Test using SAS. The *P* values of less than 0.05 were adopted as statistically significant.

Results and Discussion

Proximate and mineral composition

In the present study, phytochemical contents and antioxidant capacity of different parts of *M. oleifera* at different maturity stages were conducted. Results demonstrated that, nutrient contents were well distributed in all tested parts of the plant. As demonstrated in table 1, significantly higher protein content (24.66 ± 0.27%) was observed in *M. oleifera* leaves compared to bark and root. Observed results of higher protein content in leaves is in agreement with previous findings [14-16]. Moreover, the significantly higher total fibre content and calcium content were observed in roots followed by bark and leaves respectively. Our results of fibre and magnesium contents of different parts, of *M. oleifera* are in-line with results obtained [16] (Table 1).

Plant Part	Nutrient content (mg/g)				
	Crude protein (%)	Crude fibre (%)	Ca	Mg	Fe
Leaf	24.66 ± 0.27^{a}	20.99 ± 1.00^{b}	41.92 ± 1.0^{a}	15.55 ± 0.8^{a}	1.06 ± 0.2^{b}
Bark	7.65 ± 0.18^{b}	22.95 ± 0.98^{b}	43.82 ± 1.3^{a}	15.00 ± 1.0^{a}	1.61 ± 0.1^{b}
Root	6.19 ± 0.13^{c}	46.07 ± 0.64^{a}	42.43 ± 0.5^{a}	14.76 ± 1.2^{a}	3.27 ± 0.4^{a}

Table 1: *Crude protein, crude fibre, Ca, Mg and Fe contents of different parts of M. oleifera. Means denoted by the same letters in a column represent non-significant differences ($p < 0.05$).*

Total antioxidant capacity (TAC) and total phenolic contents (TPC)

Total antioxidant capacity and total phenolic contents play an important role in human metabolism. As exhibited in table 2, significantly higher content of TAC was observed in flowers followed by leaves and roots. The order of increase of TAC was flower > leaf > root > immature pods > bark > seeds > mature pods. The TPC variation also displayed an approximately similar pattern except the TAC in mature pods. The order of increase was flower > leaf > root > immature pods > mature pods > seeds > bark. Our results are comparable with Vyas., *et al*. [17], who reported ascending order of antioxidant activity of different tissues of *M. oleifera* as flower>leaf>root> gum>bark>seed. The highest TAC of flower might be due to composed of antioxidant compounds such as anthocyanin, reductions etc. in flowers. On the other hand, leaf having longer life span in comparison with other plant parts and being site of energy production faces high magnitude of oxidative damage, therefore, requires greater production of antioxidants and their capacity to act as a protection [18].

Plant Part	TAC (mg TE/g DW)	TPC (mg GAE/g DW)
Leaves	53.19 ± 2.69[b]	10.07 ± 0.21[b]
Barks	5.26 ± 0.11[d]	3.19 ± 0.04[f]
Roots	38.38 ± 1.15[c]	8.36 ± 0.4[c]
Flowers	60.26 ± 2.69[a]	11.44 ± 0.33[a]
Seeds	3.70 ± 0.19[d]	4.40 ± 0.35[e]
Immature pods	6.58 ± 0.19[d]	6.20 ± 0.19[d]
Mature pods	2.51 ± 0.27[d]	4.06 ± 0.47[ef]

Table 2: *Total Antioxidant Capacity (TAC) and Total Phenolic Contents (TPC) of Different Parts of M. oleifrea. Means Denoted by the same letters in a column represent non-significant differences ($p < 0.05$); TAC: Total Antioxidant Capacity; TPC: Total Phenolic Contents; TE: Trolox Equivalent; GAE: Gallic Acid Equivalent; DW: Dry Weight.*

Significantly the highest TPC was recorded in flower extracts (11.44 ± 0.33 mg GAE/g DW) of *M. oleifera* followed by leaf and root. Bark extract of *M. oleifera* had the lowest TPC (3.19 ± 0.04 mg GAE/g DW). Phenolic compounds and TAC showed strong positive correlation ($R^2 = 0.94$, $p < 0.001$), indicating that polyphenols may be due to their hydroxyl groups, were the major contributors to antioxidant activity of the methanolic extracts of *M. oleifera* (Figure 1). A positive correlation between phenolic compounds and antioxidant activities of *M. oleifera* extracts was also reported by Vyas., *et al* [17].

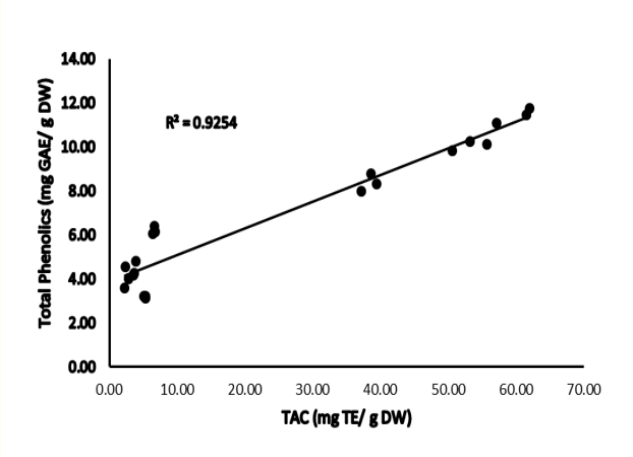

Figure 1: *The Correlation between Total Antioxidant Capacity (TAC) and Total Phenolics of M. oleifera extracts.*

Conclusion

According to the findings of the present study, all the tested plant parts contained marked amounts of phytochemicals and antioxidant capacity. The results of the current study also scientifically validate the traditional claims of extensively use of leaf, flower and immature pods of *M. oleifera* for cooking purposes. Due to presence of high nutrients, minerals, secondary metabolites and antioxidant actives, all parts of *M. oleifera* could be effectively used for elimination of malnutrition and production of pharmaceuticals and nutraceuticals.

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