Experimental exploration of minerals in *Moringa oleifera* leaves as a potential dietary supplement

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Abstract
Moringa oleifera is a botanical marvel, rich in minerals. In plants, minerals are present in the form of different complexes making it difficult to extract them effectively. A comparison of the mineral composition of leaves and widely used leaf extracts has been reported. Experimentation has also been carried out to concentrate the minerals present in Moringa leaves via incineration. The determination of minerals was performed on a Flame atomic absorption spectrophotometer (AAS). The mineral contents of the extracts prepared were not much greater than those of the leaf powder indicating that the traditional extraction method is not beneficial for maximum mineral retention. The ash values and mineral contents of the crude ash of the leaves are significantly high and can be utilized for human and animal nutrition via dietary supplementation.

Additionally, no traces of heavy metals are detected in the samples. The investigations presented here may bridge the gap in our understanding of the extraction of essential minerals from Moringa leaves. Further research is needed to explore ways for dietary interventions to utilize the maximum benefits of the rich mineral content of leaves.

Keywords: Ash, leaves, mineral, moringa, spectroscopy.

Introduction

Moringa oleifera, commonly referred to as the "drumstick tree" or "horseradish tree," is a botanical marvel, rich in minerals. This plant, native to the Indian subcontinent, has attracted the attention of scientists, nutritionists and health enthusiasts for its remarkable nutritional properties[25]. The plant has been utilized since 150 BC for various health purposes. Owing to its nutritional and pharmacological properties, *M. oleifera* is the most commonly used species among the 13 cultivars of Moringa[11]. It has been proven to possess antioxidant, anti-inflammatory, antifungal and antimicrobial properties. It also protects against cardiovascular diseases, liver diseases and DNA damage[2,7,12,21,24,26,28].

Almost all parts of the tree of *M. oleifera* contain nutrients and minerals in good amounts, however, its leaves are widely consumed for their nutritional properties. It has been estimated that dried plant leaves contain 9 times more protein than yogurt does, 10 times more vitamin A than carrot does, 7 times more vitamin C than orange does, 25 times more iron than spinach does, 17 times more calcium than milk does and 15 times more potassium than banana does[14,29]. Minerals are fundamental to human health and contribute to various physiological functions and processes. Data available on the mineral composition of different parts of the *M. oleifera* plant suggest that Moringa's leaves, in particular, are rich and impressive sources of mineral concentrations[6,14,23]. Therefore, it has great potential for use as a potential dietary supplement.

The leaves of the plant are consumed in the form of either dried leaf powder or leaf extract for nutrient supplementation. Various studies have reported the mineral contents of the dried leaf powder and only a few have focused on the mineral composition of leaf extracts which are widely utilized[1,6,10,14,15,18-20,23,29]. Through this experiment, we aimed to elucidate the intricate mineral composition of the dried leaf powder and different extracts of *M. oleifera*. Additionally, an attempt has been made to accumulate the minerals in a concentrated form by incinerating the dried leaves into crude ashes. Heavy metal analysis was performed to determine the safety of human beings.

Material and Methods

Procurement of plant material: The mature leaves of *Moringa oleifera* were collected from Aval Poondurai in the Erode district in Tamil Nadu. The collected leaf samples were authenticated by the National Institute of Science Communication and Policy Research (CSIR). The voucher specimen was kept in the Herbarium for future reference (MDU/PhSc/phcog/2022/101).

Instrumentation: The analysis was performed on a Flame atomic absorption spectrophotometer (AA-6880F) with an air-ethylene flame at a specific burner height[14]. Different known concentrations of the standard solutions of the elements of interest [iron (Fe), calcium (Ca), magnesium (Mg), zinc (Zn), copper (Cu), lead (Pb), cadmium (Cd) and arsenic (As)] were prepared and fed to the AAS for plotting of the calibration curves. All the measurements were carried out in triplicate. The data obtained from the calibration curves of the elements are provided in table 1.

Preparation of samples: The collected leaves were washed with water and then dried under shade. The dried leaves were preserved in air-tight containers for further processing to prepare different samples for evaluation. A total of four samples were prepared for the analysis of the mineral content of moringa leaves. *Moringa oleifera* sample 1

(MOS1) was simply prepared by grinding the dried leaves into a powder. *Moringa oleifera* sample 2 (MOS2) was prepared by macerating the leaves with distilled water. The extract obtained was concentrated via a rotary evaporator at 45°C and stored for evaluation. *Moringa oleifera* sample 3 (MOS3) was prepared by macerating the leaves with 70% ethanol. The hydroalcoholic extract thus obtained was concentrated via a rotary evaporator at 45°C and stored for evaluation. *Moringa oleifera* sample 4 (MOS4) was prepared by incinerating the dried leaves in an incinerator at 400°C. The ash thus obtained was stored for evaluation.

The total ash value was determined on the basis of methods prescribed in 'The Ayurvedic Pharmacopoeia of India' by the Ministry of Ayush, Government of India[32].

Elemental analysis: The elements were analyzed on the basis of the official methods of the AOAC-Association of Official Analytical Chemists[5]. All the samples were digested separately by treating 0.20 g portions of each sample with concentrated nitric acid. The samples were heated until brown-colored fumes disappeared and became transparent. The remainder was filtered and the volume was adjusted to 100 ml with Milli-Q purified water. The prepared samples were then analyzed via an Atomic absorption spectrophotometer (AAS) and all the measurements were recorded in triplicate.

Results and Discussion

The ash content determines the purity and quality of a drug. The proximate analysis of the crude ash revealed a total ash value of 12.076%. The results of mineral analysis of different samples are provided in table 2. The analysis reported the following concentrations of minerals (mg/100g) in the samples MOS1, MOS2, MOS3 and MOS4: calcium (Ca) (3553.2, 3441.8, 3614, 15129.3), magnesium (Mg) (414.96, 416.7, 431.66, 2315.7), iron (Fe) (232.08, 243.9, 268.1, 2480.54), zinc (Zn) (10.47, 11.32, 15.31, 104.31) and copper (Cu) (1.3,1.2,1.6,10.2). Heavy metal analysis revealed that lead (Pb), cadmium (Cd) and arsenic (As) were below the detection limit in all the four samples.

In the present study, calcium was present in abundance in all the samples followed by magnesium and iron. Zinc and copper are present in trace amounts. The concentration of minerals reported in the study is remarkably high. The mineral composition of MOS1 is in agreement with the range of minerals present in dried leaf powder of Moringa reported by Mashamaite et al[13].

Owon et al[23] reported high amounts of Mg (382 mg/100 g) and Zn (6.45 mg/100 g) in dried leaf powder of *M. oleifera*. Al-Juhaimi et al[4] reported similar values for Mg (381.13 mg/100 g) and Cu (0.841 mg/100 g), but the values of Fe (127.4 mg/100 g) and Ca (1763.841 mg/100 g) were lower than the estimated values. El-Massry et al[8] reported remarkably high amounts of Fe (277.6 mg/100 g) and Mg (403.6 mg/100 g). Other mineral compositions, such as Ca (2078.98 mg/100 g), Zn (5.43 mg/100 g) and Cu (0.76 mg/100 g) were also similar to those in the present work. Gopalakrishnan et al[9] reported similar values for the mineral composition in moringa leaves. However, the values reported by Asekunowo et al[6] were much lower than those in the present study.

Table 1
Characteristic data of calibration curves of different elements

Element	Calibration equation	Correlation coefficient	Wavelength (nm)
Ca	y = 0.017418x + 0.018862	0.9933	422.7
Mg	y = 0.0044801x + 0.052222	0.9942	285.2
Fe	y = 0.066836x – 0.0031250	0.9961	248.3
Zn	y = 0.23393x + 0.10919	0.9888	213.9
Cu	y = 0.070154x + 0.25268	0.9860	324.8
Pb	y = 0.021015x – 0.0034391	0.9997	283.3
Cd	y = 0.21564x + 0.057662	0.9944	228.8
As	y = 0.0070109x – 0.0019070	0.9995	193.7

Table 2
Mineral compositions of different samples

Element	MOS1	MOS2	MOS3	MOS4
Ca	3553.2	3441.8	3614.8	15129.3
Mg	414.96	416.7	431.66	2315.7
Fe	232.08	243.9	268.1	2480.54
Zn	10.47	11.32	15.31	104.31
Cu	1.3	1.2	1.6	10.2

All the values are expressed in mg/100g.

In the present research, the amount of calcium reported was greater than that reported in other studies but in agreement with the range of 1932 - 3914 mg/100 g reported by Nouman et al[18] in a study of mineral composition in seven different cultivars of *M. oleifera* in different parts of the world. This could be due to the variations in the soil, climate and cultivation practices used. A comparison of mineral compositions of MOS2 and MOS3 revealed that the aqueous extract (MOS2) has a slightly lower concentration of minerals than the hydroalcoholic extract (MOS3). The amount of minerals in the extracts is in agreement with that reported in the study by Nkechinyere et al[17]. Moringa leaf extracts are widely employed in the prevention and treatment of various diseases related to nutrition such as anemia[16,27,30,31].

Despite the wide use of extracts, no other studies in the literature have reported the mineral content of Moringa leaf extract. An analysis of the results obtained in the present work revealed that methods such as maceration, which are widely applicable for the extraction of phytochemicals, are not very effective in the extraction of minerals. The low extractability of minerals may be because, in plants, minerals are generally present in the form of various complexes whose bonds are sufficiently strong not to be broken by simple extraction processes. For example, iron is translocated in plants as ferric citrate and in upper oxidation states such as cytochrome, ferredoxin, ferritin etc.[3] An amount of calcium is present in the form of calcium oxalate crystals in leaves, which are insoluble in water and ethanol[33]. Therefore, the extracted minerals may get reduced during the extraction procedure.

Ooi et al[22] suggested that a high ash content corresponds to a high mineral content. The reported total ash value of 12.076% of Moringa leaves is significantly high and is correlated with that reported by Mubeena et al[15] (12.78%). Therefore, efforts have been made to determine the mineral content of the crude ash of Moringa leaves. The results of the MOS4 analysis reveal the high amount of minerals present in the crude ash. The heavy metal content in all four samples is below the detectable limit which indicates that the samples are devoid of any toxic elements. Thus, crude ash of *M. oleifera* might be utilized as a source of human and animal nutrition providing greater amounts of minerals through dietary supplementation. However, further research is needed to optimize the extraction methods for maximum retention of minerals. A comprehensive analysis of the mineral content of Moringa might be useful for future researchers to gain a deeper understanding of its potential for dietary supplementation and its role in promoting human health. This understanding holds the key in realizing the full potential of Moringa, not only in fortifying human diets.

Conclusion

This study presented an attempt to determine the mineral contents of crude leaf ash and proposed that it should be utilized for human and animal nutrition purposes via dietary supplementation. The distribution of minerals across moringa leaves, leaf extracts and leaf ash revealed interesting variations. These variations have significant implications for dietary supplementation and the potential health benefits associated with consuming Moringa.

The distribution and extractability of these minerals within moringa leaves provide valuable insights into the nutritional value and potential health benefits associated with their consumption. Further research can focus on optimizing extraction methods for Moringa leaves to maximize mineral retention and exploring specific dietary applications to address mineral deficiencies in different populations.

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