Quantitative analysis of the nutritional components in leaves and seeds of the Persian *Moringa peregrina* (Forssk.) Fiori

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ABSTRACT

Background: *Moringa peregrina (M. peregrina)* is an important tropical tree recognized for its nutritional and medicinal properties. The objective of this study was to investigate the nutritional component in the leaves and seeds of the Persian *M. peregrina* (Forssk.) Fiori. **Materials and Methods:** The *M. peregrina* leaves and seeds of wild cultivated trees were collected from the areas of arid environment located in the South-East of the Iran. The leaves and seeds of *M. peregrina* were dried and grounded to a fine powder and kept in dark for the day of experiment. The acidic digested leaves and seeds were analyzed for Vitamins C and A, calcium, and potassium using atomic adsorption and flame emission spectrophotometer. **Results:** The analytical data revealed that the leaves and seeds of the Persian *M. peregrina* (Forssk.) Fiori contain sufficient amounts of Vitamin C: 83 ± 0.5 and 14 ± 0.6 mg/100 g/DW; and Vitamin A: 6.8 ± 0.7 and 24.8 ± 0.7 mg/100 g/DW, respectively. The elemental analysis in the leaves and seeds showed that the calcium content are 764.8 ± 1.6 and 1164.8 ± 43.4 mg/100 g/DW and for potassium content are 900.2 ± 14 and 572 ± 10 mg/100 g/DW, respectively. **Conclusions:** The nutritional characteristics of the Persian *M. peregrina* (Forssk.) Fiori, investigated in this study revealed that, daily use of leaves and seeds of this plant could significantly provide the recommended dietary allowance for the Vitamins C and A, and minerals, such as calcium and potassium.

Key words: Calcium, *Moringa peregrine*, Potassium, Recommended dietary allowance, Vitamin A

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INTRODUCTION

Moringa peregrina (*M. peregrina*) belongs to the family of flowering plant *Moringaceae* that have only one genus called *Moringa,* which contains 14 species from tropical and subtropical climates.[1,2] The *M. peregrina* and *M. oleifera (M. oleifera)* are the most popular between them. The *M. peregrina* seed oil has antimicrobial activity.[3] It has straight trunk, 3–10 m high, and white bark with high aridity adaptation. The bipinnate leaves are 30–40 cm long, the axes persistent, and deciduous leaflets. Flowers appear before leaves in May. Flowers are pentamerous, zygomorphic, hermaphrodite, and pinkish-white with white sepals. This tree bears, silique similar to pod, cylindrical fruits with 20–40 cm length. Seeds are un-winged, triquetrous, and 25 mm in length and 10–12 mm in diameter.[4] The *M. peregrina* flowering and fruiting time is in the months of February to April. The geographical distribution of *Moringa* is in tropical and non-tropical climate. Therefore, *Moringa* is adaptable to a wide range of environmental conditions from hot and dry to hot, humid, and wet conditions. The tree produces a tuberous tap root, which explains its tolerance to drought conditions. Originally *M. peregrina* is a desert species and considered a tree of hot semi-arid regions. In Iran *M. peregrina* is distributed in West and Central parts of Baluchestan Province. The vernacular names are Gas-e-Rooghani and Gaz Rokh.[4,5] The *Moringa* plant has been consumed by humans throughout the century in diverse culinary ways.[4] Almost all parts of the plant are used culturally for its nutritional value,

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purported medicinal properties and for taste, and flavor as a vegetable and seed. The leaves of *M. oleifera* can be eaten fresh and cooked.[5] The leaves contain all essential amino acids and are rich in protein and minerals.[6,7] The *M. peregrina* seed kernel is rich in fixed oil (42–54%) with up to 23% protein. The edible oil is used medicinally as diuretic, rubefacient, and astringent.[7] The seeds of the plants contain edible oil which is used for cooking, frying, and as a salad oil for dressing. Newer applications include the use of *Moringa* powder as a fish food in aqua cultural systems.[8,9] The *Moringa* leaves as a protein supplement are used for animals, like cows. Furthermore, *Moringa* seeds contain active coagulating compounds in the seeds for water treatment.[10,11] The whole seeds can also be eaten green, roasted or powdered. There is medical evidence that the *M. oleifera* has therapeutic and prophylactic properties. The pods and seeds, often referred to as *M. kernels*, have a taste that ranges from sweet to bitter and are most popularly consumed after frying to get a peanut-like taste.[12,13] *M. oleifera* leaf has been purported to be a good source of nutrition and a naturally organic health supplement that can be used in many therapeutic ways.[14,15] Identification of these vitamins would be a great advantage to the nutritional attributes of the *M. peregrina*. It was reported that *M. peregrina* showed anti-hyperglycemic effect of the ethanolic extract from which quercetin, quercetin-3-O-rutinoside, chryseriol-7-O-rhamnoside, and 6, 8, 3', 5'-tetramethoxy apigenin were isolated.[16] The aerial parts of *M. peregrina* yielded lupeol acetate, β-amyrin, α-amyrin, β-sitosterol, β-sitosterol-3-O-glucoside, apigenin, rhamnetin, neochlorogenic acid, rhamnetin-3-O-rutinoside, and 6-methoxy-acacetin-8-C-β-glucoside.[17] Occurrence of isothiocyanates in seed extracts of *M. peregrina* growing in Iran and elsewhere was reported.[7,18] Even though various studies have been done on the *M. oleifera*, there are few experimental studies regarding the potential nutritional value of the *M. peregrina*. These include the total vitamins, mineral, protein, and phenolic content.[14] The ethanolic *M. oleifera* leaf extract could prevent testicular toxicity of rats exposure to high dose of chromium.[19] Although must people in some countries have used the *M. oleifera* plant as food, there is a little evidence about its chemical contents and nutritional characteristics. In a study revealed that *M. oleifera* leaves contain considerable amount of crude protein, whereas nearly 30% of total proteins are digestible.[20] The research conducted in this study seeks to assess the nutritional and medicinal values of *M. peregrina* leaves and seeds collected from the province of Bluchestan/Iran. Our objectives are to quantitatively analyze the Vitamin C, Vitamin A, calcium, and potassium in the leaves and seeds samples to get an overarching idea of the nutritional value of this species located in Iran.

MATERIALS AND METHODS

Sample preparations

The *M. peregrina* leaves and seeds of wild cultivated trees were collected from the city of Nikshahr/Kerman under authorization of the Jahad Keshavarzi and Agriculture Organization of Sistan and Bluchestan Province/Iran from the areas of arid environment. The leaves and seeds of *M. peregrina* (No. 2025) were kept in the Herbarium of Pharmacy Faculty/Isfahan University of Medicine. The leaves and seeds were dried in clean, cool, and dark area at room temperature for 20 days. The dry leaves and seeds of *M. peregrina* were grounded to a fine powder and kept in dark at room temperature for the day of analysis.

Phytochemical tests

Tests for alkaloids

Most alkaloids are precipitated from neutral or slightly acid solution by Mayer's reagent (potassium-mercuric iodide solution), by Wagner's reagent (solution of iodine in potassium iodide). The Mayer's and Wagner's tests were performed to detect the total alkaloids content of *M. peregrina* and compared with *Datura stramonium* alkaloid content.[21-24]

Tests for tannins

The tannin compounds are widely distributed in plants. It plays a role in protection from predation and growth regulation. The ferric chloride ($FeCl_3$) and lead acetate tests were applied to measure the total tannin content of *M. peregrina* and compared with tannins content from the leaves of *Punica.*

Test for cardenolide

Many plants contain sugar-steroid derivatives known as cardenolides. Cardenolide glycosides are often toxic with heart-arresting. The Baljet reaction and the 3,5-dinitrobenzoic acid color reaction with cardenolides were used to clarify the total cardenolides content of *M. peregrina* and compared with the digitalis nervosa L as control.[25-30] The Kodde-reaction was performed in alkaline pH with 2 mL KOH (1N) and 3,5-dinitrobenzoic acid.

Tests for steroids and terpenoids

The Liebermann–Burchard or acetic anhydride test was used for the estimation of cholesterol contents in *M. peregrina*. The formation of a reddish ring at the junction of two layers after a few minutes was taken as positive. The Salkowski's test was also used for semi-quantification of terpenoids content. When concentrated sulfuric acid was added to the chloroform solution of *M. peregrina*, at the lower of the chloroform layer a yellow with deep red color at the junction of two layers were taken as positive terpenoids content and compared with *Glycyrrhiza glabra* L. as control.[31]

Tests for saponins

The powdered sample of leaves and seeds (5 g) were boiled with 20 mL of distilled water and the filtered. The filtered of each sample (10 mL) was mixed with distilled water (5 mL). The frothing was then mixed with three drops of olive oil for the formation of emulsion that was taken positive, if saponins present comparing with *Zizyphus spina-christi* (sedr.).

Test for anthraquinones

Anthraquinones may be detected by the Borntraeger's reactions. 0.2 g of leaves and seeds were added to sulfuric acid solution (5 mL, 2N) and boiled for 2 min. After cooling, 10 mL toluene solvent was added and decanted to stay for 10 min. The yellow color of the toluene layer turns to red in alkaline pH if the anthraquinones present. The *Cassia angustifolia L.* was applied as a positive control.

Test for flavonoids

A few drop of 1% NH_3 or NaOH solution was added to the aqueous extract of each plant sample in a test tube. A yellow coloration was observed if flavonoids compound are present. It should be colorless with a few drop of diluted acid.[32,33]

Chemical analysis

Atomic adsorption/flame emission spectroscopy

Atomic adsorption/flame emission spectroscopy (AA/FES, Shimadzu AA-670) were used for determination of elements in samples after digestion of dry sediment in 7 M HNO_3.

Quantitative analysis of calcium and potassium

The *Moringa* sample (5 g) with concentrated nitric acid (20 mL, 7N) was placed in a small dish and heated slowly to boil for 2 h. After cooling, 10 mL deionized water was carefully added and the mixed boiled for 3 min. After cooling, the samples were filtered with filter paper in volumetric (1000 mL) flasks and then the volume made to 1 L with de-ionized water.[19] The pure potassium chloride and calcium carbonate were incubated in oven (110°C) to dehydrate for 1 h and then transferred to a desiccator. The compounds were dissolved in 1000 mL double distilled water to make a serial concentration of standards, 1 ppm, 2 ppm, 3 ppm, 4 ppm, 5 ppm, and 10 ppm. The *M. peregrina* leaves and seeds were dissolved in HNO_3 (10 mL, 7N) and then diluted 10 times with double distilled water. FES was used for determination of calcium and potassium by standard curves.

Quantitative analysis of Vitamin C

To measure the Vitamin C contents of the samples a standard method was used with a little modification.[25] All analysis was performed in triplicates. Ascorbic acid is highly sensitive to various modes of processing. There are factors that can influence the degradation of Vitamin C.[34] This water soluble compound is extracted from the *M. peregrina* (powdered seeds and leaves 1 g) with acidic solution (water 75 mL and phosphoric acid 25 mL) over a shaker for 1 h. Ascorbic Acid was purchased from Merck (Germany). Ascorbic acid (50 mg) was dissolved in 100 mL of metaphosphoric acid to make a stock solution. 2,4-dinitro hydrazin (10 g) was added to 500 mL of sulfuric acid (4.5 M). Tioreh (5 g) was added to 100 mL of water and anhydrous copper sulfate; (0.6 g) was added to 100 mL of water and stirred to dissolve. Tiore solution (5 mL) and $CuSO_4$ solution (5 mL) were added to 2,4-dinitro hydrazin solution (100 mL) while stirring to get a reagent. All the solutions were sealed and refrigerated before use. A blank, containing only meta-phosphoric acid and the reagent of 2,4-dinitro hydrazin, were similarly prepared. The absorbance of each sample was read at 520 nm. Using the standard stock solution of ascorbic acid, a calibration curve was established of three dilutions across a range of concentrations 0.1–2 g/mL. The stand curve equation was, $y = 0.02356 \times -0.009$ ($r^2 = 0.9988$).

Quantitative analysis of Vitamin A

The experimental methods described by Sebrell, *et al.* were used to measure the Vitamin A contents of the sample.[35] All analysis was done in triplicates. Standard compounds, Vitamin A vial (Darupakhsh, Iran). Vitamin A (50,000 IU/mL, Batch No. 12 OSVE) were dissolved in 330 mL of a mixture of Et-OH: KOH (300–30) to obtain the standard stock solution which was then diluted to make work solutions of five concentration ranging from 500 IU/mL to 5 IU/mL for the standard curve with an equation, $y = 105 \times +0.0123$, $r^2 = 0.991$. After saponification by heating the solution (30 min) and extraction with diethyl ether (3X), the reagent (Antimony tri chloride 10 mL) was added to make solution 100 mL with $CHCL_3$. A blank containing only solutions and the reagent was similarly prepared. The absorbance of each sample was read at 620 nm.

RESULTS

The *M. peregrina* leaves and seeds were collected from the city of Nikshahr/Iran and the plant originality and the specification were confirmed by botanic specialist from the Jahad-e-Keshavarzi and Agriculture Organization of Sistan and Bluchestan Province and by an Isfahan University Pharmacognosy Professor. The vernacular names were Gas-e-Rooghani and Gaz Rokh. The leaves and seeds were dried in clean, cool, and dark area at room temperature to protect vitamins. The dry leaves and seeds of *M. peregrina* were ground to a fine powder and kept in dark at room temperature for the day of analysis. Semi-quantitative

analysis of phytochemicals of *M. peregrina* leaves and seeds are shown in Figure 1. As shown in Figures 2 and 3, the Vitamin C as ascorbic acid in the leaves and seeds are 83 ± 0.5 and 14 ± 0.6 mg/100 g/DW and Vitamin A 6.8 ± 0.7 and 24.8 ± 0.7 mg/100 g/DW, respectively. The compounds of leaves and seeds measured, and the nutritional standards were compared with the Vitamin A and Vitamin C content of carrot, liver, orange, and kiwi per serve. Figures 4 and 5 show the calcium and potassium content of the leaves and seeds which are 764.8 ± 1.6 and 1164.8 ± 43.4 mg/100 g/DW and for potassium content are 900.2 ± 14 and 572 ± 10 mg/100 g/DW, respectively. The nutritional standards as shown in Figure 6 were compared with the calcium and potassium in whole milk and banana per serve.

DISCUSSIONS

The results were expressed for the macronutrients as mg of element per 100 g on a dry weight bases (% g DW). The *Moringa* leaves and seeds samples contained relatively high amounts of examined minerals. As the data shows for these samples, the highest value of calcium was obtained from the seeds in which it was found to be a good source of calcium (1.16 g/100 g DW). It was reported *M. oleifera* leaves contain calcium (2003 mg/DW) and potassium (1324 mg/g/DW).[35] This research suggests that seeds of *M. peregrina* like leaves of *M. oleifera* were excellent sources for the recommended daily requirements of calcium as shown in Figure 6. Our findings also confirm and support the traditional idea that the use of *Moringa* could be a good source for our diet to provide vitamins and minerals. This plant is safe for human consumption even in a concentrated formula, as traditionally seeds of the plant are eaten in the way peas and seeds are fried or roasted like groundnuts.[7,8] The results from the analysis of compounds showed that the leaves and seeds of *M. peregrina* contain Vitamin C and Vitamin A, with higher amounts of Vitamin C than A. The significant difference in Vitamin C content was observed among plant parts. There was no direct relationship between the values obtained for Vitamin C and Vitamin A in the leaves and seeds. That is clear that, leaves that exhibited high concentrations of Vitamin C did not necessarily have high concentrations of Vitamin A. Although Vitamin A content in *M. peregrina* leaves (6.8 mg/100 g DW) is less than Vitamin A content of *M. oleifera* leaves but *M. peregrina* seeds showed higher concentrations of Vitamin A (24.8 mg/100 g DW) than seeds of *M. oleifera* (18.9 mg/100 g DW).[35,36] The seed kernel is rich in fixed oil and Vitamin A is a soluble in oil. The oil is edible therefore, it is important to note that the *M. peregrina* seeds can serve as a valuable source of Vitamin A.[37] Similarly, *M. oleifera* leaves are a very rich source of nutrients and contain the essential Vitamins A

Phytochemical Tests	Name of Test	Positive Control	Responce of Positive Control	Leaves Test Results	Seeds Test Results
Alkaloids	Mayer's Test	Datura stramonium	Whit-Yallow Percipitation	+++	+++
	Wagner's Test	Datura stramonium	Dark Brown Percipitation	+++	++++
Tannins	Ferric chloride	Punica granatum	Green to Blue Color	Negative	Negative
	Lead Acetate	Punica granatum	White to Yallow Percipitation	+	++
Cardenolides	Baljet's Test	Digitalis nervosa L	Orange Color	Negative	Negative
	Kellar Killani's test	Digitalis nervosa L	Pink Color	Negative	Negative
Steroids	Liebermann–Burchard Test	Glycyrrhiza glabra L	Reddish ring	Negative	Negative
Terpenoids	Salkowski's test	Glycyrrhiza glabra L	The lower layer a yellow color	Negative	Negative
Tests for Saponins	Foam test	Zizyphus Spina Christi (Sedr)	Foam formation	Negative	Negative
Anthraquinones	Borntraeger's test	Cassia angustifolia L	Red color	Negative	Negative
Flavonoids	Flavonoids Test	Glycyrrhiza glabra L	Yeloow color	Negative	Negative

Figure 1: Semi-quantitative analysis of phytochemicals of *Moringa peregrina* leaves and seeds

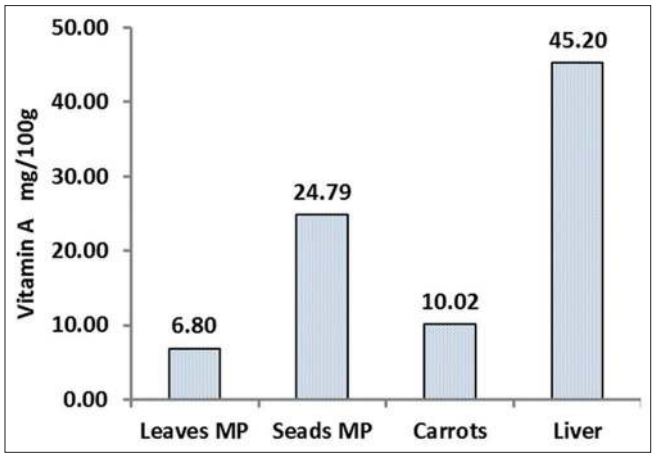

Figure 2: The Vitamin A content in 100 g dry weight of *Moringa peregrina* leaves and seeds. The figure is comparing the Vitamin A content of 100 g of carrot and liver per serve (data taken from FDA and published reports for Vitamin A source reference) with the vitamin contents of *M. peregrina* leaves and seeds

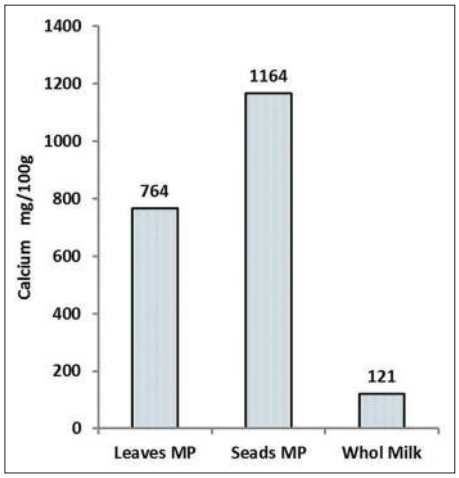

Figure 4: The calcium content in 100 g dry weight of *Moringa peregrina* leaves and seeds. The figure is comparing the calcium content of 100 g of whole milk per serve (data taken from FDA and published reports for calcium source reference) with the calcium contents of *M. peregrina* leaves and seeds

Nutrition component supplied	Recommended dietary allowance (RDA)		Percent of Daily Value(DV)	
	Adults	Child <4	Leaves MP/100g (Percent DV)	Seeds MP/100g (Percent DV)
Calcium	1000mg	200-1000mg	%76.4 Adults % 372 Child	%116.4 Adults %582 Child
Potassium	4700mg	3000mg	%12.2 Adults %19 Child	%19.2 Adults %30 Child
Vitamin A	0.900mg	0.600mg	%755 Adults % 1133 Child	%2666 Adults %4000 Child
Vitamin C	60mg	60mg	%138	%40

Figure 6: The Recommended dietary allowance of various nutrients

and C. Leaves are rich in biologically active carotenoids and Vitamin C have health-promoting potential in maintaining a balanced diet and preventing free-radical damage that can initiate many illnesses.[37] Other reports show fresh

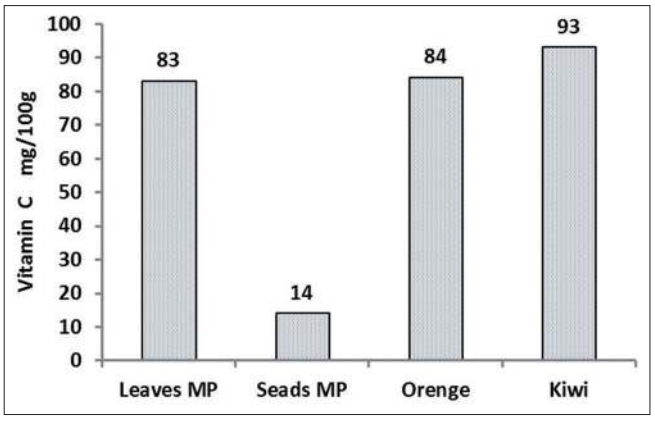

Figure 3: The Vitamin C content in 100 g dry weight of Moringa peregrina leaves and seeds. The figure is comparing the Vitamin C content of 100 g of Orange and Kiwi per serve (data taken from FDA and published reports for vitamin C reference) with the vitamin contents of *M. peregrina* leaves and seeds

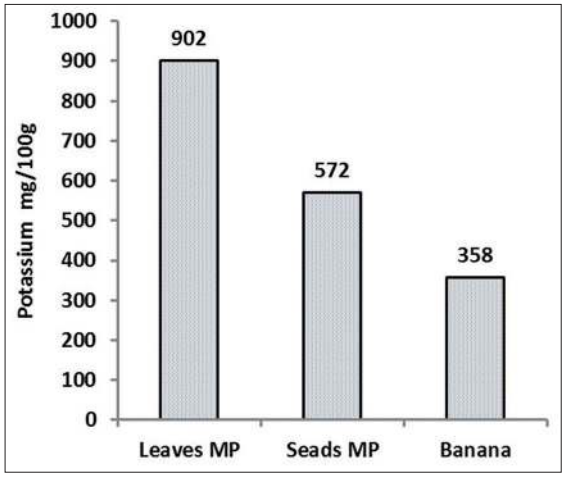

Figure 5: The potassium content in 100 g dry weight of *Moringa peregrina* leaves and seeds. The figure is comparing the potassium content of 100 g of banana per serve (data taken from FDA and published reports for potassium source reference) with the potassium content of *M. peregrina* leaves and seeds

M. oleifera leaves as having high concentrations of ascorbic acid.[38,39] Researchers used fresh leaves. In this research, the dried leaves gave lower concentration of ascorbic acid (83 mg/100 g DW) compared to values as higher as 5500 mg/100 g DW determined from fresh materials.[6] In addition, other reports indicated that the Vitamin C content ranges around 204 mg/100 g of fresh weight.[39,40] Previous research on *M. oleifera* showed, with one fresh *Moringa* leaves contain approximately 220 mg/100 g of ascorbic in the leaves.[14] Studies have shown that ascorbic acid is highly sensitive to a number of factors, including temperature and light. The lack of ascorbic acid in processed food has been explained mostly by heat, oxygen, and light samples.[6,39] Therefore, as ascorbic acid has a very short half-life and the leaves collected in this analysis were dried, the ascorbic acid should be far lower than that which would be found in

fresh tissue. Another reason comes from the variation of species in the *Moringa* plant. To the best of our knowledge, there is no report on the vitamins of *M. peregrina*.

The *M. oleifera* leaves are rich of Vitamin C and phenolic compounds which are responsible for its antioxidant activity.[40,41] Antioxidant systems have evolved to help defend the body against free–radicals, which are commonly produced within the body under normal circumstances. Vitamin C or ascorbic acid acts as an antioxidant that, along with other vitamins, protects the body from oxidative stress, maintains the immune system. In addition, ascorbic acid neutralizes any radicals, damaged lipids, and DNA in the blood that may cause illnesses to emerge.[41] These findings demonstrate that *Moringa* is a potential plant candidate to be used to improve the health and nutrition. Together, the macronutrients and micronutrients provide energy, structure, and regulation which are needed for growth, maintenance, repair, and reproduction.

The results of the present study are consistent with previous finding that the *M. peregrina* (Forssk.) Fiori has valuable nutritional components and could be a complementary food for individuals with vitamins and mineral deficiencies. The bioavailability of the nutrients demands more studies such as digestibility of the proteins and fats and existence of anti-nutrients, like metal chelators (e.g., phytates oxalates), and protease inhibitors. Exploration of these issues could provide a more complete picture of the nutritional significances of the seeds and leaves of *M. peregrina* (Forssk.) Fiori.

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