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Research article

Elemental composition, total fatty acids, soluble sugar content and essential oils of flowers and leaves of *Moringa oleifera* cultivated in Southern Portugal

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ABSTRACT

The evaluation of the elemental content of moringa leaves and flowers by Energy Dispersive X-Ray Fluorescence Spectrometry revealed that the leaves are a good source of some macro (Ca and K) and micronutrients (Mn) beyond the presence of important polyunsaturated fatty acids (PUFAs), essential in human nutrition. Total soluble sugars prevail in the flowers which may be linked to insect attraction and the pollination process. *M. oleifera* leaves, flowers and seeds essential oils (EOs) were isolated by hydrodistillation. Gas chromatography and gas chromatography-mass spectrometry analysis (GC-MS) showed EOs dominated by alkanes and fatty acids in diverse ratios in the analyzed plant parts. The nutritional characterization of *M. oleifera* cultivated in Portugal showed some important nutrients to human physiology. Further studies will allow determining if its consumption may overcome the nutritional imbalances of daily modern households, preventing the emergence of hypertension and diabetes.

1. Introduction

The search for natural products, particularly in developed countries, has created a boom of unconditional supporters who believe that "*what is natural is good*". For example, the production and consumption of algae in Europe in the form of pills, powdered samples or even dried samples of macroalgae, microalgae, and/or the cyanobacteria *Spirulina*, is in agreement with the present EU political priorities favouring the movement towards a sustainable economy and natural resources protection [1]. Nevertheless, an extreme caution is needed regarding the quality of these type of products since they can accumulate some harmful contaminants to human health such as As, Pb, Cd or Hg [2, 3, 4]. Also, several supplements with terrestrial origin might contain some of these elements whose risk to public health is amplified by the often very high daily intake suggested by the manufacturer [5] as seen for Camu-Camu (*Myrciaria dubia*), Goji berries (*Lycium barbarum*), Maca (*Lepidium meyenii*), or even Moringa (*Moringa oleifera*).

Developing regions have a long tradition of using what nature offers in most of the cases to compensate several and profound nutrient imbalances although it must be referred that some of these products contain in fact chemical compounds and mineral elements with useful properties to human health. For example, edible parts of *Moringa stenopetala* and *Moringa oleifera* are commonly used in households in southern Ethiopia and Kenya, respectively, due to their richness in amino acids, vitamins and specially selenium [6]. All *Moringa* growing households in southern Ethiopia and 79% in Kenya used Moringa leaves as food - boiled fresh, and powder used in tea or mixed with other dishes.

M. oleifera has medicinal, nutritional, and socio-economic value in Senegal and Benin, particularly the powdered leaf to treat moderate malnutrition in children. Several medicinal uses of *M. oleifera* leaves were established in Uganda, mainly to treat hypertension and diabetes, which is related with the phytochemicals present such as reducing sugars, flavonoids, anthraquinones, tannins, steroids and triterpenoids, saponins, and alkaloids [7] although the authors emphasize the need to standardize *M. oleifera* leaf use in nutrition and herbal medicine.

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It is well recognized that major nutritional imbalances occur worldwide related with mineral elements such as Fe, Zn, Mg among others, leading to biofortification programs of several crops [8, 9, 10]. Thus, some wild species may contribute to achieve this goal due to its richness of some particular elements as is the case of *Corema album* white berries which are rich in Ca, Fe and Zn [11]. Likewise, *M. oleifera* leaves show a particularly relevant elemental composition in Ca, K and P, plus a protein content that can reach approximately 30% [12]. Other authors mention the high Mg and Se concentrations in *M. oleifera* leaves [13] with average concentrations of 0.5% and 363 mg/kg on a dry weight basis, enhancing the importance of both elements in human nutrition.

The reported carbohydrate content of *M. oleifera* dried leaves and leaf powder was 41.2 and 38.2 g/100 g of plant material, while reaching only 8.67 g in the seeds [14]. In most plants, the major unsaturated fatty acids (UFAs), are the three C18 compounds, oleic (18:1), linoleic (18:2), and α-linolenic (18:3), whose roles are related with biotic and abiotic stresses. Particularly linoleic (18:2), and α-linolenic (18:3) are dietary essential polyunsaturated fatty acids (PUFAs), because humans are incapable of their biosynthesis [15] and some oil crops are rich in these C18 compounds. Using gas chromatography-mass spectrometry, eleven fatty acids were identified and quantified, in the seeds oil of *M. oleifera*, collected in the arid southwestern of Ecuador. From the poly unsaturated fatty acids (PUFAs) detected, oleic was the most abundant, ranging between 72.4 to 75.5% [16]. Also, *M. oleifera* leaf contains high amounts of total monounsaturated fatty acids (MUFA) 4.48%, and total PUFAs 52.2% [13], while the remaining percentage come from total saturated fatty acids (SFA) 43.3%.

Plants produce a great range of secondary metabolites that may be extracted by different, not often comparable, extraction techniques. Whereas vegetable oils are mainly fatty acids rich, the essential oils (EOs) are a part of plant volatiles, usually dominated by terpenes and phenylpropanoids, among others [17]. EOs are only obtained by one of two procedures, by either steam-, hydro- or dry-distillation of any plant part or by expression (a mechanical process without heating) of the epicarp of citrus fruits [18]. EO isolation allows also obtaining an important co-product, the hydrolate [19]. EOs are complex mixtures whose composition is dependent of several factors [20] and that show a wide range of both therapeutic and non-therapeutic uses [17].

Hexacosane (13.9%), pentacosane (13.3%) and heptacosane (11.4%) were the main components, determined by GC and GC-MS, in the EO of *M. oleifera* leaves grown in Mozambique. The EO antimicrobial activity was assayed against two Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*), and two Gram-positive strains (*Bacillus cereus*, *Staphylococcus aureus*), and five fungal strains (*Aspergillus niger* spp., *Penicillium aurantiogriseum*, *P. citrinum*, *P. digitatum*, and *P. expansum*). Only *B. cereus*, *P. aeruginosa*, and all fungal strains showed sensitivity to the leaf EO [21].

The present study aimed to determine the elemental composition (Ca, Cu, Fe, K, Mg, Mn, P, S and Zn) of *M. oleifera* leaves and flowers, their lipidic profile and soluble sugar content, plus the characterization of the essential oils in the same plant organs as well as in the mother seeds, from samples cultivated for the first time in Southern. Portugal.

2. Materials and methods

2.1. Soil characterization, plant material and growth conditions

Moringa oleifera flowers and leaves used in the experiments were obtained from a greenhouse plantation, in Sousel (Portalegre, Portugal).

The greenhouse with 64 m length x 17 m width x 3.5 m high, occupies 1088 m^2 and uses a drip irrigation system. The soil has clay characteristics with pH values ranging between 6.47 and 6.91, electrical conductivity of 243 μS/cm, 3.17% of organic matter and cation exchange capacity of 15.2 $cmol_c$/kg. According to Ferreira [22], the Sousel greenhouse is located in Luvisoils/Cambisoils.

The seeds used in the plantation were purchased in 2017 from a company that produces and sells certified seeds in the region of Matões (Brazil). Sowing occurred in mid-April 2018 and the growth was very fast which forced a first pruning at the end of July 2018, as the specimens were reaching the top of the greenhouse. In August 2018 leaves were harvested for the first elemental analysis. From October onwards, the plants grew more slowly, the leaves began to turn yellow, an obvious symptom of senescence only returning to new shoots in March 2019. From this date, growth continued and at the end of April flowering began which continued until the end of November when leaves and flowers were collected.

2.2. Elemental evaluation by Energy Dispersive X-Ray Fluorescence Spectrometry

All of the samples that were previously dried, were powdered in an agate mortar and pressed under 10 tons in a hydraulic press for 2 min. This procedure results in a cylindrical pellet of 20 mm diameter and 1 mm thick which was glued onto a mylar foil, supported by a plastic frame that is directly placed under the X-ray beam.

Three replicate pellets were fabricated for each sample. The average concentration values and the corresponding standard deviation, for each set of pellets, are presented in Table 1. The X-ray fluorescence spectra were acquired with a custom built tri-axial setup that features a secondary target for polarization of the X-ray tube's radiation. This polarization of the Bremstrahlung radiation at the secondary target and sample, allows the removal of the Compton and Rayleigh scattered radiation thus lowering the limits of detection of the technique [23, 24].

The spectrometer is composed of a Philips PW1400 X-ray tube with a tungsten anode, water cooled, that can reach 100 kV and 80 mA. Although the secondary target can be changed between measurements in order to excite more efficiently the element of interest, in this work a 99.999% pure Mo disc was used. An Ag filter is placed between the X-ray tube's exit window and the secondary target in order to absorb the low energy scattered radiation that contributes only to the background noise. The radiation is collected by a, liquid nitrogen cooled, Si (Li) solid state detector which features a 50 mm2 active area, 8 μm beryllium window and 135 eV resolution at 6.4 keV. Two silver collimators are used to collimate the radiation that reaches the detector in order to restrict the effective area and lower the angular dispersion. Each spectrum was acquired during 1000 s at operating conditions of 50 kV and 20 mA. The

Table 1. Elementar composition of flowers and leaves of *Moringa oleifera*.

Plant organ	Ca*	Cu	S*	Fe	P*	Mn	K*	Zn	Mg
Flower	0.5 ± 0.01	8.4 ± 0.2	1.05 ± 0.02	88.9 ± 3.61	0.6 ± 0.20	30 ± 1.4	3.2 ± 0.12	43 ± 1.9	501 ± 18.5
Leaf[1]	2.1b ± 0.11	7.4a±0.4	0.8b ± 0.02	137a±13.0	0.2b ± 0.02	64b ± 7.1	1.1b ± 0.04	27a ±4.3	257b ± 15.9
Leaf[2]	2.7a±0.17	7.7a±0.3	1.1a ±0.02	142a ±6.40	0.4a ±0.03	96a ±5.1	1.4a ±0.08	32a ±1.9	522a±10.2

* Mean values are expressed in %//Kg ± standard deviation (Ca, S, P and K) and mg/Kg ± standard deviation (Cu, Fe, Mn e Zn). Leaf[1] = August 2018 sampling; Leaf[2] = November 2019 sampling; Mean values not followed by a common letter are significantly different at the 0.05 significance level.

elemental quantification was performed using the built-in software that features the fundamental parameters method.

Detection limits, similar to those referred in references [10, 23] were the following: Ca = 105 mg/kg; Cu = 3 mg/kg; Fe = 6 mg/kg; K = 180 mg/kg; Mn = 9 mg/kg; P = 1000 mg/kg; S = 3500 mg/kg; Zn = 3 mg/kg. Plant reference materials were used for data validation: Bush branched and leaves (GBW 07603), Orchard leaves (NBS 1571) and poplar leaves (GBW 07604); the recovery values ranged between 91% and 104%.

2.3. Magnesium determination by atomic absorption spectrometry

One gram of each dried sample, was placed in a 50 mL Erlenmeyer and submitted to an acid digestion with HNO_3–$HClO_4$ (4:1) according the methods described elsewhere [25, 26]. Two sequential steps were performed - the first one using 10 mL of HNO3, and the second one using 2 mL of HNO_3 plus 3 mL of $HClO_4$. In both steps, samples were digested until total evaporation. The residue (final digestion) was diluted in a 2% HCl solution and filtered into a 50 mL volumetric flask. The standard solution and the blank were also prepared with 2% HCl and then analysed. The concentration of the element was evaluated, using an atomic absorption spectrophotometer (Perkin Elmer AAnalyst 200) fitted with a deuterium background corrector, with the AAWinLab software program.

2.4. Fatty acids analysis

Fatty acids analysis was performed in leaf and flower samples (*ca.* 1 g FW) previously frozen in liquid nitrogen and stored at -80 °C. Samples were boiled in 10 mL MQ water (2 min) to inactivate lipolytic enzymes. Total lipids were extracted using a mixture of chloroform/methanol/water (1/1/1; v/v/v), following the method of Allen et al. [27]. To study fatty acid (FA) composition of total lipids, aliquots of extracts were saponified and methylated with BF_3-methanol (Merck), after addition of a known amount of heptadecanoic acid (C17:0) as an internal standard. Methyl esters were separated by GC-FID (CP-3380, Varian, CA, USA) using a DB-Wax capillary column (0.25 mm *i.d.* x 30 m, 0.25 μm, J&W Scientific) with programmed column temperature (80 °C–200 °C, 12 °C min^{-1}, after 2 min at initial temperature; injector: 200 °C; detector; 250 °C). Hydrogen was used as carrier gas (flow rate of 1 mL min^{-1}, split ratio of 1:100 of the sample), as described previously in Scotti-Campos et al. [28] FAs were identified using fatty acid standards (Sigma). Unsaturation of TFA was estimated through the calculation of double bond index (DBI), according to the formula: DBI = (% monoenes + 2 × % dienes + 3 × % trienes)/% saturated FAs [29].

2.5. Soluble sugars composition

Soluble sugars were extracted from frozen leaf and flowers samples (*ca.* 1 g), according to Damesin & Lelarge [30]. Samples were ground to a fine powder in a mortar using liquid nitrogen and immediately transferred to centrifuge tubes containing 5 mL of cold MQ water. Samples were then homogenised with a vortex (1 min) prior to sonication on ice (1 min), left for 20 min on ice to allow extraction and centrifuged (12 000 g, 5 min, 4 °C). The supernatant was boiled (3 min) to achieve protein denaturation, cooled in ice (6 min) and centrifuged again, as mentioned above. Clear samples were then further purified used nylon filters (0.45 mm). For sugars analysis 50 ml aliquots were injected in a HPLC system coupled to a refractive index detector (Model 2414; Waters, Milford, MA, USA). Sugars separation was performed using a Sugar-Pak 1 column (300 × 6.5 mm; Waters) at 90 °C, with H_2O as eluent (containing 50 mg EDTA-Ca L^{-1} H_2O) at a flow rate of 0.5 mL min^{-1}. Known sugar standards were used for sugars identification and quantification in samples.

2.6. Statistical analysis for fatty acids and sugars

A one-way ANOVA ($p < 0.05$) was applied, using Statistix 9 (Analytical Software, 2009), followed by a Tukey test for mean comparison (95% confidence level).

2.7. Essential oil isolation

The essential oils (EOs) were obtained by hydrodistillation (HD) using a Clevenger apparatus according to the European Pharmacopoeia [31]. The EOs were recovered from the Clevenger apparatus graduated tube by rinsing with in-laboratory distilled *n*-pentane when the distillation procedure was over and allowed to cool (10–15 min). This was accomplished by introducing the distilled *n*-pentane in the filling funnel after flowing out part of the hidrolate of the connecting tube until just below the filling funnel. The residual heat of the distillation flask evaporated the distilled *n*-pentane, which then condensed, and dissolved the volatiles, above the aqueous phase in the graduated tube. The mixture of distilled *n*-pentane and volatiles was then recovered in a vial and concentrated to ≈ 100 μl, using a blow-down evaporator system, at room temperature under nitrogen flux. Until analysis, the volatiles were stored in the dark at −20 °C.

2.8. Essential oil composition analysis

The EOs were analysed by Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS) for component quantification and identification, respectively.

2.9. Gas chromatography (GC)

GC analyses were run in a Perkin Elmer Clarus 400 gas chromatograph with two flame ionization detectors (FIDs), a data handling system and a vaporizing injector port into which two columns of different polarities were installed: a DB-1 fused-silica column (polydimethylsiloxane, 30 m × 0.25 mm i.d., film thickness 0.25 μm; J & W Scientific Inc., Rancho Cordova, CA, USA) and a DB-17HT fused-silica column [(50% phenyl)-methylpolysiloxane, 30 m × 0.25 mm i.d., film thickness 0.15 μm; J & W Scientific Inc.]. Oven temperature was programmed at 3 °C/min from 45 to 175 °C, then at 15 °C/min up to 300 °C, and finally held isothermal for 10 min. The temperatures of the injector and detector were 280 °C and 300 °C, respectively. The carrier gas was hydrogen (30 cm/s). The split sampling technique ratio was 1:50. The injection volume was 0.1 μL of a 1:1 distilled *n*-pentane-essential oil solution. The EOs percentage composition was determined using the normalization method from the GC peak areas, calculated as average of two injections *per* sample, without using the response factors, in accordance to ISO 7609 [32].

2.10. Gas chromatography-mass spectrometry (GC-MS)

A Perkin Elmer Clarus 600 gas chromatograph, equipped with a DB-1 fused-silica column as detailed above, and interfaced with a PerkinElmer 600T mass spectrometer (software version 5.4.2.1617, Perkin Elmer, Shelton, CT, USA) was used in GC-MS analyses. Oven and injector temperatures were as for GC analyses. Transfer line at 280 °C. Carrier gas, helium (30 cm/s). Ion source at 220 °C. Split ratio, 1:40. Ionization energy, 70 eV. Scan range at 40–300 u and scan time of 1 s. Components identity was established by comparing their retention indices, calculated as in ISO 7609 [32], relative to *n*-alkane indices and GC-MS spectra from a lab-made library, created with laboratory-synthesized and isolated components, reference essential oils, and commercially obtainable standards.

3. Results and discussion

3.1. Elemental composition

M. oleifera leaves (collected in August 2018 and November 2019), showed a great variability in the concentration of macronutrients, such as Ca that ranged from 2.1 % to 2.7 %, or P where the concentration

doubled from 2018 to 2019. The concentrations of S and K also increased from 2018 to 2019 although slightly (Table 1).

Micronutrients (Cu, Fe and Zn) showed very small increases, although not significantly different ($P \leq 0.05$) except Mn where the concentration increased from 64 mg/kg in 2018 to 96 mg/kg in the following year (Table 1).

It should be noted that the flowers, only collected in 2019, showed much more P, K, and Zn, than the leaves. Conversely, the leaves had much higher levels of Ca, Fe and Mn. For Cu and S, the maximum contents found in the leaves were 7.7 mg/kg and 1.1%, while the concentrations observed in the flowers were relatively close, *i.e.*, 8.4 mg/kg and 1.05% respectively (Table 1).

The reported data on the elemental analysis of *Moringaás* flowers is scarce. Kumssa et al. [33], reported Ca: 0.3%, Cu: 6.2 mg/kg, Fe: 4.7 mg/kg and Zn: 31.7 mg/kg, while Lanjwani et al. [34], registered 0.2% of Ca, 19.1 mg/kg Cu, 461 mg/kg Fe and 14.6 mg/kg Zn. In the current study higher levels were noted particularly for Ca and Zn *i.e.,* 0.5 % and 43 mg/kg, respectively.

Leaf reported data shows that the levels of Ca may range between 0.5% [35] and 2.6% [5], in plants from Egypt, although several authors pointed out to near 2.0%, regardless the origin [36, 37, 38]. Agamou et al. [39] reported that *Moringa* young leaves from Cameroon may reach a maximum of 1% Ca, while the concentration of Ca in mature leaves ranged between 1.2% and 1.8%. This might explain in part, the variability of the results, although the geographic origin, where soil characteristics are included, and the climatic factors influence decisively the uptake of minerals by the plants [40].

Leaf samples collected in 2018 exhibited 0.2% P, like what was observed in leaves from Uganda [37] and Nigeria [38], although in 2019 the concentration doubled. In the case of K, the concentrations ranged between 1.1% and 1.4%, which are generally in agreement with the levels observed by other authors [5, 37, 38], for samples from Mozambique, Malawi and Nigeria, respectively. The S concentrations reported in this work which ranged from 0.8% in 2018 to 1.1% for the following year's sampling, agreed with the levels observed in samples from Mexico [36].

Copper (Cu) content varied between 7.4 and 7.7 mg/kg, which in general agrees with the data of several authors who reported concentrations always < 10 mg/kg [33, 35, 36, 37]. The foliar Mn contents show great variability. In *M. oleifera* leaves from Mexico an average of 59 mg/kg Mn was observed [36], like what was found in the present work for the 2018 campaign *i.e.*, 64 mg/kg. Nevertheless, in the 2019 sampling the Mn content increased to 96 mg/kg, a concentration relatively close to that reported [37] for samples from Uganda (116 mg/kg).

For Zn, the concentrations in leaves, ranging from 27 to 32 mg/kg (Table 1), agree with those observed by other authors [5, 33, 36]. The Fe content in the leaves is similar regardless the sampling year and the values concur with those referred for similar samples from India and Thailand [37]. The concentration of Mg double from the first to the second year reaching 522 mg/kg, a level clearly lower than the values referred by [14, 33, 36].

As previously mentioned, only Agamou et al. [39] reported differences in elemental composition (macro and micronutrients) between young and adult leaves of *M. oleifera* collected in eight different regions of Cameroon, pointing out that this composition is strongly influenced by location and stage of plant development. Likewise, the change of seasons may be responsible for significant changes in Ca concentration, as was reported for *M. oleifera* leaves collected in Taiwan [41], where Ca content increased from 0.87% in summer to 1.86% in winter.

Young leaves of *M. oleifera* presented higher concentrations of K, P and Zn than the adult leaves, occurring the reverse for Ca, Cu, Fe and Mn. The increase of the micronutrient levels (except Zn) during foliar development is particularly relevant for Fe and Mn with increases from 126 to 207 mg/kg and 32.8–58.6 mg/kg, respectively [39]. Regarding Ca, the levels almost tripled from young to adult leaves, while for the remaining elements the levels observed are more alike [39].

Table 2. Elemental concentration of *Moringa* leaves assuming a daily intake of 5 g and the correspondent percentage of the Recommended Daily Intake.

Element	RDI	Average concentration (mg/g)	Concentration (5 g daily intake)	% of RDI (5 g)	% of RDI (30 g)
Ca	800 mg	24.0 mg	140 mg	17.5	105
Cu	1 mg	0.00755 mg	0.03775 mg	3.78	22.68
Fe	14 mg	0.1395 mg	0.6975 mg	4.98	29.88
K	2000 mg	12.5 mg	62.5 mg	3.12	18.72
Mg	375 mg	0.3895 mg	1.9475 mg	0.52	3.12
Mn	2 mg	0.080 mg	0.4 mg	20.0	120
P	700 mg	0.30 mg	15.0 mg	2.14	12.84
S*	1.0 g	9.5 mg	0.0475 g	4.75	28.5
Zn	10 mg	0.0295 mg	0.1475 mg	1.48	8.88

RDI = Recommended Daily Intake levels adopted by Official Journal of the European Union.
for adults [51]. The RDI for Sulfur was based on Young and Borgonha [50].

M. oleifera leaves seemed to reach a plateau of accumulation of Cu, Zn and Fe which is in line with the adequate concentrations in dry tissues of 6 mg/kg, 20 mg/kg and 100 mg/kg, respectively [42]. Furthermore, the levels observed in 2018 and 2019 were not significantly different ($P \leq 0.05$). Regarding the macronutrients the adequate concentrations are much behind the levels observed in the present study and the concentrations observed are significantly different at the 0.05 significance level.

These apparent contradictory findings in which some elements increase from 2018 to 2019, and others remain more and less stable, does not mean necessarily that we are using young leaves in 2018 and mature leaves in 2019. The plant is in a rapid growing process and the appearance of flowers in 2019 probably require important resources particularly carbohydrates and amino acids and Ca for example is one of the most important elements in the flowering process [43].

As previously referred the variability of the data in the different *Moringa* depends of the geographic origin, where soil and the climatic factors characteristics are included [40]. According the Harmonized World Soil Database from 2009 [44] Cambisoils are predominant in the Mediterranean area covering 26% of the total, while Luvisoils cover 10%, only. Despite Cambisoils are considered productive, in our case they develop on granites, leading to Mg imbalance mainly due to the high levels of Mn in soil solution [45]. Moringa is well adapted to different type of soils although the above-ground biomass of Moringa seedlings was higher in clayey and sandy soils than calcareous and loamy soils [46]. In Saudi Arabia, Moringa is highly tolerant to drought living in a diverse type of soils and pH values (5.0–9.0) but prefers a well-drained sandy loam soil or even a loam soil soils [47] thus indicating that the plant itself has a long adaptative plasticity.

Regarding our soils, the influence of Mn on the content of the *Moringa* leaves was well expressed on the fact that the Mg content of the leaves was scarce compared with other data [14, 33, 36], while the Mn content was within the range observed by several authors [13, 35, 37].

3.2. The elemental contribution to the daily intake

For determining the contribution of *Moringa* leaves to the daily intake, the average concentration of each studied element, considering the two sampling years *i.e.,* August 2018 and November 2019, was determined.

We assume a daily *Moringa* leaf consumption of 5.0g/person. Our choice was based on the results of the interview of fifty average-income families conducted in Nigeria, which concluded that an average culinary preparation contains about 300 g of fresh leaves of *Moringa*

Table 3. Total fatty acids (TFA) (mg g^{-1} DW), relative abundance of individual fatty acids (mol %) and unsaturation index (DBI, *double bond index*) in lipids extracted from leaves and flowers of *Moringa oleifera*.

	Leaves				Flowers			
TFA (mg g^{-1} DW)	1.44	±	0.16	a	1.32	±	0.15	a
C14:0 (mol %)	0.76	±	0.13	a	0.59	±	0.03	a
C16:0 (mol %)	13.33	±	0.68	b	19.30	±	1.56	a
C16:1 (mol %)	0.19	±	0.03	a	0.09	±	0.00	b
C18:0 (mol %)	2.13	±	0.32	b	4.61	±	0.76	a
C18:1 (mol %)	1.44	±	0.10	b	13.15	±	1.49	a
C18:2 (mol %)	19.67	±	2.24	b	39.19	±	3.89	a
C18:3 (mol %)	62.43	±	1.63	a	23.06	±	1.31	b
DBI	14.29	±	0.79	a	6.75	±	0.56	b

Results are means ± SE (n = 6). Different letters (a, b) correspond to significant differences between leaves and flowers for each parameter (one-way ANOVA, $p \leq 0.05$). Myristic (C14:0), palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3) acids.

oleifera, Adansonia digitata, Colocasia esculenta, Corchorus tridens, Cassia tora, and *Amaranthus spinosus*, and this would result in an intake of 30 g dry weight leaves per serving portion [48]. If we take into account the number of species involved (6) and the intake (30 g dry weight) we might well admit a value of 5 g for each species, which validates our choice.

From the results here presented it can be emphasized the high contribution to the Ca and Mn supply to the human diet, near 20% of the RDI, only based on a small intake of 5 g (Table 2). This agrees with several studies that stress the importance of *Moringa* leaves in alleviating human macro/micronutrient deficiencies in several areas worldwide [14, 33, 47]. Nevertheless, a huge variation in the data exists which is related with factors previously discussed [40]. For example, Peñalver et al [49], observed an average Mn concentration of 72.1 mg/kg, a value very close to ours, while their Ca levels do not reach 15000 mg/kg. Kumssa et al [33] measured 18300 mg/kg Ca while our data has an average value of 24000. Whatever the variability, both works reflect a particular enrichment in some nutrients which is a useful help to mitigate malnutrition.

Regarding the other elements, the contribution is generally < 5% of the RDI, although Fe and S, had percentual values near that threshold (Table 2). No recommended daily intake has been proposed for S. Daily S needs are met when adequate amounts (13 mg/kg of body weight) of methionine and cysteine are consumed [50]. Therefore, a person weighing 70 kg, requires the consumption of around 1.0 g of methionine/cysteine per day. In our case the intake of 5 g will provide 0.0475 g, *i.e.,* 4.75% of the above-mentioned level of 1.0 g. Iron, has a requirement of 14 mg per day, and 4.98% of the RDI can be satisfied by the ingestion of 5 g *Moringa* leaves (Table 2). If the daily dish is only based in *Moringa* instead of other plant leaves, and assuming the 30 g dry weight per serving portion as referred by Barminas et al. [48], the percentages of RDI largely increase overcoming 100% of the cases of Ca and Mn (Table 2). Although *Moringa* flowers can be eaten, their contribution to the household menus is insignificant.

3.3. Fatty acids analysis

Leaves and flowers presented similar amounts of total fatty acids (TFA), despite the distinct fatty acids (FAs) profiles in the two different organ tissues (Table 3). In leaves linolenic acid (C18:3) was the most abundant FA (62.4%), followed by linoleic (C18:2) and palmitic (C16:0) acids. In flowers C18:2 prevailed (39.2%), followed by C18:3, C16:0 and oleic acid (C18:1). C18:2 and C18:3 are polyunsaturated fatty acids (PUFAs), considered essential FAs in human nutrition, and were largely represented in leaves (82.1 %) and flowers (62.2%).

Table 4. Soluble sugar content (mg g^{-1}DW) of *Moringa oleifera* leaves and flowers. Total soluble sugars correspond to the sum of individual ones. Results are mean ± SE (n = 3).

	Leaves	Flowers
Sucrose	3.3 ± 0.6b	8.90 ± 0.7a
Glucose	1.2 ± 0.4b	120.7 ± 10.6a
Frutose		75.6 ± 4.7
Arabinose	2.4 ± 1.1	
Mannitol	5.0 ± 1.3	
Total	12.0 ± 0.7b	205.2 ± 10.9a

Such profile differs from those reported the same species grown in Africa [52]. PUFAs prevailed in *M. oleifera* flowers collected in Southern Portugal, which may be beneficial for health.

A higher availability of unsaturated FAs over saturated ones in diets has been related to the prevention of obesity, cardiovascular diseases, and inflammatory processes [53]. Lipids unsaturation, expressed as a double bond index (DBI) (Table 3), was higher in leaves due to higher abundance of PUFAs, in particular C18:3, in relation to flowers.

No Dietary Reference Value for the intake of total cis-polyunsaturated fatty acids is established. The European Food Safety Authority (EFSA) do not set specific values for the n-3/n-6 ratio due to insufficient data on clinical and biochemical endpoints in humans [54]. However, EFSA proposes to set an Adequate Intake for alpha-linolenic acid of 0.5 E%, based on the lowest estimated mean intakes of the various population groups from different European countries, while the Adequate Intake for linoleic acid was 4 E%, given as percentage of total energy intake [54].

Table 5. Percentage composition of *Moringa oleifera* essential oil components, isolated from flowers, leaf and seeds.

Peak #	Components	RI	Flowers	Leaf	Seeds
1	*n*-Octane	800			5.9
2	2-*trans*-Hexenal	866		11.8	
3	Benzaldehyde	927			t
4	Benzene acetaldehyde	1002		1.6	
5	Benzyl cyanide * (= Phenylacetonitrile)	1077			12.8
6	Geranyl acetone	1434		1.5	
7	*n*-Dodecanol	1468		t	
8	*trans*-Nerolidol	1549		t	
9	*n*-Hexadecane	1600		t	t
10	Palmitic acid (= Hexadecanoic acid)	1908	15.0	37.5	37.9
11	*n*-Heneicosane	2100		t	
12	Phytol acetate	2101		5.1	
13	Oleic acid [= (9-*cis*)-Octadec-9-enoic acid]	2250		t	28.6
14	*n*-Tricosane	2300	0.6	t	
15	*n*-Pentacosane	2500	16.0	9.2	t
16	*n*-Hexacosane	2600	0.9	t	
17	*n*-Heptacosane	2700	36.4	13.8	1.4
18	*n*-Octacosane	2800	1.2	t	t
19	*n*-Nonacosane	2900	29.2	17.6	6.5
	% Identification		99.3	98.1	93.1
	Grouped components				
	Oxygen-containing sesquiterpenes			t	
	Fatty acids		15.0	37.5	66.5
	Alkanes		84.3	40.6	13.8
	Others			20.0	12.8

RI: Retention index relative to C_8–C_{29} *n*-alkanes on the DB-1 column. * Identification based on mass spectra only. t: traces (<0.05%).

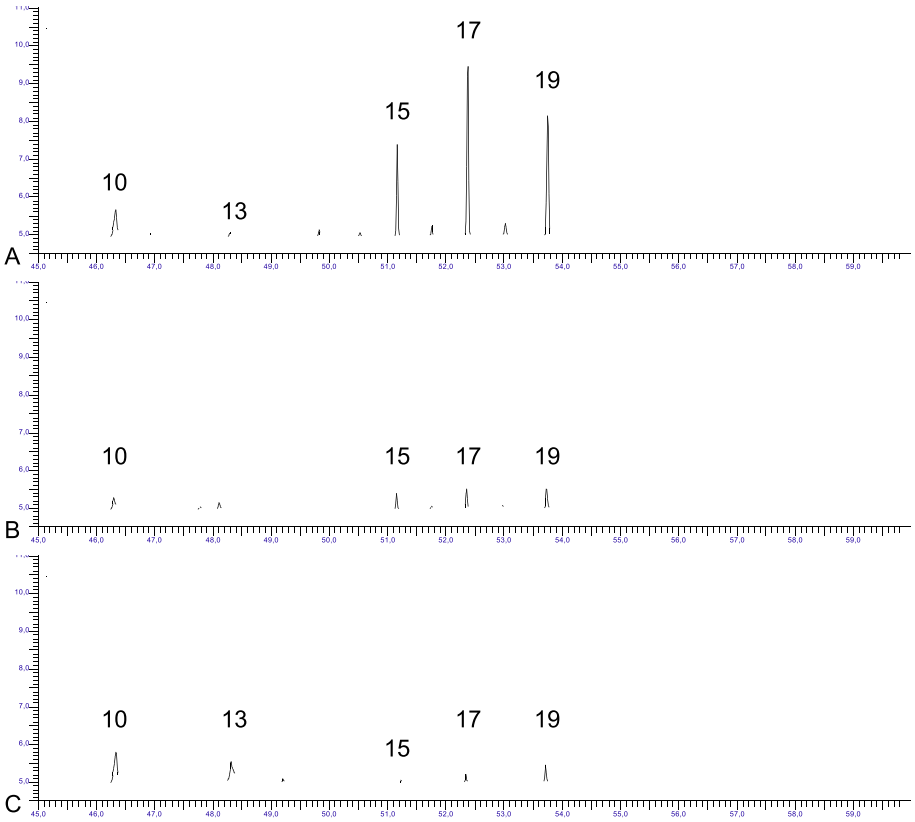

Figure 1. Details of the gas chromatography profiles of *Moringa oleifera* essential oils isolated from the flowers (A), leaves (B) and seeds (C), showing some of the main components (≥10%). Total run time 61 min. For peak number identifications see Table 5.

3.4. Soluble sugars composition

The highest levels of soluble sugars were found in flowers which presented a substantially higher content (16-fold) than leaves (Table 4). Glucose accounted for 58.8% of the total sugar content in flowers, followed by fructose (36.8%) and sucrose (4.3%). Leaves analysis enabled the detection and quantification of four soluble sugars, namely mannitol (41.7%), sucrose (27.5%), arabinose (20.0%), and glucose (10.0%), whereas fructose was not detected. Several authors [52, 55] detected fructose in leaf samples, although these variances could be ascribed to edaphoclimatic variables that can impact biochemical and physiological processes involved in plant sugar synthesis. Edaphoclimatic factors may also underly the lower sugar amounts (20.5 g/100g DW) of *Moringa oleifera* flowers collected in Southern Portugal when compared to values obtained in other countries such as in Guinea-Bissau (65.5–67.2 g/100g), as reported by Fernandes et al. [52].

Regarding glycaemia carbohydrates (GC) which include our soluble sugar content, data from dietary surveys show that intakes in European countries varied between 38 and 56 E% in adults [54]. The daily requirement for GC is not precise, since protein and fat also contribute to the energy needs. Generally, an intake of 50–100 g per day will prevent ketosis, while 130 g per day will be sufficient to cover the needs of brain glucose [54].

3.5. Essential oil composition

Moringa oleifera essential oils (EOs) achieved a yield < 0.05% (v/w). *M. oleifera* EOs chemical composition is detailed in Table 5, in accordance to their elution order on the DB-1 column, and their representative chromatographic profiles in Figures 1A–1C. Despite the EO low yield, the EO designation was kept, because it was obtained by a specific EO isolation procedure, hydrodistillation, and to avoid ambiguities with the vegetable oil, obtained by solvent extraction.

Alkanes were the main group (84%) in flowers EOs, being dominated by *n*-heptacosane (36%) and *n*-nonacosane (29%) - Table 5. Alkanes (41%) and fatty acids (38%) dominated in the leaf EOs, with palmitic acid (38%) and *n*-nonacosane (18%), as major compounds. The seeds EOs were dominated fatty acids (67%), palmitic acid (38%) and oleic acid (29%) being the main components. The occurrence of high levels of benzyl cyanide (= phenylacetonitrile) (13%) in the seeds may be due to the presence of the nitrogen- and sulphur-containing secondary metabolites, glucosinolates, in the plant tissues. Benzyl cyanide is considered a glucosinolate hydrolysis product. The presence of isothiocyanates has been reported in *M. oleifera* flower volatiles [56], although they were not found in the current study.

Although rich in vegetable oils, mainly isolated by cold pressing or Soxhlet, *M. oleifera* is less rich in volatile compounds and in EOs. Even so, some studies have addressed different *M. oleifera* plant part EOs (Table 6), isolated by either hydrodistillation or steam-distillation, or their volatile oils, obtained by simultaneous distillation-extraction (SDE), solvent-free microwave extraction (SME) or headspace-solid phase microextraction (HS-SPME). As any EO-poor species, *M. oleifera* EOs were mostly constituted by alkanes and fatty acids, and not the usual terpene and/or phenylpropanoid-rich composition that characterize an EO-rich species.

Only two studies addressed *M. oleifera* flower volatiles (Table 6) and there were some differences between them, although Barreto et al. [57] also reported an alkane as main component. Again, the studies on *M. oleifera* leaf EOs showed some variability (Table 6), though alkanes and fatty acids were reported in most works. *M. oleifera* seeds EOs showed variability and even a solvent was reported as main component in one case. The reduced number of studies and their variability highlights the need for more comprehensive studies on this species volatiles.

Table 6. Previous studies on *Moringa oleifera* essential oils or volatile oils.

Plant part	Country of origin	EP	AP	Main components ≥ 10%	Reference
Flowers	Brazil	H	GC-MS	Tetracosane 27, hexyl acetate 21, palmitic acid 18, linoleic acid 12, nonacosane 10	[57]
	Cuba	SDE	GC-FID, GC-MS	*trans*-Nerolidol 13	[56]
Leaves	Brazil	H	GC-MS	Phytol 22, palmitic acid 14, thymol 10	[57]
	China	HS-SPME	GC-MS	Acetic acid 13, 3,3-dimethyl-cyclohexanol 10	[58]
	Mozambique	H	GC-FID, GC-MS	Hexacosane 14, pentacosane 13, heptacosane 11 tetracosane 10, octacosane 10, nonacosane 10	[59]
	Nigeria	H	GC-FID, GC-MS	α-Phellandrene 25, α-terpinene 25	[60]
	Rwanda	HS-SPME	GC-MS	Hexanoic acid (= caproic acid) 20	[58]
	South Africa	H	GC-MS	Hexacosane 16, pentacosane 12, heptacosane 10	[61]
	Taiwan	SD	GC-MS	Pentacosane 17, hexacosane 11	[62]
Seeds	India	H	GC-MS	Dimethyl sulfoxide 72, oleic acid 11	[63]
	Nigeria	SME	GC-MS	Cyclopentane 52, hexadecanoic acid 11	[64]
		H	GC-MS	Tetracosane 34, heptadecane 22, eicosane 20	[64]

EP: Extraction procedure. AP: Analysis procedure. H: Hydrodistillation. SDE: Simultaneous distillation-extraction. HS-SPME: Headspace-solid phase micro-extraction. SD: Steam-distillation. SME: Solvent-free microwave extraction. GC-MS: Gas chromatography-mass spectrometry. GC-FID: Gas Chromatography with flame ionization detector.

4. Conclusions

The nutritional value of flowers and leaves of *M. oleifera* grown in Portugal is in agreement with other studies emphasizing such fact. Leaves were shown to be a good source of Ca, K and Mn as well as linolenic acid, the most abundant fatty acid (62.4%), followed by linoleic (19.7%) *i.e.,* the precursors of other important omega-3 and omega-6 fatty acids. In flowers the levels of K were approximately 3-fold higher than the levels found in the leaves, linoleic predominated over the remaining fatty acids (39.2%) while glucose and fructose were the dominant soluble sugars, which were almost absent in the leaves. Essential oil isolation confirmed the low aromatic profile of moringa plant parts, which were dominated by alkanes, fatty acids and some characteristic nitrogen- and sulphur-containing secondary metabolites.

Declarations

Author contribution statement

Josélia Monteiro: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Paula Scotti-Campos, A.Cristina Figueiredo, Fernando Reboredo: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Isabel Pais: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Dulce Viegas: Performed the experiments; Contributed reagents, materials, analysis tools or data.

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Data availability statement

Data included in article/supp. material/referenced in article.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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