Nutritional and phytochemical characterisation of *Tunisian Moringa* oleifera Lam. aerial parts

Maha Bergaoui[1] ✉
Mouna Boulares[1]
Imen Mahmoudi[1]
Youkabed Zarroug[2]
Nawres Dachraoui[1]
Mnasser Hassouna[1]

University of Carthage[1], Research Laboratory: "Technological Innovation and Food Security LR22-AGR01" Higher Institute of Food Industries of Tunisia (ESIAT)
El Khadhra City, Tunis, Tunisia

[2] Filed Crops Laboratory
National Agronomic Research Institute of Tunisia (INRAT)
Ariana, Tunisia

✉ CORRESPONDING AUTHOR:
Maha BERGAOUI
Phone: +21698754188
E-mail: mahabergaoui263@gmail.com

Received: September 11, 2023
Accepted: December 6, 2023

This work aimed to investigate the composition of various aerial parts (leaves, flowers, and seeds) of the Tunisian *Moringa oleifera* Lam as well as their extracted oils.
The *Moringa* seeds showed the highest level of protein (33.45±0.07%) and fat (30.32±0.15%). However, flowers and leaves were rich in carbohydrates, and minerals. Furthermore, *Moringa* leaves extract revealed the highest levels of polyphenols (58.77±0.59 mg GAE/g DE) and flavonoids (77.13±0.005 mg QE/g DE), while the highest tannin levels was that of the flower extract (82.90±0.07mg CE/g DE). Sixteen amino acids were identified in the different *Moringa* aerial parts with a richness of seeds in essential amino acids. This study showed the dominance of unsaturated fatty acids in cold pressed seed oil when compared to that extracted by Soxhlet method. Cold pressed *Moringa* seed oil was rich in beta sitosterol (50.56±0.65%), stigmsterol (25.04±1.06%) compesterol (23.16±0.81%), alpha tocopherol (243.51±0.49 mg/kg), gamma tochopherol (112.6±0.1 mg/kg) and delta tocopherol (11.65±0.2 mg/kg) for the production of specific antioxidants for health promotion in cosmetics, food and pharmacological industries.
Keywords: *Moringa oleifera* Lam, aerial parts, oils fractions, biochemical composition, phytochemical quality.

1. INTRODUCTION

Moringa is considered as the most beneficial tree in the world. It is a native plant of the Himalayan region of Northeastern India. It was cultivated in many other places around the world such as North and South America, Italy, Greece, Africa, and Egypt [1]. *Moringa* is the only genus of the *Moringaceae* family, having 13 species [2]. *Moringa oleifera* Lam (*MO*) is a specie known for its nutritional, agronomic and medical benefits [3], pharmacological, biological, immunomodulatory, antispasmodic, hepatoprotective, anticancer, hypotensive, hypoglycemic, cholesterol lowering effects [4]. In addition, all *MO* parts have been used in traditional foods and dishes for human consumption to prevent malnutrition challenges [3]. Added to this, a high level of proteins, ash, calcium, potassium, sodium, iron, moisture, fat, crude fibre, carbohydrates, β-carotenes, vitamin C, were identified in *Moringa oleifera* [1]. It is a main source of phenolic contents that are mainly found in their leaves [2]. *Moringa oleifera* organs are also known as good sources of secondary metabolites and bioactive compounds, such us terpenoids, flavonoids, tannins, alkaloids, phenolic compounds, anthocyanins and proanthocyanidins with interesting biological activities. While *Moringa* seed oil composition is comparable to olive oil one, that's why it has been used as culinary oil in salads [5]. Recently *Moringa oleifera* has been cultivated in Tunisia [6]. In this context, this study was performed to charaterise the nutritional and phytochemical compounds of various aerials parts of *Moringa oleifera* collected from Northern Tunisia and identify their antioxidant activity.

2. MATERIAL AND METHODS

2.1. CHARACTERISATION OF *MORINGA OLEIFERA* AERIAL PARTS

2.1.1. Preparation of plant material

Leaves, flowers, and seeds of *Moringa oleifera* were collected from the North of Tunisia (Morneg, Ben Arous) between August and October 2022. Fresh material of *Moringa oleifea* samples were cleaned and washed under tap water to remove dust then air-dried for one week at room temperature until constant weight and ground to fine powders. All samples were stored in an airtight container at -20°C until further use.

2.2. CHEMICAL CHARACTERISATION OF *MORINGA* AERIAL PARTS

2.2.1. Proximate analysis of *Moringa oleifera*

The Ash, moisture, and fat contents of various powders of *MO* aerial parts were determined according to AOAC (1998) methods. Protein content was determined using the AOAC (1990) procedure using the conversion factor of 6.25 as mentioned by [7]. The carbohydrate was determined according to [8].

2.2.2. Energetic value

The energetic value was calculated according to the Regulation (EC) No. 1169/2011 of the European Parliament and Council as follows:
Energy (kcal/100 g (DW)) = 4 × (g protein+g carbohydrate) + 9 × (g fat).

2.2.3. Determination of amino acids composition

The *MO* samples were hydrolysed using chlorydric acid for 24h at 105°C. After cooling, sodium hydroxide was added to neutralise the extract. Finally, the filtrated extract was injected to the HPLC-FLD (Agilent Technology.USA). The online amino acid derivatisation reaction was carried out with O-Phthaldialdehyde reagent solution (OPA). The column, used in this work, was column ZORBAX C18 (250×4.60 nm) with 5µm pore size. Due to the wide range of amino acids, it is necessary to perform a gradient to be able to separate them. The analysis was realised in gradient composed of an organic phase A (acetonitrile, methanol, water at 45/45/10 ratio) and an organic phase B (Na_2HPO_4 adjusted with phosphoric acid to pH = 6.5). The flow rate was 1ml/min. The HPLC conditions were adopted as described in the study of [9] with some modifications.

2.2.4. Determination of mineral composition

Mineral elements Copper (Cu), Iron (Fe), Manganese (Mn), Zinc (Zn) were identified by acid mineralisation according to the method described by [10] using an Atomic Absorption Spectrometer. In fact, 2.5 g of raw material was heated in two steps. First, calcination was performed at 500°C to obtain a constant mass. Second, the resulting ash was burned at 600°C. The obtained ash was dissolved in 10 mL of 40% nitric acid and mixture was heated to obtain wet salts. Subsequently, the solute was dissolved in 15 mL of nitric acid (1 N) and transferred to a 25 mL volumetric flask for analysis.
After mineralisation, potassium (K) and sodium (Na) were determined using the flame photometer. Calcium (Ca) and magnesium (Mg) were determined by atomic absorption. Phosphorus (P) was assayed at 880 nm by the molybdenum blue method.

2.3. PHYTOCHEMICAL CHARACTERISATION

2.3.1. Preparation of *Moringa oleifera* extracts

The *MO* aerial parts seeds, flowers and leaves extracts were prepared according to [11] method. Thirty grams of each sample were mixed with 300 ml of solvent (ethanol Sigma Aldrich) and kept for 24 h à 4°C. The extracts were filtrated with Whatman filter paper No.4 then concentrated at 40°C-50°C using a rotary vacuum evaporator [6].

2.3.2. Determination of total phenolic, flavonoid, and condensed tannins contents

Quantification of total phenolic content (TPC) was determined using the Folin-Ciocalteu reagent according to [12]. Briefly 100 µl of extract were mixed with 500 µL of the Folin–Ciocalteu reagent and 1.5 mL of sodium carbonate (20% w/v). Then, 10 ml of distilled water were added. After keeping the mixture in the dark for 2 h the absorbance was determined at 765 nm using a spectrometer (Jenway 6352 spectrophotometer). The total phenolic content was expressed as mg of gallic acid equivalents per g of dry matter (mg GAE/g DM) according to the standard curve prepared with different concentrations of gallic acid (0.3 M).
Flavonoids content (FVT) was evaluated in each part following the colorimetric method described by [12]. For this, one mL of each extract was mixed with 1 mL of ethanolic solution of aluminium trichloride ($AlCL_3$; 2% w/v in ethanol). The mixture was incubated for 15 min at room temperature before measuring the absorbance at 430 nm. Total flavonoids were expressed as mg of quercetin equivalents per gram of dry matter (mg QE/g DM), through the calibration curve of quercetin. The absorption of standard quercetin solution was measured under the same conditions.
The determination of condensed tannins (TTC) was carried out using the vanillin method as described by [13]. Two mL of each extract were added to 4 mL of vanillin (1% w/v) in 7 M sulfuric acid [H_2SO_4]). The mixture was maintained at 25°C for 15 min before the assay. The absorbance was measured at 500 nm against a blank. The results were expressed in mg of catechin equivalent per gram of dry matter (mgCE/g DM).
Determination of free radical scavenging of *Moringa oleifera* aerial parts

The free radical scavenging potency of different ethanolic *Moringa* extract was determined by using DPPH according to the method proposed by [14]. A fresh solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was prepared with absolute ethanol at a concentration of 0.2 mM. Briefly, *Moringa* ethanolic extracts with different concentrations was mixed with the DPPH solution in 1:1 ratio. Finally, the mixture was placed in the dark for 30 min and the absorbance was determined at 517 nm. The control samples were prepared using the DPPH solution mixed with ethanol. The results were determined using the standard curve and expressed as IC50 (µg/ml) as the effective concentration of the sample that is required to reduce 50% of the initial DPPH free radicals as mentioned by [15].

3. CHARACTERISATION OF EXTRACTED OILS OF *MORINGA OLEIFERA* AERIAL PARTS

3.1. OIL EXTRACTION

The extraction of lipid fractions from *MO* aerial parts was carried out using two methods. Seed, leaf, and flower oils were extracted by the soxhlet method using hexane according to [5]. The temperature was set at 50°C for 6 hours than the solvent was removed from oil recovered in the rotary evaporator. The residue oil was kept in opaque vials at -20°C for further investigation.

Moringa seed oil was also obtained by cold press extraction using a Komet DD 85 G vegetable oil screw press (IBG Monforts Oekotec GmbH & Co. KG, Monchengladbach, Germany). The remaining oil was filtrated to remove plant debris and stored under the same conditions until analyses as mentioned by [16].

3.2. DETERMINATION OF FATTY ACIDS COMPOSITION

Fatty acid composition of *Moringa* oils involved their esterification determined using a gas chromatography system (Agilent HP 6890) equipped with flame-ionisation detector (FID) set at 260°C and an Rtx-2330 capillary column (90% biscyanopropyl /10% phenylcyanopropyl polysiloxane, 30 m × 0.32 mm, 0.2 µm film thickness) as explained by [16]. Nitrogen served as the carrier gas with a flow rate of 1.2 mL/min. A total of 1µL of the sample was injected in splitless mode at a temperature of 240°C. The oven temperature was programmed to increase from 100 to 230°C at a rate of 4°C/min.

Fatty acids' peak identification was conducted referred to the retention times of a mixture of pur standards (CRM47885, Supelco 37 Component FAME Mix).

3.3. CHEMICAL COMPOSITION OF COLD PRESSED MORINGA SEED OIL

The cold pressed seed oil having the best yield of extraction and the more interesting fatty acid composition was analysed for its chemical composition. Peroxide value (PI), iodine value, saponification value, specific gravity (using a 10 mL pycnometer at 25°C), and the refractive index at 20°C as described by [16]. Specific absorptivity values K_{232} and K_{270} were determined by using an ultraviolet (UV) spectrophotometer, chlorophylls and carotenoids content were determined respectively as described by [17].

3.4. DETERMINATION OF TOCOPHEROL COMPOSITION

The analytical procedure for determination of tocopherols in cold pressed *Moringa* seed oil involved the use of high-performance liquid chromatography (HPLC) procedure as described by [5] in which 4g oil sample were dissolved in 25 ml of n-Heptane. The detector was set at 295 nm excitation wave lengths whereas the emission wavelength was 330 nm. The injection volume was 20 µl. The rate flow was set at 1 ml/min. n-Heptane and tetrahydrofurane (3.85%) were used as mobile phase. Standard solutions of α, β, γ and δ-tocopherols, formulated using 3–6 µg / mL standard concentrations, were used for quantification purposes.

3.5. DETERMINATION OF STEROL COMPOSITION

The estimation of sterol content involved saponification of 250 mg oil sample using a potassium hydroxide ethanolic solution by boiling under reflux according to [5]. The unsaponifiable matter are derivatized into trimethylsilyl ethers and analysed by capillary DB-5 Agilent column (5% phenyl methyl polysiloxane, 30 m x 0.32 mm internal diameter, 0.25 mm film thickness) gas chromatography (Agilent, HP 6890 series) with split injection and flame ionisation detector according to (COI, 2017) using. Helium was used as a carrier gas at a flow of 2 ml/min. The injector/detector temperatures were held at 280 and 290°C, respectively. The column temperature was set to 240°C and it was next raised to 260°C at a rate of 4°C /min**.**

4. STATISTICAL ANALYSIS

All the assays were carried out in triplicate. The results were presented as mean values and standard deviation. A Duncan test was used to assess significant differences among plant samples with $\alpha = 0.05$. The analysis was carried out using SPSS V.22.0 IBM SPSS Statistics.

5. RESULTS AND DISCUSSION

5.1. CHEMICAL CHARACTERIZATION OF *MORINGA OLEIFERA* AERIAL PARTS

5.1.1. Proximate composition

The proximate composition of Tunisian *Moringa oleifera* Lam flowers leaves and seeds is summarised in Table I.

In this study it was shown that carbohydrates were

Table I - Proximate composition of *Moringa oleifera* aerial parts

Parameters	*Moringa oleifera* aerial parts		
	Leaves	Flowers	Seeds
Moisture (%)	4.43 ± 0.31[a]	7.02 ± 0.30[b]	4.12 ± 0,33[a]
Ash (%)	15,15 ±0,03[c]	9,53±0,02[b]	4,44±0,03[a]
Crude fat (%)	4,36 ±0,11[b]	3,2±0,23[a]	30,32±0,08[c]
Crude protein (%)	25,21 ±0,03[b]	21,37±0,03[a]	33,45±0,04[c]
Carbohydrate (%)	50,85 ±0,03[b]	57,4 ±0,03[c]	25,62±0,03[a]
Energetic value (Kcal/100g)	343,48 ±0,27[a]	343,88±0,15[a]	509,16±0,05[b]

[a,b,c]: mean values with lowercase letters show a significant difference (P<0.05) between the analysed different samples.

the major compounds in all *Moringa* aerial parts with the major content registered in flower powder (57.4±0.03%). The carbohydrate content was estimated at about 50.85±0.03% and 25.62±0.03%, respectively in leaves and seeds. More seed powder is a very rich source of proteins with about 33.45±0.04% followed by leaf (25.21±0.03%) and flower (21.37±0.03%) powders. This finding was partially in concordance with those 32.9% - 38.3% reported in other studies showing that *Moringa oleifera* seeds are considered as a good source of protein and amino acids [18]. The results obtained on *Moringa oleifera* leaf powder were higher than those (24.84%) found by [19]. The protein content of flowers was lower than that (47.97%) reported by [20].

In this study, the lipid content in leaves, flowers, and seeds of *Moringa oleifera* were respectively about 4.36±0.11%, 3.2±0.23% and 30.32±0.08%. These results agreed with those reported by [21]. However, seed fat content was lower than that (41.7%) reported by [16].

In fact, it was shown that aerial parts were rich in mineral. In fact, leaves showed the highest content (15.15±0.03%) followed by flowers (9.53±0.02%) and seeds (4.44±0.03%). These results were similar to those by [22] showing similar mineral content varying between 3.9% and 4.4% in *MO* seeds. Concerning the caloric value of leaf, flower, and seed powder of *Moringa oleifera,* it was around 343.48±0.27 Kcal, 343.88±0.15 Kcal and 509.16±0.05 Kcal, respectively. These values were higher than those of some conventional fruits such as mango (73.9 Kcal), pomegranate (80.6 Kcal), kiwi (60.5 Kcal) and papaya (42.2 Kcal) (Anses, 2020). This variation in term of *MO* composition is assigned to different factors, such as the climatic conditions, growing sites, agricultural practices, harvesting period, and genetic characteristics [3].

5.2. AMINO ACIDS COMPOSITION

The results of the essential and non-essential amino acid composition of *Moringa oleifera* aerial parts are shown in Table II.

These results reveal the highest amount of glutamic acid as non-essential amino acid in *Moringa* seed powder with a content of about 2103.24 mg/Kg followed by arginine, threonine, and glycine (1202.01 mg/kg). However, the dominant amino acids in leaf powder were tyrosine 1527.71 mg/kg, leucine 903.14 mg/kg, glutamic acid 782.88 mg/kg. Similarly,

Table II - Amino acids composition (mg/Kg) of *Moringa oleifera* aerial parts

Amino Acids (mg/Kg)	Aerial parts of *Moringa Oleifera*		
	Seed powder	leaf powder	flower powder
Aspartic Acid	498.96 ±0.73[b]	595.87 ±0.68[c]	325.4 ±0.91[a]
Glutamic Acid	2103.24±0.65[c]	782.88±0.67[b]	408.32±0.80[a]
Serine – Histidine- glutamine	518.69±0.76[c]	508.13±0.68[b]	228.6±0.76[a]
Arginine- Threonine-glycine	1202.01±0.83[c]	754.36±0.56[b]	387.1±0.79[a]
Alanine	816.75±0.67[c]	516.71±0.56[b]	352.12±0.87[a]
Lysine	ND	ND	306.39±.0.58
Tyrosine	ND	1527.54±0.87[b]	1234.5±0.56[a]
Isoleucine	415.96±0.86[c]	262.7±0.90[b]	170.53±0.84[a]
Phenylalanine	246.72±0.45[c]	164.66±0.68[b]	121.22±0.55[a]
Leucine	1187.98±0.83[c]	903. 14±0.91[b]	580.72±0.40[a]
Valine+ Methionine	319.96± 0.82[b]	354.31±0.47[c]	173.12±0.27[a]

ND: not detected; [a,b,c]: mean values with lowercase letters show a significant difference (P<0.05) between the analysed different samples.

11 amino acids were identified in the flower powder with a high amount of tyrosine of about 1234.5 mg/kg. Lysine was only detected in the flower powder. Moreover, the essential amino acids contents were significantly higher in leaves and seeds when compared to flowers. These finding confirmed that the different parts of *MO* are valuable sources of essential amino acids showing their importance for human or animal nutrition.

5.3. MINERAL COMPOSITION

Data in Table I show that *MO* dry leaves had a high amount of ash, resulting in a high content of P (2565 mg/kg), Mg (4308 mg/kg), Ca (9220 mg/kg), Zn (233 mg/kg), K (16584 mg/kg), Na (1443 mg/kg), Fe (310 mg/kg), and Mn (60.60 mg/kg) as shown in Table III.
This result showed that the leaves are a good source of mineral mainly Ca, Mg and K which was in agreement with results published by [23] who found remarkable high amounts for Mg (4036 mg/kg) and K (14988 mg/kg). However, the mineral content in seeds was significantly lower than of leaves. Minerals composition of *MO* flower powder was partially in accordance with that reported by [20]. The high amount of minerals found in this study in the *MO* aerial parts suggested that they can contribute to maintaining the body regulatory functions.

5.4. TOTAL PHENOLIC, FLAVONOIDS AND CONDENSED TANNINS CONTENTS

The phytochemical composition of *Moringa oleifera* was shown in Table IV. In this study, the content of total phenolic compounds in the *M. oleifera* leaf extract was 58.77±0.59 mg GAE/g DM. This result was slightly lower than that found (62.33 mg GAE/g DM) by [24] and higher than the value (49.29 mg GAE/g DM) reported in *Moringa* leaves extract by [19]. For the total polyphenols content in seeds extract it was about 19.51±0.21mg GAE/g DM. This content was quite similar to that noted by [25]. This variation was attributed to various factors specific to the plant such as the degree of maturation as well as genetics, climatic and environmental conditions [26]. Indeed, the quantity of extracted polyphenols was directly affected by their location in the plant and by the polarity of solvents [27].
Moreover, the concentration of flavonoids in the leaves of *M. oleifera* was around 77.13±0.005 mg QE/g DM. This finding was significantly important than that (29.90 mg QE/g DM) found by [24]. The obtained flavonoids content in *Moringa* seeds (26.75±0.41 mg QE/g DM) was significantly lower than that (144.07 mg QE/g DM) indicated by [28]. Same results were found by [1] in *MO* extracts showing that total phenol and flavonoid contents were significantly ($P < 0.05$) higher in the leaf extract when compared to seed one. Concerning the condensed tannins content Tunisian *Moringa* seeds had a high content of condensed tannins of around 40±1.76 mg EC/g DM when compared to the value (27.62 mg EC/g DM) [27]. This variability in terms of phytochemical composition of *Moringa oleifera* aerial parts could be attributed to the variation in the environment where the plant was collected, the season and the physiological stage of the plant [29].

Table III - Mineral composition (mg/Kg) of aerial parts of *Moringa oleifera*

Minerals composition (mg/Kg)	Aerial parts of *Moringa oleifera*		
	Leaves	Flowers	Seeds
K	16584±1.21	-	-
Na	1443±0.52	-	-
Ca	9220±0.69^{c}	4370±0.36^{b}	1950±1.09^{a}
Mg	4308±0.35^{c}	2280±0.43^{a}	2750±0.46^{b}
P	2565±0.17	-	-
Fe	310±0.33^{c}	172.4±0.36^{b}	52.25±0.40^{a}
Mn	60.60±0.51^{c}	30.20±0.36^{b}	11.65±0.52^{a}
Cu	2.65±0.35^{a}	6.75±0.17^{b}	ND
Zn	31.80±0.07^{c}	12.55±0.36^{a}	31.60±0.11^{b}

^{a,b,c}: mean values with lowercase letters show a significant difference ($P<0.05$) between the different analysed samples.

Table IV - photochemical composition of *Moringa oleifera*

Extract	PPT (mg GAE/g DM)	FVT (mg QE/g DM)	TC (mg CE/g DM)
Leaves	58.77 ±0.59^{b}	77.13 ±0.005^{c}	41.95 ±0.04^{a}
Flowers	17.82 ±0.04^{a}	22.85 ±0.03^{b}	82.90 ±0.07^{c}
Seeds	19.51 ±0.21^{a}	26.75 ±0.41^{b}	39.33 ±0.55^{c}

PPT: total polyphenols, FT: total flavonoids, TC: condensed tannins, GAE: gallic acid equivalent, CE: catechin equivalent, QE: quercitin equivalent, DM: dry matter, ^{a,b,c}: mean values with lowercase letters show a significant difference ($P<0.05$) between the different analysed samples.

Table V - Antioxydant activity of *Moringa oleifera* aerial parts

Samples	IC_{50} µg/ml
Moringa oleifera leaf extract	39 ±0.03[a]
Moringa oleifera flower extract	94.42 ±0.04[c]
Moringa oleifera seed extract	83.59 ±0.59[b]

[a,b,c]: mean values with lowercase letters show a significant difference (P<0,05) between the different samples analysed.

ANTIOXIDANT ACTIVITY

For an effective and complete evaluation of the antioxidant potential of *Moringa oleifera* seed, leaf and flower extracts, the results of scavenging free radicals are shown in Table V.

These results obtained showed anti-radical capacity of leaves with a value of IC50 about 39±0.03µg/ml. This result was in disagreement with that of [19] showing a lower leaf anti radical capacity with IC50 value of around 60.07µg/ml. More, the antioxidant activity of *Moringa* leaves was higher than that of the seeds and flowers. In fact, the result indicated that the antioxidant activity of the ethanolic extract of *Moringa* seeds showed a good capacity on the free radical DPPH (IC50 = 83.59±0.59µg/ml) when compared to that (280±0.05 µg/ml) found by [25]. In this study, ethanolic flower extract showed an important anti radical activity (IC50 = 94.42µg/ml). These finding were significantly lower than those obtained by [1] and [30] and they were attributed to the difference in geographical locations and climatic factors [6]. In this context natural antioxidants are always extremely important for health. They prevent any bad effects of free radicals [14].

6. CHARACTERISATION OF *MORINGA OLEIFERA* EXTRACTED OILS

6.1. FATTY ACIDS COMPOSITION

The main fatty acids composition in different extracted oils from *M.oleifera* aerials is shown in Table VI.

In this study 10, 8 and 11 major fatty acids were identified respectively in *MO* leaf, seed and flower extracted oils. It was shown that the leaf oil was mainly composed of 44.68% of linolenic, followed by 18.39% of palmitic and 10.4% of linoleic acids. The result obtained was in accordance with those found by [21] reporting the same fatty acid composition with respective close levels. Concerning flower oil, the fatty acid composition showed the dominance of unsaturated fatty acids (58.63%) with C18:1 the prominent fatty acid (40.31%). Concerning seed oils extracted with cold press and soxhlet method, they showed the same acidic composition with respective high level of monounsaturated fatty acids about (81.54%) and (80.64%). Besides, the predominant poly-unsaturated fatty acids were linoleic acid, linolenic acid, followed by palmitic acid. These results were similar

Table VI - Fatty acids (%) composition of *Moringa oleifera* oil

	Cold press extraction	Soxhlet extraction		
Fatty acids (%)	**Seed oil**	**Seed oil**	**Leaf oil**	**Flower oil**
Lauric Acid: $C_{12:0}$	Nd	Nd	1.33±0.06	Nd
Myristic Acid: $C_{14:0}$	0.18±0.005[a]	0.15±0.01[a]	2.47±0.11[c]	1.07±0.06[b]
Myristoleic Acid:$C_{14:1}$	Nd	Nd	Nd	0.54±0.02
Palmitic Acid:$C_{16:0}$	6.65±0.46[a]	6.03±0.46[a]	18.39±0.06[c]	13.23±0.11[b]
Palmitoleic Acid:$C_{16:1}$	1.78±0.46[a]	2.25±0.06[b]	2.22±0.03[b]	1.58±0.11[a]
Margaric Acid: $C_{17:0}$	0.08±0.11[a]	Nd	2.01±0.17[b]	0.1±0.006[a]
Margaroleic Acid :$C_{17:1}$	0.07±0.006[a]	Nd	Nd	0.52±0.006[b]
Stearic Acid: $C_{18:0}$	5.07±0.28[b]	5.30±0.11[b]	3.22±0.06[a]	6.07±0.03[c]
Oleic Acid:$C_{18:1}$	81.54±0.11[d]	80.64±0.17[c]	7.92±0.11[a]	40.31±0.06[b]
Linoleic Acid: $C_{18:2}$	0.86±0.02[a]	0.82±0.01[a]	10.4±0.17[c]	6.47±0.11[b]
Linolenic Acid:$C_{18:3}$	0.32±0.02[a]	0.45±0.02[a]	44.68±0.35[c]	9.21±0.11[b]
Arachidic Acid: $C_{20:0}$	2.27±0.06[a]	2.41±0.03[a]	7.33±0.11[b]	20.87±0.17[c]
Gadoleic Acid :$C_{20:1}$	1.20±0.13	Nd	Nd	Nd
MUFA	84.59±0.30[d]	82.89±0.23[c]	10.14±0.14[a]	42.95±0.17[b]
PUFA	1.18±0.04[a]	1.27±0.03[a]	55.08±0.52[c]	15.68±0.23[b]
ω_6 /ω_3	2.68±0.14[d]	1.82±0.07[c]	0.23±0.002[a]	0.70±0.004[b]
SAFA	14.24±0.80[a]	13.89±0.62[a]	33.42±0.36[b]	41.34±0.38[c]
Total	100.01±1.14[a]	98.05±0.89[a]	99.97±1.08[a]	99.97±0.80[a]

ND: not detected; SAFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids;. Values are means± S three determinations. [a,b,c,d]: mean values with lowercase letters show a significant difference (P<0,05) between the different samples analysed.

to those reported by [3] confirming that seed oil was rich in unsaturated fatty acids and that it was similar to olive oil due to its richness mainly in oleic acid [31] which makes it more desirable and more stable when cooking and frying [7]. Furthermore, it should be highlighted that there is no significant difference in terms of fatty acids composition between the extracted seed oil using a different method. This finding agreed with that of [7]. But it should be noted that the cold press process has several advantages over solvent based systems, such as being environment friendly and requiring lesser energy [5]. Furthermore, saturated fatty acids were also detected with minor levels in cold pressed (14.09%) and in soxhlet (9.53%) extracted seed oils. In fact, myristic acid (C14:0) was identified in seed oils extracted using the two methods. However, margaric acid (C17:0) was detected only in cold pressed *Moringa* seed oil with a minor level (0.08%). This result was in perfect agreement with another study [36]. However, these contents did not exceed 15% of the total fatty acid composition which prevent LDL cholesterol, cardiovascular diseases, improve digestion and modulate arterial pressure and blood viscosity [7]. In addition, ω6 / ω3 ratio was around 2.68 which must not exceed 4 as recommended by AFSSA in all analysed samples as reported by [32].

6.2. CHARACTERISATION OF COLD PRESSED MORINGA SEED OIL

6.2.1. Tocopherol composition

The relative result to tocopherol composition (Table VII) of cold pressed *Moringa oleifera* seed oil showed the presence of alpha, gamma, and delta tocopherols. Beta tocopherol was not detected in the analysed oil.

Considering these results, the alpha tocopherol was the predominant form of vitamin E in this oil (243.51 mg/kg). This has been reported in most studies. In fact, [33] and [7] found alpha tocopherol contents of *Moringa* seed oil in order of 168.2 and 226.9 mg/kg, respectively. Also, the obtained gamma tocopherol (112.6 mg/kg) and delta tocopherol contents (11.65 mg/kg) were higher than those reported in literature. Additionally, most vegetable oils contain alpha, beta, and gamma tocopherols.

Delta tocopherol exists only in a few vegetable oils such as cotton seed, wheat germ, soybean, castor, and peanut oils. This richness of *Moringa oleifera* oil in tocopherols gives it some protection during storage and processing [34].

6.2.2. Sterol composition

The analysis of the sterol fraction of *Moringa* seed oil revealed the presence of 10 compounds consisting mainly of β-sitosterol, stigmasterol and campesterol representing 90% from total sterols as shown in Table VIII**.**

These sterols are part of the class of phytosterols known for their hypocholesterolemic and beneficial effect on human health [7]. It was noticed that *Moringa* seed oil has a remarkable similarity with the soybean oil in terms of major sterol fractions (β sitosterol, stigmasterol, campesterol, avenasterol). Knowing that β-sitosterol is the most representative phytosterol of vegetable oils [35], this study confirmed the observations reported by [16] on the richness of *Moringa* oil in phytosterols. In contrast, the other detected sterol fractions were only present in trace amounts. These findings reported the very interesting nutritional quality of cold pressed *Moringa oleifera* seed oil showing a good climatic, cultivation and storage conditions of seeds.

6.2.3. Physicochemical characterisation of *Moringa* cold pressed seed oil

The physicochemical characterisation of *M.oleifera* cold pressed seed oil is illustrated in Table IX.

Table VII - Tocopherol composition (mg/Kg) of *Moringa* cold pressed seed oil

Tocopherols (mg/Kg)	Cold pressed seed oil
Alpha tocopherol	243.51±0.49
Gamma tocopherol	112.6±0.1
Delta tocopherol	11.65±0.2

Table VIII - Sterols (%) composition of cold pressed seed oil

Sterols (%)	Cold pressed *Moringa* seed oil
Stigmasterol	25.04±1.06
Campesterol	23.16±0.81
β-sitosterol	50.65±0.65
Chlerosterol	0.395±0.01
Δ 7 avenasterol	0.29±0.01
Δ 5.23 stigmastadienol	0.275±0.01
Δ δ7stigmastenol	0.215±0.004
Erythrodiol	0.11±0.01
Uvaol	0.05± 0.01

Table IX - Physicochemical characterization of *M.oleifera* cold pressed seed oil

Parameters	Values
Refractive index (20°C)	1.466±0.03
Acid value(mg KOH/ g oil)	0.84±0.03
Saponification value(mg KOH/ g oil)	187.3±2.10
Iodine value (g I_2 / 100g oil)	64.42 ±0.21
Peroxide value (meq O_2/kg oil)	2.45±0.32
Chlorophyll mg/Kg	1.56±0.01
Carotenoid mg/Kg	3.26±0.03
K_{232}	1.22±0.02
K_{270}	0.059±0.01

In this study, the IR value obtained (1.466±0.03) was close to that of olive oil which varies between 1.467 and 1.470. In fact, this result was in agreement with those observed by [16] and [36] reporting RI of about 1.467 and 1.462 respectively for *Moringa oleifera* seed oils. This value was also similar to those recorded on avocado oil (1.465 - 1.474). The analysed specific extinction coefficients K232 (1.22±0.02) and K270 (0.059±0.01) showed an oxidative stability of cold pressed *Moringa* seed oil thanks to its natural antioxidants. The results registered were comparable to those found by [16] who highlighted K232 and K270 values of 1.17 and 0.043 respectively. The low acid value of this oil (0.84±0.03 mg KOH/g oil) showed its good stability. It was also lower than that (1.33±1.15 mg KOH/g of oil) found by [36]. This difference could be explained by the hydrolysis reaction of lipids during the grinding of seeds [7]. The peroxide index (PI) was related to storage conditions and extraction methods. It was noted that PI value of *Moringa* oil was in order of 2.47±0.32 meq O2/Kg. This result was lower than (10 meq O2/Kg) required by the Codex Alimentarius, (1992) for most conventional oils. Also, the work carried out by [37] recorded peroxide index value ranging from 3.3 to 4.5 meq O2/Kg for the oil extracted from pomegranate seeds of different Tunisian varieties. This lower value registered for the PI in *MO* seed oil could be attributed to the richness of this oil in natural antioxidants such as tocopherols and fat-soluble vitamins [7]. The saponification index was related to the length of the fatty acids. The saponification index recorded for the analysed *Moringa* oil was around 188 ± 2.14 mg of KOH/g of oil. This value was close to olive oil ranging between 184 and 196 mg KOH/g oil. This result was like that (185 mg KOH/g oil) found by [36]. The iodine value indicates the level of oleic and linoleic acids present in the oil. Indeed, this index increased with number of long chain fatty acids [7]. In this study the iodine index (64.53±0.25 g/100 g) of *Moringa* seed oil was lower than that (67.42 g/100 g) showed by [16]. The carotenoid and the chlorophyll contents of the cold pressed *Moringa* seed oil was about 3.26 mg/kg and 1.56 mg/kg respectively. These levels were partially in accordance with those reported by [38] showing carotenoid content of about 4 mg/Kg. However, the chlorophylls were not detected. It should be noted that chlorophylls and carotenoids were the major pigments of cold pressed *Moringa* seed oil and they were responsible for the oil colour [16]. Moreover, these pigments were known by their antioxidant activities and their capacity to prevent cardiovascular and eye diseases [17].

7. CONCLUSIONS

In short, the characterisation of Tunisian *Moringa oleifera* aerial parts revealed that seeds are considered as a good source of protein, and fat. Leaves are rich in ash and mineral. Moreover, *Moringa* leaves and seeds contain good amount in essential amino acids, which confirm their higher nutritional value. The *Moringa* aerial parts showed also good antioxidant activities due to their richness in natural bioactive compounds. On the other hand, the chemical composition of cold pressed seed oil was better than that extracted using the soxhlet method. Oleic acid, beta-sitosterol, and alpha-tocopherol were the dominant compound in *Moringa* cold pressed seed oil which may lead to interesting biological and therapeutic properties when used. This promising result encourages the use of *Moringa* powder and extracted seed oil in cosmetic, pharmaceutical medicinal and food industries.

REFERENCES

[1] M. Prabakaran, S.H. Kim, A. Sasireka, M. Murugesan Chandrasekaran, M. Chung, Polyphenol composition and antimicrobial activity of various solvent extracts from different plant parts of Moringa oleifera, Food Bioscience, (2018).

[2] E. L. Gómez-Martínez, A. L. San Martín-López, M. J. García-Vásquez, A. García-Rocha, L. V. Mendoza-García, A. San Martín-López, Impacto psicosocial de l'estética dental en alumnos con maloclusiones del Telebachillerato Coxquihui, Veracruz. *Revista Mexicana de Medicina Forense y Ciencias de la Salud*, *4*(S1), 54-57, (2020).

[3] A. Fernandes, A. Bancessi, J. Pinela, M. Inês Dias, A. Liberal, R.C. Calhelha, A. Ćirić, M.Soković, L. CATARINO, I. C.F.R. Ferreira, L. Barros, Nutritional and phytochemical profiles and biological activities of *Moringa oleifera* Lam. edible parts from Guinea-Bissau (West Africa), *Food Chemistry,* (2020).

[4] A. Sodamade, O.S. Bolaji, O.O. Adeboye, Proximate Analysis, Mineral Contents and Functional Properties of Moringa oleifera Leaf Protein Concentrate. Journal of Applied Chemistry, 4, 47-51, (2013).

[5] M. M. Özcan, K. Ghafoor, F. Al Juhaimi, I. A. M. Ahmed, E. E. Babiker, Effect of cold-press and soxhlet extraction on fatty acids, tocopherols and sterol contents of the Moringa seed oils. South African Journal of Botany, 124, 333-337, (2019).

[6] N. Bennour, H. Mighri, H. Eljani, T. Zammouri, A. Akrout, Effect of solvent evaporation method on phenolic compounds and the antioxidant activity of Moringa oleifera cultivated in Southern Tunisia, South African Journal of Botany, Volume 129, 2020, Pages 181-190, ISSN 0254-6299, (2020).

[7] S. Louni, Extraction et caractérisation physico-chimique de l'huile de graines de *Moringa Oleifera*. E.N.S.A d'El- Harrach, Algérie. (Mémoire en

vue de l'obtention du diplôme de Magister en Sciences Agronomiques), (2009).
[8] A. Korus, D. Gumul, M. Krystyjan, L. Juszczak, J. Korus, Evaluation of the quality, nutritional value and antioxidant activity of gluten-free biscuits made from corn-acorn flour or corn-hemp flour composites. European Food Research and Technology, 243, 1429-1438, (2017)
[9] X. Huang, S.J. Kao, J. Lin, X. Qin, C. Deng, Development and validation of a HPLC/FLD method combined with online derivatization for the simple and simultaneous determination of trace amino acids and alkyl amines in continental and marine aerosols. PLoS ONE 13(11): e0206488, (2018) https://doi.org/10.1371/journal.pone.0206488
[10] Akzhigitova, Zhanna, Aizhamal Baiseitova, Moldyr Dyusebaeva, Yang Ye, and Janar Jenis, "Investigation of Chemical Constituents of Artemisia Absinthium". International Journal of Biology and Chemistry 11 (1):169-77,(2018).
[11] J. L. Mau, G. R. Chao, K. T. Wu, Antioxidant properties of methanolic extracts from several ear mushrooms. Journal of Agricultural and Food Chemistry, 49 (11), 5461-5467, (2001).
[12] A. Kumaran, R.J. Karunakaran, In Vitro Antioxidant Activities of Methanol Extracts of five Phyllanthus Species from India. LWT—Food Science and Technology, 40, 344-352, (2007).
[13] S. Toda, Antioxidative effects of polyphenols from leaves of Artemisia princeps Pamp. on lipid peroxidation in vitro. J Food Biochem; 29:305–12,(2005).
[14] N. Bhalla, N. Ingle, V. Srilakshmi Patri, D. Haranath, Phytochemical analysis of Moringa Oleifera leaves extracts by GC-MS and free radical scavenging potency for industrial applications, Saudi Journal of Biological Sciences, Volume 28, Issue 12, Pages 6915-6928, (2021).
[15] I. Mahmoudi, O. Ben Moussa, M. Hassouna, Symbiotic, hypocholesterolemic and antioxidant effects of potential probiotic lactobacilli strains isolated from Tunisian camel milk. *Advances in Microbiology*, *7*(4), 328-342, (2017).
[16] K. Gharsallah, L. Rezig, K. Msaada, A. Chalh, T. Soltani, Chemical composition and profile characterization of Moringa oleifera seed oil. *South African Journal of Botany*, *137*, 475-482, (2021).
[17] M. Boulares, A. Bezzezi, M. Arfaoui, A. Boulares, M. Ghrab, O. Ben Moussa, M. Hassouna, S.Boudiche, Improvement of Tunisian 'Chemlali' extra virgin olive oil stability with rosemary and laurel herbs and essential oils, (2021).
[18] Granella, An approach to recent applications of Moringa oleifera in the agricultural and biofuel industries, (2020)
[19] D.E. Páramo-Calderóna, A. Aparicio-Saguilána, A. Aguirre-Cruza, J. Carrillo-Ahumadaa, J.P.Hernández-Uribeb, S. Acevedo-Telloc, J.G. Torruco-Uco, Tortilla added with Moringa oleífera flour: Physicochemical, texture properties and antioxidant capacity. Food Science and Technology, (2019).
[20] M. Ndong, S. Wade, N. Dossou, A.T. Guiro, R.D. Gning, Valeur Nutritionnelle du Moringa Oleifera, Étude de la Biodisponibilité du fer, Effet de L'enrichissement de Divers Plats Traditionnels Sénégalais Avec la Poudre des Feuilles. *African Journal of Food, Agriculture, Nutrition and Development*, (2007).
[21] W. C. J. Sagona, P. W. Chirwa, S. M. Sajidu, The miracle mix of Moringa: Status of Moringa research and development in Malawi. South African Journal of Botany, 129, 138-145, (2020).
[22] S. Boukandoul, S. Casal, F. Zaidi, *Moringa Oleifera* seed oil: Production, uses and health benefits. In: Hong, N.Kh.D. (Ed.), Seed oil: Production, uses and benefits. NovaScience Publishers, New York, 2018. 1–27 , (2019).
[23] [F. H. El-Massry, M. E. MOSSA, S. M. YOUSSEF, Moringa oleifera plant. *Egyptian Journal of Agricultural Research*, *91*(4), 1597-1909,(2013).
[24] T. Prabsattroo, J. Wattanathorn, S. Iamsaard, *Moringa oleifera* extract enhances sexual performance in stressed rats. *J. Zhejiang Univ. Sci. B* 16, 179–190, (2015).
[25] H. Barakat, G.A. Ghazal, Propriétés physico-chimiques de *Moringa Oleifera* Graines et leur huile comestible cultivées dans différentes régions d'Égypte. Sciences de l'alimentation et de la nutrition, 7, 472-484, (2016).
[26] S. Mohamed, A. Youssef, M. Issa, H. Abdel Salam, A. El-Ansary, Validated HPLC Method for Quantitative Analysis of Gallic Acid and Rutin in Leaves of Moringa Oleifera Grown in Egypt. *Egyptian Journal of Chemistry*, *64*(3), 1583-1591,(2021).
[27] S. Djermoune, N. Henoune, Composition chimique et teneur en composés phénoliques des graines de Moringa oleifera. Mémoire de Fin de Cycle En vue de l'obtention du diplôme de Master of science. Département des Sciences Alimentaires, Université A. MIRA-Bejaia, Algérie 89 p, (2015).
[28] W. R. Compaoré, P. A. Nikièma, H. I. N. Bassolé, A. Savadogo, J. Mouecoucou, Chemical composition and antioxidative properties of seeds of Moringa oleifera and pulps of Parkia biglobosa and Adansonia digitata commonly used in food fortification in Burkina Faso. *Current Research Journal of Biological Sciences*, *3*(1), 64-72, (2011).
[29] M. Saadoudi, L. Hambaba, M. Abdeddaim, A. Lekbir, A. Bacha, S. Boudraa, S. Zidani, nutritional composition, physical properties and sensory evaluation of biscuit produced from jujubes (fruits of Zizyphus lotus L.) Annals. Food Science and Technology, (2017).

[30] S. Vats, T. Gupta, Evaluation of bioactive compounds and antioxidant potential of hydroethanolic extract of Moringa oleifera Lam. from Rajasthan, India. Physiol Mol Biol Plants 23, 239–248, (2017).
[31] R.S. G. Singh, P. S. Negi, C. Radha, Phenolic composition, antioxidant and antimicrobial activities of free and bound phenolic extracts of *Moringa Oleifera* seed flour. Journal Of functinal Foods. 5: 1883 –1891, (2013).
[32] M. Boulares, M. Mankai, S. El Adab, O. Ben Moussa, R. Teber, M. Hassouna, Anti-Listerial Activity of Oregano and Cinnamon Essential Oils in Vacuum Packed Ground Ovine Meat during Refrigerated Storage. Journal of Agricultural Science and Technology, 25(1), 61-73, (2023).
[33] M. A. Salama, M. A. Owon, M. F. Osman, A. I. Esmail, B. Matthäus, Characterization of Egyptian Moringa oleifera lipids (whole seeds and kernels). *Journal of Food and Dairy Sciences*, *9*(12), 379-384, (2018).
[34] M. E. Cuvelier, M. N. Maillard, "Stabilité des huiles alimentaires au cours de leur stockage", *Oléagineux. Corps Gras Lipides*, 19(2), 125132, (2012).
[35] M. V. Aïssi, S. Mohamed, F.P. Tchobo, Etude comparative de la qualité des huiles végétales alimentaires raffinées en usage au Bénin. Bulletin d'Informations de la Société Ouest Africaine de Chimie N°06 ; 25 – 3, (2009).
[36] A. Leone, A. Spada, A. Battezzati, A. Schiraldi,J. Aristil ,S. Bertoli, *Moringa Oleifera* Seeds and Oil: Characteristics and Uses for Human Health: An overview. Int. J. Mol. Sci, 17, 2141.édition Maurizio Battino université de milan, Italie, (2016).
[37] M. Khemakhem, Y. Zarroug, K.Jabou, S. Selmi, N.Bouzouita, Physicochemical characterization of oil, antioxidant potential, and phenolic profile of seeds isolated from Tunisian pomegranate (Punica granatum L.) cultivars. Journal of Food Science Vol. 0, Iss. 0, (2021).
[38] N. Foidl, H. P. S. Makkar, K. Becker, The potential of *Moringa oleifera* for agricultural and industrial uses. In: What development potential for Moringa products. October 20th - November 2nd 2001. Dar Es Salaam, (2001).