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Consumer Nutrition Knowledge of *Moringa oleifera* New-Installed in Southern Tunisia: Preservation of Mineral and Phytochemical Contents

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ABSTRACT

Background and Objective: Amid global agricultural sustainability concerns, *Moringa oleifera*'s potential in Southern Tunisia is explored. The region's challenges include aridity, limited land and water scarcity, hindering farmers. This study evaluates the suitability of the exotic plant for local cultivation to address these issues and enhance regional agriculture's resilience and productivity. **Materials and Methods:** Ethnonutritional data were gathered via a modified questionnaire shared online, yielding 450 responses. Proximate analysis and mineral quantification were conducted using standard methods. Preliminary phytochemical screening was performed on *M. oleifera* leaf extracts obtained via methanol, ethanol, hexane and water. **Results:** As 69% of respondents were aware of *M. oleifera*, with 40% using it. Leaves (88%), seeds (24%) and flowers (14%) were commonly used. Infusion was prevalent (67%) for cancer, digestive disorders and inflammation. Leavesrichness: 28.65% fiber, 46.87% dry matter, 18.17% protein, 20.73% minerals and 79.14% organic matter. Phytochemical screening identified alkaloids, cardiac glycosides, saponins, diterpenes, triterpenes, phenols, phytosterols, tannins, flavonoids, amino acids and quinones. **Conclusion:** This study showed that *M. oleifera* retained its nutritional values and its rich compositional with the new planting conditions in an arid environment.

KEYWORDS

Moringa oleifera, ethnonutritional survey, proximate analysis, mineral composition, phytochemical screening, Southern Tunisia

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INTRODUCTION

The global quest for sustainable agricultural practices has gained significant momentum in recent years, driven by the pressing need to address food security, environmental conservation and economic development challenges. In this context, *Moringa oleifera*, commonly known as the "miracle tree", has

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emerged as a promising crop due to its exceptional nutritional value, adaptability to diverse climates and multiple applications in various industries[1].

Southern Tunisia faces unique challenges in its agricultural sector, primarily attributed to its semi-arid climate, water scarcity and limited arable land[2]. These factors have limited the options for local farmers, impeding their ability to sustainably produce nutritious crops and contribute to the region's socio-economic development[3]. However, recent studies have suggested that *M. oleifera* may hold significant potential for addressing these challenges, offering a versatile and resilient solution to enhance agricultural productivity in this region[4]. Since 1990, besides the next top strategic and traditional crops such as olive, citrus and cereal, the Tunisian Ministry of Agriculture has undertaken to introduce new exotic varieties such as *M. oleifera* Lam. Tunisian farmers had intensified the culture of this species to take advantage of its medicinal and nutritional properties.

Moringa oleifera, native to the Indian subcontinent, is renowned for its extraordinary nutritional profile, containing a rich array of essential vitamins, minerals and proteins[5]. Moreover, its fast growth rate and ability to thrive in marginal soils make it suitable for cultivation in harsh environments, where other crops struggle to survive. The tree's multiple uses, such as food, fodder, medicinal products and biofuel, further contribute to its desirability as a sustainable agricultural option.

Hearing about the benefits of treating many types of diseases and considering also as a dietary supplement rich in vitamins and iron[6-9], Tunisians tried to use the leaves of the *Moringa* species. Till now, no scientific information and no published data on the feeding habits of the population with this new exotic variety recently installed. The present study is considered a part of the evaluation of the leaves of *M. oleifera* cultivated in Tunisia. An ethnonutritional survey published online over three months interesting the cultural and nutritional values of *M. oleifera*, the eating habits and culinary techniques used by arborists, will be identified. In addition, the physicochemical of the *M. oleifera* leaves will be carried out to quantify the mineral and chemical composition of this tree and check if it retains its nutritional value and its rich chemical composition with the climate and planting conditions in Southern Tunisia.

MATERIALS AND METHODS

Study area: This research was conducted at the Arid Regions Institute and Higher Institute of Biotechnology of Monastir, Tunisia, spanning from September, 2021 to February, 2022. The *M. oleifera* leaves (2 kg) were collected from Menzel Lahbib (Gabes-Tunisia) and authenticated by Pr. Ahmed Akrout from the Arid Regions Institute, Medenine, Tunisia. Voucher specimens (MO-2) were deposited in the laboratory of natural products' separation, analysis and valorization at the Arid Regions Institute. The plant materials were air-dried for a period of 18 days at room temperature, following which the dried leaves were finely powdered for all subsequent analyses.

Ethnonutritional survey: The ethnonutritional study is based on a modified questionnaire shared via social networks and submitted to respondents. After conducting evenly direct interviews with most local herbalists, obtained data was entered on the form distributed in Google Forms. A total of 450 responses were collected. A prepared database concerning the users (sex, age, study level, habitat,...) and plants (origin, price, used part, preparation mode), was organized for further analysis.

Proximate analysis: *Moringa oleifera* leaves were analyzed for their moisture, extractible substances by water and extractible substances by 80% ethanol, ash, sulfuric ash and chlorydric ash, using standard analytical methods[10].

Quantification of mineral composition by atomic absorption spectroscopy (AAS): Powdered dried *Moringa* leaves (1 g) were placed in a previously weighed porcelain crucible and heated in an oven for 24 hrs at 105°C to delete the moisture and then in a furnace (PROLABO) at 550°C for 4 hrs. The resulting

white ash was weighed, dissolved in ultrapure water (4 mL) and concentrated chlorydric acid (1 mL) then filtered and diluted with ultrapure water in a 100 mL calibrated flask. Under the same conditions, a blank test is carried out[11]. The obtained solutions were used to determine sodium, potassium, calcium, magnesium, cuvier, zinc and iron. Samples are prepared in triplicate. An atomic absorption spectroscopy (AAS) model thermo-Scientific iCE3000 AA equipped with hollow-cathode lamps was used to determine the mineral contents of the *M. oleifera* leaves. The results are established by comparing the atomic spectroscopic signal of each metal ion with a corresponding standard solution of the ion. This assessment relies on the principles of the Beer-Lambert Law[12]. Standard stock solutions of various minerals were utilized and suitably diluted to create a series of concentrations, thus obtaining the calibration curve (absorbance/concentration) for each metal ion[13].

Preliminary phytochemical screening: The methanol extract was obtained with powdered dried leaves (5 g) extracted by maceration with 100 mL methanol (70%) for 72 hrs at room temperature with occasional shaking. The solution was filtered with Whatman No.1 filter paper. The water extract was obtained with powdered dried leaves (5 g) boiled in 100 mL distilled water for 15 min, kept at room temperature overnight, filtered and 100 mL distilled water was added to the filtrate. For the ethanol and hexane extracts, powdered dried leaves (25 g) were extracted in Soxhlet apparatus (BRAND w-Germany, 100 mL) with ethanol or hexane as a solvent for 6 hrs. The extracts were used for the preliminary screening of phytochemicals. The freshly prepared crude methanol, ethanol, hexane and water extracts of leaves were also, subjected to qualitative chemical tests to detect the presence of certain phytochemicals using standard procedures based on the data reported in previous studies[14,15]. The presence of particular phytochemicals is indicated by the development of coloration or precipitation, which is observed during the screening of alkaloids, carbohydrates, cardiac glycosides, anthranol glycosides, cyanogenic glycosides, saponins, diterpenes, triterpenes, phenols, phytosterols, tannins, flavonoids, amino acids, proteins, phlobatannins, quinines and oxalates.

Statistical analysis: All tests used in proximate analysis and quantification of mineral composition, were prepared in triplicate. Differences were considered significant at $p<0.05$. Results were expressed as Mean±Standard Deviation (SD).

RESULTS AND DISCUSSION

Ethnonutritional survey: Most participants in the ethnonurtitional survey (70%) are ranging in the age group between 20 and 40 years as long as the older ones (40-60 years) which haven't exceeded 22.5% (Fig. 1). Although that experience gained with age is considerate as the primary source of information, in this survey, older people shown fairly reliable best information about the harvesting, drying, storage and use of plants and more informed about diseases. Based on the obtained results, women are more interested in medicinal plants (62%). Those indicated that they have greater phytotherapeutic knowledge, according to cultural traditions[16].

As in the world, the most used parts are the leaves (88%), followed by seeds (24%) and flowers (14%), against 10% for the stems and 5% for the roots. Most users of this plant (51%) indicate that they obtained the plant material through direct collection. Of Tunisian consumers 65% of people are interested in planting a *Moringa* tree and 16% have already planted a tree at home. Those can explain the high spread and availability of this plant after the success of its first installation in all the regions of Tunisia. The plant is used fresh and dry (60 and 58%, respectively). Due to its impressive nutritional and medicinal value, the infusion is the most cited preparation method (67%) followed by direct consummation (40%).

The survey results revealed that the different parts of *M. oleifera* are used against several pathologies and the most important cited are cancer, digestive disorders, inflammation, diabetes and hypertension (Table 1).

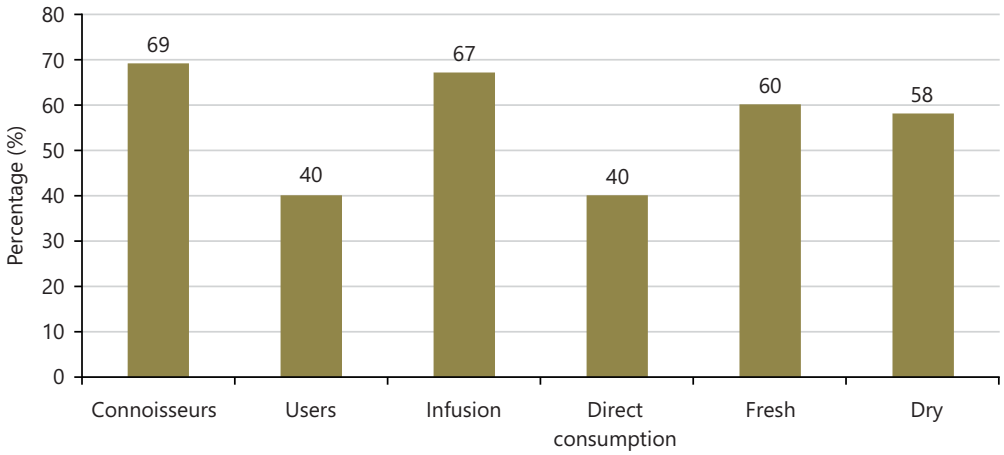

Fig. 1: Results of the ethnonutritional survey of *Moringa oleifera* used by Tunisians
Y-axis: Ethnonurtitional

Table 1: Area of therapeutic indication

Pathologies	Number of citations
Cancer	18
Digestive disorders	18
Inflammation	10
Diabetes	09
Hypertension	07
Coronavirus	06
Food supplement	05
Cosmetic	04
Obesity	02
Allergy of the eyes	02

This ethnonutritional survey's findings can provide insights into the cultural practices and dietary habits of Tunisians, shedding light on their utilization of *M. oleifera* as a nutritional resource. Those can contribute to expanding the understanding of the nutritional value of this plant within the Tunisian context, potentially leading to better dietary choices and practices. If *M. oleifera* is found to have significant nutritional benefits, it could promote its cultivation and use in local agriculture, potentially leading to economic and agricultural development. The study's results can be utilized in public health campaigns to promote the consumption of *Moringa* and raise awareness about its nutritional benefits. Especially in regions with limited access to diverse foods, this plant could be integrated into food security initiatives to provide a sustainable source of essential nutrients. In fact, to provide more comprehensive insights and consider cultural nuances and preferences when integrating *M. oleifera* into local diets, ensuring that it aligns with traditional culinary practices, further research on its nutritional composition, potential health benefits and optimal ways of consumption, must be encouraged.

Proximate analysis: To investigate the pharmacological value of Tunisian *M. oleifera*, the phytochemical properties of the plant were performed in order to confirm whether it retains its different nutritional values and its richness compositional with the climate and the new planting conditions. Table 2 were presented average values of moisture, fiber, dry matter, total nitrogen, mineral matter, organic matter, total ash, sulphuric ash, hydrochloric ash, water-extractable substances and 80% ethanol-extractable substances contained in the leaves of *M. oleifera*.

The results showed that the powder of leaves had a low water content (>10%) compared to the standard established by the international pharmacopeia (9.21%) which is recommended for the good conservation of plant raw materials[17]. This value closely resembles this found by Leone *et al.*[18] for *Moringa* cultivated

Table 2: Proximate analysis of *Moringa oleifera* leaves

	Percentage
Humidity	9.21±0.11
Fibers	28.65±0.24
Dry matter	46.87±0.45
Total nitrogenous matter	18.17±0.36
Mineral matter	20.73±0.10
Organic material	79.14±0.20
Total ash	11.07±0.13
Hydrochloric ash	1.74±0.08
Sulfuric ash	13.93±0.09
Water extractable substances	22.63±0.64
Ethanol extractable substances	11.63±0.91

Data expressed as Mean±Standard Deviation (n≥3)

Table 3: Mineral content in the *Moringa oleifera* leaves

Macro-elements	(g kg^{-1} DM)
Na (Sodium)	1.03±0.01
K (Potassium)	11.83±0.06
Ca (Calcium)	15.53±0.11
Mg (Magnesium)	3.36±0.03
Micro-element	(mg kg^{-1} DM)
Cu (Copper)	9.80±0.20
Zn (Zinc)	22.40±1.10
Fe (Iron)	227.20±3.30
Mn (Manganese)	51.20±0.70

Data expressed as Mean±Standard Deviation (n≥3)

in Algeria (8.6%). A compositional richness in *M. oleifera* leaves containing 28.65% fiber, 46.87% dry matter, 18.17% protein, 20.73% mineral and 79.14% organic matter. This analysis confirms the richness of the plant in inorganic minerals. In fact, the total ash rate is of the order of 11.07%. Sulphuric ash and hydrochloric ash, which provide information on siliceous materials, are in order of 1.74% and 13.93%, respectively. The low level of hydrochloric ash indicates the absence or low level of impurities in the plant material. Substances extractable by water and substances extractable by hydro-ethanol (80%) are in the order of 22.63 and 11.6%, respectively. According to Makkar and Becker[19], *Moringa* leaves are an excellent source of protein with average levels ranging from 17 to 35% of dry matter. These translate to its impact on digestion by promoting intestinal transit through fiber. The current study results are similar to *Moringa* leaves growing in Haiti while it is lower than those recorded in Algeria (27.98%), India (35.03%), Chad (31.47%) and Haiti (20.80%)[18,20]. The fiber content of Tunisian *Moringa* is slightly lower than those in Algeria (31.88%) and Chad (33.29%)[18]. The high ash content confirms that minerals occur in a modest proportion in the dry matter of *M. oleifera* leaves. This value is similar to those found by Leone *et al.*[18] and Andriambelo *et al.*[20] in their studies on *Moringa* leave growing in Algeria (13.38%), India (11.39%), Chad (10.79%) and Haiti (9.62%).

Mineral composition: The mineral composition analysis (Na, K, Ca, Mg, Cu, Zn, Fe and Mn) of *M. oleifera* leaves performed by atomic absorption spectrometry, is shown in Table 3.

The *M. oleifera* leaves can be considered rich in these minerals. Indeed, the calcium amount reached 15.53 g kg^{-1} DM in the leaves of this plant which is 4 times more than milk[21]. This high content is slightly lower than those recorded in leaves collected in India (17.50 g kg^{-1} DM), Chad (18.39 g kg^{-1} DM), Haiti (21.50 g kg^{-1} DM) and Algeria (27.43 g kg^{-1} DM)[18,20]. Magnesium content is equal to 3.36 g kg^{-1} DM in Tunisian *M. oleifera* leaves which was about 30 times higher than the content found in leaves collected in India (0.11 g kg^{-1} DM), whereas it was similar to Algerian *Moringa* (4.90 g kg^{-1} DM), Chad (5.62 g kg^{-1} DM) and Haiti (5.34 g kg^{-1} DM). This mineral is considered by recent studies to be a natural

Table 4: Phytochemical screening of *Moringa oleifera* leaves extracts

Alkaloids	Methanol	Ethanol	Hexane	Aqueous
Dragebdorff	+	-	++	+
Bouchardat	+	-	+	+
Mayer	-	+	+	+
Carbohydrates	-	-	-	-
Cardiac glycosides	++	-	±	+
Anthranol glycosides	-	-	-	-
Cyanogenic glycosides	-	-	-	-
Saponosides	+	-	-	±
Diterpenes	+	+	+	+
Triterpenes	+	-	-	+
Phenols	+	+	-	-
Phytosterols	+	+	+	-
Tannins	-	-	+	-
Flavonoids	+	+	-	-
Aminoacids	++	-	+	-
Proteins	-	-	-	-
Phlobatannins	-	-	-	-
Quinones	±	±	-	+
Oxalates	-	-	-	-

++: Very abundant, +: Abundant,±: Not abundant and -: Not detected

anti-stress[22,23]. The potassium content reached 11.83 g kg^{-1} DM, according to Anwar *et al.* (2007)[24], it is 3 times higher than in bananas. The sodium value is equal to 1.03 g kg^{-1} MS which is in agreement with that detected in the leaves collected in India (1.16 m kg^{-1} DM) and slightly lower than those recorded in the *Moringa* leaves native to Haiti, Chad and Algeria (2.63, 3.08 and 7.91 m kg^{-1} DM, respectively)[18,20]. This mineral salt preserves normal fluid balance and plays a key role in nervous functioning and muscle contraction[25,26]. The iron content reached 227.20 mg kg^{-1} DM which is higher than those detected in the leaves collected in Haiti (119.10 mg kg^{-1} DM) and Chad (170.3 mg kg^{-1} DM). This trace element is considered a primary source of body energy[27]. Manganese is present at 51.20 mg kg^{-1} DM. Manganese is one of the essential trace elements involved in the defence against free radicals[28,29]. Zinc (22.40 mg kg^{-1} DM) and copper (9.80 mg kg^{-1} DM) contents were similar to those found in the collected leaves in Algeria, India, Chad and Haiti with contents ranging from 13.50 to 30.90 mg kg^{-1} DM and 6.60 to 12.20, respectively[18,20]. This remarkable difference in the mineral content may be related to multiple factors that may influence this composition. These factors include climatic and geographic conditions, the stage of development of the plant and the experimental conditions of the analyses[30]. The Tunisian *M. oleifera* retains its nutritional value. This tree keeps in fact, high contents of various essential minerals for growth and body development which explains its consideration as a common food plant and its use in combating malnutrition, particularly among infants and nursing mothers worldwide[31].

Phytochemical screening: Qualitative phytochemical screening methods detected the presence of particular compound families in methanoic, ethanoic, hexatonic and aqueous extracts of the leaves of Tunisian *M. oleifera*. Depending on the case, the change in color and the formation of a precipitate or foam during handling indicate the presence of the desired phytochemicals. The results obtained at the end of the preliminary tests are presented in Table 4.

The qualitative tests showed the presence of eleven chemical compound families in the different *M. oleifera* leaf extracts which are: Alkaloids, cardiac glycosides, saponosides, diterpenes, triterpenes, phenols, phytosterols, tannins, flavonoids, amino acids and quinones. Under the used operating conditions, carbohydrates, anthranol glycosides, cyanogenic glycosides, proteins, phlobatanins and oxalates were not detected in the prepared extracts. Phytosterols were absent from the water extract due to their insolubility in water, attributed to their substantial molecular size. Amino acids were undetectable

in both the ethanolic and aqueous extracts. Despite the proximate analysis indicating the existence of extractable proteins in *M. oleifera* leaves, ethanol and water might possess higher polarity compared to these proteins, hence inadequately solvating the larger and less polar protein structures within the leaves. This is in agreement with the observations made by Shahriar *et al.*[32], who identified the presence of flavonoids, tannins, saponins, alkaloids, glycosides, carbohydrates and triterpenoids in *Moringa* leaves native to Bangladesh. In addition, Al-Owaisi *et al.*[33] confirmed the richness of the Nigerian *M. oleifera* leaves by the presence of tannins, saponins, phenols, alkaloids and phlobatannins as major secondary metabolites present in the leaf of this plant. The presence of different detected phytochemicals may be the origin of the therapeutic properties and medicinal value of this plant. Several studies highlight the importance of these detected compounds. Indeed, polyphenols could prevent many pathologies such as cancer[34], cardiovascular and degenerative diseases[35]. Flavonoids were reported as antioxidants, anti-allergic, antispasmodic, antibacterial, antiviral, hepato-protective, anti-inflammatory and anti-carcinogen[36-39]. Tannins were known for their antiseptic, antibacterial and antifungal effects[40]. Saponins were usually hemolytic. It was also quite frequently reported that these molecules provide defense against microbial or fungal attacks and moreover have been shown to have hypotensive and cardio-depressive properties[41]. Alkaloids are often presented as anti-hyperglycemic and anti-inflammatory[42,43]. Phytochemical screening has been shown to be quite useful in detecting the presence of the plant's bioactive chemical constituents, which can then be identified, quantified and tested for new pharmacological uses.

Based on these findings, this study highlights *Moringa oleifera*'s potential as an innovative solution for Southern Tunisia's agricultural challenges due to its adaptability and nutritional value. It could enhance food security, economic growth and environmental sustainability. The tree's adaptability and various uses offer avenues to address malnutrition and foster sustainable farming. Collaboration between makers and farmers is recommended for integration, raising awareness and diversifying income sources. While limitations include regional focus and short-term analysis, further research should explore cultivation techniques, processing and market potential. The study fills a void in research by examining *M. oleifera* consumption, highlighting the necessity for validation and cultural understanding. It suggests strategic implementation and continued investigation for optimal utilization.

CONCLUSION

The survey on Tunisian consumer nutrition knowledge of newly introduced *M. oleifera* in Southern Tunisia highlighted its significance due to its medicinal and nutritional properties. The plant is now integrated into local diets, often consumed through infusion. Proximate analysis revealed substantial fiber content, beneficial for intestinal transit and notable mineral richness in arid-cultivated *M. oleifera* leaves. Phytochemical screening identified alkaloids, phenols, tannins, flavonoids, carbohydrates, saponins and terpenes among seventeen tested compounds. This study underscores *M. oleifera*'s retained nutritional value and compositional richness in Tunisia's specific climate and planting conditions.

SIGNIFICANCE STATEMENT

This study explores the potential of *Moringa oleifera* cultivation in Southern Tunisia, to understand its suitability in arid climates. The purpose is to assess its nutritional and cultural significance, thus providing insights into the feasibility of integrating it into local agricultural practices. The research unveils that *M. oleifera* is gaining popularity among Tunisians due to its medicinal and nutritional properties. The ethnonutritional survey highlights its integration into local diets and culinary practices. Moreover, physicochemical analyses confirm the tree's robust retention of nutritional value. This pioneering study uniquely bridges the gap between the scientific evaluation of *M. oleifera* and its incorporation into dietary practices, offering essential insights into its role as a sustainable crop for addressing agricultural challenges in resource-constrained regions.

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