

Research Article

Nutritional Content and Elemental and Phytochemical Analyses of *Moringa oleifera* Grown in Mexico

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Moringa oleifera is a tree distributed in Mexican semiarid and coastal regions. *M. oleifera* is used in practice in the treatment of various diseases and is available without a medical prescription, often in the form of an herbal infusion for everyday use. The aim of the present study was to evaluate the chemical composition and nutritional values of dried *M. oleifera* leaf powder collected from two different regions in Mexico. All samples of *M. oleifera* exhibited moisture levels varying from 3.06 to 3.34%, lipids from 10.21 to 10.31%, fiber from 7.29 to 9.46%, ashes from 10.71 to 11.18%, crude protein from 10.74 to 11.48%, and carbohydrates from 54.61 to 57.61%. The predominant mineral elements in the leaf powder according to ICP-MS were Ca (2016.5–2620.5 mg/100 g), K (1817–1845 mg/100 g), and Mg (322.5–340.6 mg/100 g). The HPLC analysis indicated the presence of phenolic acids (gallic and chlorogenic acids) and flavonoids (rutin, luteolin, quercetin, apigenin, and kaempferol). We concluded that Lombardia *M. oleifera* samples could be employed in edible and commercial applications. Our results showed that the highest mean value of As from the San Pedro samples exceeds the recommended level and may constitute a health hazard to consumers.

1. Introduction

Plants have been an important source of medicine for thousands of years. Even today, the World Health Organization (WHO) estimates that up to 80% of people still rely primarily on traditional remedies such as herbs for their medicines [1]. The medicinal value of these plants is due to the presence of a variety of phytochemicals and their elemental composition. The role of medicinal plants in disease prevention or control

has been attributed to the antioxidant properties of their constituents, usually associated with a wide range of amphipathic molecules that are broadly referred to as polyphenolic compounds [2]. There is a growing interest in the development and evaluation of natural antioxidants from medicinal plant materials in the food industry and the field of preventive health care. Among those herbs, one promising species is *Moringa oleifera* Lam. (*Moringa* or drumstick tree), which is native to the sub-Himalayan regions of Northwest India.

It is widely distributed throughout Africa, Saudi Arabia, Southeast Asia, the Caribbean Islands, and South America. Every part of *M. oleifera* has medicinal properties and is commercially exploitable for the development of medicinal and industrial byproducts [3].

Traditionally, the leaves, fruits, flowers, and immature pods of this tree are edible; they are used as a highly nutritive vegetable in many countries, particularly in India, Pakistan, the Philippines, Hawaii, and some African nations [4–6]. In developing nations, *M. oleifera* is used as an alternative to imported food supplements to treat and combat malnutrition, especially among infants and nursing mothers, by virtue of its chemical constituents [7].

Several valuable reviews of the ethnobotanical uses of *M. oleifera* are available [8–11]. Moringa has been found to be a good source of polyphenols and antioxidants [11]. Phytochemicals such as vanillin, omega fatty acids, carotenoids, ascorbates, tocopherols, beta-sitosterol, moringine, kaempferol, and quercetin have been reported in its flowers, roots, fruits, and seeds. The leaves, in particular, have been found to contain phenolics and flavonoids [12, 13]; these compounds have various biological activities, including antioxidant, anticarcinogenic, immunomodulatory, antidiabetic, antiatherogenic, and hepatoprotective functions and the regulation of thyroid status [14–16]. Moreover, leaves contain trace elements that are essential to human health. For instance, magnesium, iron, selenium, and zinc play an important role in metabolism, and interest in these elements is increasing together with reports relating trace element status and oxidative diseases [17, 18]. However, a recent study has shown that dried *M. oleifera* leaves contain lead at very high values of 352.0 mg/L [19]. Therefore, it is very important to identify the mineral composition of *M. oleifera* leaves that are widely consumed by humans and animals.

In Mexico, *M. oleifera* is widely cultivated in different zones of the country and is found in more than ten states from Sonora to Oaxaca on the Pacific side. Few studies have been conducted on nutritional and phytochemical composition [20, 21]; however, to date, a detailed composition of the leaves of *M. oleifera* that is native to Mexico has not been reported yet. In addition, it is important to bear in mind that the mineral and phenolics contents present in leaves depend on several factors such as geographical area where the plant is cultivated, type of soil, water and fertilizers, industrialization process, and storage conditions. Taking these precedents into consideration, the aim of this study was to evaluate the phytochemical constituents of methanolic extracts and trace elements and nutritional values present in *M. oleifera* leaves grown in the northeast and west regions of Mexico.

2. Materials and Methods

2.1. Reagents and Standards. The methanol and acetonitrile used in this study were of HPLC grade and were purchased from JT Baker, USA, and Caledon Lab., Canada. HPLC-grade phenolic standards (gallic and chlorogenic acids, rutin, luteolin, quercetin, apigenin, and kaempferol) were of analytical reagent grade and were purchased from Sigma-Aldrich (St. Louis, MO, USA). Demineralized water was taken from a

Milli-Q Plus water purification system (Millipore, Bedford, USA). The multielement standard mixture solutions contained Ba, Bi, Li, Cu, Co, In, Li, Ni, Pb, and V; the others contained Ba, Ca, Mg, and Sr. Analytical grade 65% w/v nitric acid (HNO_3) (JT Baker) was used in the decomposition.

2.2. Samples Preparation. *M. oleifera* leaves were collected from Mexican cultivars (Lombardia, Michoacán, located on 19°01'30"N and 102°05'39"W and San Pedro, Coahuila, located on 25°45'35"N and 102°58'54"W, Mexico, April 2011) and a voucher of the specimen was deposited in the CIIDIR-IPN herbarium, Durango, Mexico, for future references. The plants were identified and authenticated by M. Sc. Alberto González Zamora, Faculty of Biology, UJED. The leaves were cleaned and processed as described by previous researchers [22, 23]. A set of five samples from each geographical origin were randomly collected and analyzed. All materials were air-dried and powdered and protected from light until further analysis.

2.3. Chemical Analysis. Representative subsamples were dried in a forced drought oven at 105–110°C to a constant weight for moisture determination. Crude protein, crude lipid, crude fiber, and ash were analyzed by triplicate according to AOAC [24]. The total carbohydrates were determined by the difference method [100 – (proteins + fats + moisture + ash in percentage)] [25, 26]. The energy values (kcal/100 g) were determined by multiplying the values of carbohydrates, lipids, and proteins by factors of 4, 9, and 4, respectively, and taking the sum expressed in kilocalories [27].

2.4. Extraction of Phenolic Compounds. The extracts were prepared using 23 g of dry-ground sample and 260 mL of 80% methanolic aqueous solution by successive maceration. The mixtures were shaken in a magnetic grid at room temperature for 21 h. They were then filtered through Whatman filter paper number 1. The final extract was concentrated on a rotator evaporator, and the filtrate was placed in a deep freezer for 24 h and lyophilized to obtain a powdered extract. All of the extractions were performed in triplicate, and the supernatants were kept at –40°C until further analysis.

2.5. ICP-MS Analysis. The instrumentation used for digestion consisted of a closed microwave oven (MARS-Xpress, CEM). The multielemental analysis was performed by inductively coupled plasma mass spectrometry (ICP-MS) with a reaction cell (XSeries 2, Thermo Scientific). Prior to analysis, the samples were homogenized in a mortar; approximately 0.5 g of the sample was accurately weighed in an acid-washed polytetrafluoroethylene (PTFE) digestion tube, and 5 mL of HNO_3 (65% w/v) was added. The tube was heated in a microwave oven at a power setting of 50% and 150°C for 10 min, 70% and 220°C for 20 min, and 10% and 100°C for 15 min. The digest was transferred into a 50 mL acid-washed volumetric flask, which was filled with demineralized water and stored in a polypropylene container. One water blank was run with each batch of samples.

2.6. HPLC-DAD Analysis. Seven phenolic standards and samples were dissolved in the mobile phase, yielding concentrations of 25 $\mu\text{g}/\text{mL}$. The solutions were filtered through a 0.45 μm membrane filter, and the evaluation of each calibration curve was fitted by linear regression. An Agilent 1200 HPLC Series (Agilent, Palo Alto, CA, USA) was operated at 30°C, equipped with a degasser, a diode array detector (DAD), a quaternary gradient pump, and an Eternity C18 reversed-phase analytical column of 150 mm \times 4.6 mm and a 3.5 μm particle (Sigma-Aldrich, USA). The mobile phase was a binary gradient: methanol and buffer solution. The buffer contained TCA 0.1% solution in water, and its pH was adjusted to 2.1. The linear gradient began with 20% to 50% methanol over the first 30 min, followed by 70% methanol over the next 30 min. The flow rate was 0.6 mL/min, and data were collected at 270 and 352 nm. The injection volume was 20 μL . Data acquisition, peak integration, and calibrations were performed with Agilent 1200 Chemstation software. The results are reported as means \pm standard deviations of triplicate independent analyses.

2.7. Statistical Analysis. The data were expressed as means \pm SD, and the results were statistically analyzed using a Holm-Sidak test and one-way analysis of variance (ANOVA) on a SigmaPlot program v. 11.0 for Windows. The differences were considered statistically significant if $p < 0.05$.

3. Results

3.1. Chemical Analysis. The proximate and nutrient analyses of *M. oleifera* play a crucial role in assessing its nutritional significance ($p < 0.05$). The proximate analysis for both cultivars is presented in Table 1, along with the values found in the literature for identical *M. oleifera* for comparative purposes. Nonsignificant differences were observed between the proximate analyses of the samples studied in Lombardia and San Pedro. The mean moisture, lipid, fiber, and ash values found in the present study are in agreement with the values reported [28]. The result shows that Mexican cultivars contain crude protein (10.6%), crude fiber (about 8%), ash (about 11%), carbohydrates (about 56%), moisture (about 3.2%), and lipid (about 10.2%). Carbohydrates are the principal sources of energy. The ash content of about 11% indicates that the leaves are rich in mineral elements. The mean protein content found in the Mexican samples ranged from 10.74 to 11.48%. This is a lower protein level than the 27.2% reported by other authors [21, 29–31], though it may still be valuable as a protein source. The variation in chemical analyses between our cultivars and the reference may depend on seasonal variations, the plants' stages of development, and the techniques employed to collect leaf samples before the experimental analysis. The chemical composition values confirmed that *M. oleifera* leaves are an excellent food source, justifying its direct use in human nutrition or development of balanced diets for animal nutrition.

3.2. Elemental Analysis. Different soils contain a particular of mineral elements qualities and quantities whose bioavailability depends on soil properties (pH, clay and humid complex,

TABLE 1: Nutritional composition of dried *M. oleifera* leaf powder.

Parameter	Lombardia	San Pedro	[28]
Moisture (%)	3.34 \pm 1.36	3.06 \pm 1.38	7.4 \pm 2.89
Lipid (%)	10.31 \pm 1.2	10.21 \pm 1.83	6 \pm 2.5
Fiber (%)	7.29 \pm 0.84	9.46 \pm 1.14	9 \pm 7.45
Ash (%)	10.71 \pm 0.81	11.18 \pm 0.19	17.6 ^b
Protein (%)	10.74 \pm 1.3	11.48 \pm 1.4	24 \pm 5.8*
Carbohydrates (%) ^a	57.61 \pm 2.19	54.61 \pm 0.6	36 \pm 9.2*
Energy value (Kcal/100 g)	366.2 \pm 4.23	356.25 \pm 6.48	304 \pm 87

Values are mean \pm SD, analyzed individually in triplicate, and are expressed as g/100 g leaf powder.

^{a, b} Calculated by difference.

* Difference significative between Mexican samples against a reference $p < 0.018$.

TABLE 2: Measured concentration in (mg/100 g) of trace element in the *M. oleifera* leaf powder.

Elements	Lombardia	San Pedro	[28]
Calcium (Ca)	2016.5 \pm 22.6	2620.5 \pm 5.6**	1897 \pm 748.4
Magnesium (Mg)	322.5 \pm 0.0	340.6 \pm 2.8**	473 \pm 429.4
Potassium (K)	1845 \pm 7.0	1817 \pm 14.1	1467 \pm 636.7
Sodium (Na)	8.13 \pm 0.6	40.78 \pm 0.7**	220 \pm 180
Iron (Fe)	19.37 \pm 6.6	7.07 \pm 0.4*	32.5 \pm 10.78
Zinc (Zn)	1.0 \pm 0.7	1.6 \pm 0.6	2.4 \pm 1.12
Copper (Cu)	1.03 \pm 0.47	0.41 \pm 0.0	0.9 \pm 0.48
Selenium (Se)	9.55 $\times 10^{-3}$ \pm 0.0	0.107 \pm 0.0**	ND
Lead (Pb)	0.355 \pm 0.0	0.2 \pm 0.0**	ND
Arsenic (As)	5.5 $\times 10^{-3}$ \pm 0.0	0.28 \pm 0.0**	ND

Results are mean \pm SD, analyzed individually in triplicate.

ND: nondetermined.

Statistically significant differences between the means of both cultivars are denoted as ** $p < 0.001$; * $p < 0.05$.

and mineralogy) [32]. Ten elements, including toxic ones such as lead and arsenic, were determined to be present in two crops of *M. oleifera* collected from Lombardia and San Pedro locations. The elements were determined by ICP-MS after wet digestion of the dried sample with concentrated nitric acid in closed PTFE vessels using a microwave oven. Ca, Mg, K, Fe, and Na were present at levels of mg/100 g dry matter, whereas Zn, Cu, and Se were present at $\mu\text{g}/100$ g levels, in close agreement with levels previously reported [30], and these values fall within the ranges reported (Table 2, reference column). The elemental analysis of our samples revealed high contents of Ca (2016 to 2620 mg/100 g), Mg (322 to 340.6 mg/100 g), and K (1817 to 1845 mg/100 g), while Zn, Cu, and Se were 1, 1, and 0.1 mg/100 g, respectively. Iron was abundant in Lombardia cultivar, whereas the Ca, Mg, and Na levels were higher in San Pedro cultivar (Table 2). These values of *M. oleifera* leaves from Mexico agree with those found in Burkina Faso and India [29, 30]. The Fe varied among the leaf samples collected from Lombardia and San Pedro, ranging from 19.37 to 7.07, respectively. However, this level is low when compared to the contents of some results

from India [30]. The Pb level in Lombardia and San Pedro cultivars was below the limit set by the WHO acceptable daily intake of Pb for adults of 0.21–0.25 mg/day [33]. The lowest As content was observed in the Lombardia cultivar (0.0055 mg/100 g), whereas the content of As in the samples collected from San Pedro was the highest (0.28 mg/100 g), which correlates with the extremely high As level in San Pedro soil. Because the levels in the Lombardia sample were still within the prescribed limit for food at 1 mg/kg according to the WHO/FAO standard [34], this sample could be recommended as a source of essential elements. However, because the As levels in San Pedro samples were found to be higher than the limit set by the WHO, it is necessary to study them in further detail to avoid overconsumption and cumulative toxicity from long-term use.

3.3. Quantification of Phenolics. RP-HPLC with DAD detection was employed for the identification and quantification of main phenolic compounds present in the methanolic extracts. The quantifications of phenolics (mg/g dry matter) were accomplished by comparing retention times and peak areas between the standards and the samples. Curves of the standard compounds were made using serial dilutions of standards dissolved in HPLC-grade methanol. The separation conditions of the phenolic compounds were taken from a previous study [35]. A representative chromatogram of a phenolic standard mixture under optimal conditions is presented in Figure 1, demonstrating that the peaks of the phenolic standards were separated with satisfactory efficiency and resolution in 70 min of analysis time. The eluting peaks were monitored with a diode array detector at 270 nm and 352 nm, and the spectra were recorded between 0 and 62 min. Detection was accomplished by using these two wavelengths to observe a wide range of phenolic classes: phenolic acids and isoflavonoids at 270 nm and flavonoids at 352 nm. A high degree of linearity was observed in all of the phenolic compounds (Table 3); the square of correlation coefficient for the calibration curve estimates the reproducibility of the method. All of the *M. oleifera* methanolic extracts from the different varieties were injected under these conditions.

Figure 2 presents a comparison between Lombardia extract and San Pedro extract regarding the presence of bioactive compounds by HPLC-DAD analysis. The results show that their chromatographic separations were similar; however, the peak sizes were different, which determines the amount and proportion of each compound. Only seven phenolic compounds were identified: gallic acid, chlorogenic acid, luteolin, rutin, quercetin, kaempferol, and apigenin. The most abundant phenolic acid was chlorogenic acid, and rutin was the most abundant flavonoid in all analyzed samples of both cultivars. The quantitative determination of these compounds is shown in Table 4. An effect of cultivation location on five phenolics content was observed for chlorogenic acid, luteolin, and apigenin, but not for gallic acid and quercetin (Figure 2 and Table 4). The plant cultivated at Lombardia contained higher phenolics than those at San Pedro. In addition, Lombardia cultivar showed larger quantities of chlorogenic acid and luteolin than those

TABLE 3: Elution order of polyphenolic compounds separated by HPLC-DAD.

Reference compound	Retention time (min)	Linear range ($\mu\text{g/mL}$)	r^2
270 nm			
Gallic acid	4.53 \pm 0.05	1.0–9.9	0.9999
352 nm			
Chlorogenic acid	15.52 \pm 0.38	0.6–23.1	0.9995
Luteolin	46.89 \pm 0.29	0.5–4.0	0.9993
Rutin	48.25 \pm 0.25	9.9–49.5	0.9996
Quercetin	56.07 \pm 0.21	0.99–3.0	0.9999
Kaempferol	59.79 \pm 0.81	0.66–3.3	0.9998
Apigenin	60.59 \pm 0.15	0.1–1.0	0.9995

Each value is the mean \pm SD of triplicate determinations.

TABLE 4: Characterization ($\mu\text{g/g}$ dry matter) of polyphenolic compounds separated by HPLC-DAD.

Compound	San Pedro	Lombardia
Gallic acid	49.07 \pm 4.53	43.28 \pm 1.83
Chlorogenic acid	286.13 \pm 15.09	479.53 \pm 16.24**
Luteolin	44.56 \pm 2.03	94.27 \pm 7.6**
Rutin	603.35 \pm 13.48	845.25 \pm 18.83**
Quercetin	46.18 \pm 0.6	49.89 \pm 6.98
Kaempferol	46.43 \pm 2.14	67.36 \pm 7.86*
Apigenin	24.41 \pm 2.16	8.74 \pm 0.95**

The concentration is given in $\mu\text{g/g}$ of the dry plant material for triplicate injections.

Statistically significant differences between the means of both cultivars are denoted as ** $p < 0.001$; * $p < 0.05$.

of San Pedro cultivar. The average mean of apigenin in San Pedro cultivar was about 3 times greater than that found in Lombardia cultivar. Overall, both the phenolic compounds and their concentrations are generally consistent with those found in *M. oleifera* leaves by other plant researchers [13, 36]. The differences observed could be attributed to the influence of several factors, such as different climate, irrigation system, and location of the cultivars. These phenolic compounds act as potent metal chelators and free-radical scavengers; therefore, our results indicate that the methanolic leaves extracts of *M. oleifera* grown in Mexico may be used in treating diseases related to free-radical reactions.

4. Discussion

M. oleifera is perhaps the most useful traditional medicinal plant found in several African and Asian countries. Anecdotal evidence of the benefits of *M. oleifera* has fueled a recent increase in the exploitation and attention to its many healing benefits, specifically the high nutrient composition of the leaves and seeds.

Although various studies have been conducted on the *Moringa* species in India, Africa, and Pakistan, gaps and inconsistency between the information on the nutrient of this

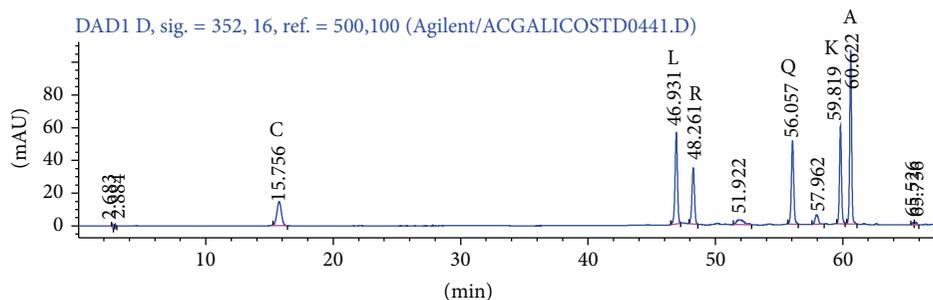


FIGURE 1: Chromatogram of a mixture of standards' polyphenols; DAD detection is at 352 nm. The chromatographic conditions are described in the text. Peak identification: C: chlorogenic acid; L: luteolin; R: rutin; Q: quercetin; K: kaempferol; A: apigenin.

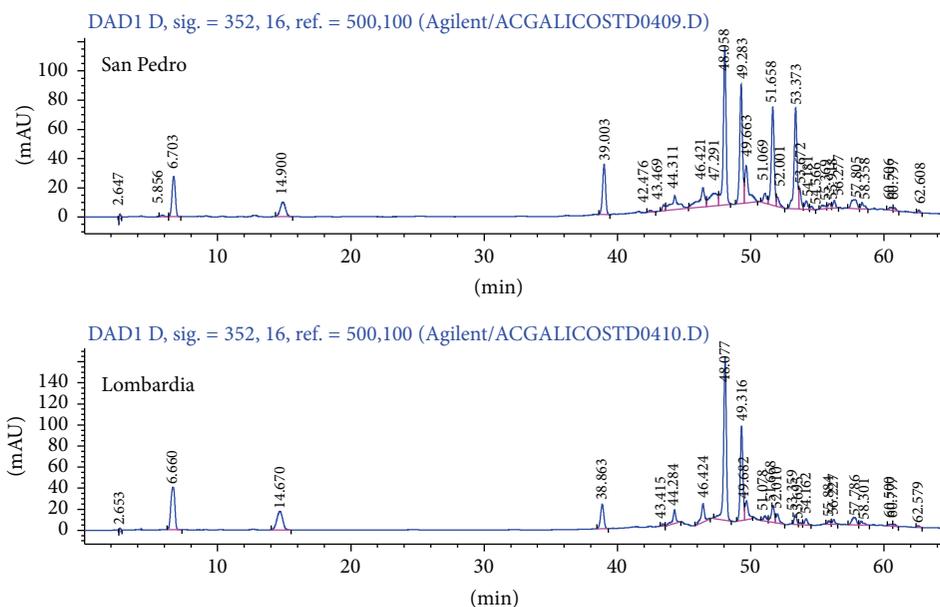


FIGURE 2: Comparison of gradient HPLC analyses of two varieties of *M. oleifera* flavonols. Detection was performed at 352 nm.

plant remain [28], and there are few experimental studies regarding the potential nutritional value of the plant grown in Central and South America [23, 37, 38] and some reports on *M. oleifera* cultivated in Mexico [20, 21]. However, in recent years, the pharmaceutical properties associated with *M. oleifera* have garnered increasing attention in the Mexican market, in part because the dietary supplements are natural compounds.

The results of our study suggest that the concentration of moisture, ash, fiber, lipid, and carbohydrates exhibited no changes in the proximate analysis because there was no statistical difference (at the level of 95%) from the two different *M. oleifera* cultivars, and their amounts were comparable to the concentration ranges found in the literature [28]. However, numerous studies have shown that *M. oleifera* leaves offer high protein content; contrary to these findings, our study did not find a high level of protein. This variation may be explained by several factors, such as climate and the geography of development of the crop. Despite this difference, the proximate analysis shows that *M. oleifera* leaves are a good source of proteins, lipids, and carbohydrates. In addition, the

high ash content indicates that the leaves are a good source of inorganic minerals.

The previous data on the characterization and quantitation of trace element studies present in *M. oleifera* leaves are rather limited. Important studies in this area were presented by [39], who characterized eleven different elements in South India leaf samples, and by [29], who identified and quantified seven diverse elements in *M. oleifera* cultivated in Africa.

The most abundant macroelements found in our analysis were Ca, Mg, and K. The San Pedro cultivar investigated in this study exhibited higher concentrations of Ca and Mg. In contrast, the K content was higher in the Lombardia cultivar. The concentrations of these macronutrients overlapped with those reported for leaves of *M. oleifera* grown in the literature. Furthermore, the concentration of Na in our cultivars was lower than the levels reported in several studies [40, 41]. Therefore, the minerals found in *M. oleifera* play both a curative and preventive role in combating human disease. For example, Ca is a multifunctional nutrient essential to the body metabolism [42], and Ca deficiency leads to osteoporosis. Thus, *M. oleifera* is considered to be a natural

cure for osteoporosis [43]. Furthermore, there is strong biological plausibility for the direct impact of Mg intake on cardiovascular disease prevention, insulin sensitivity, and diabetes [44].

The most abundant microelements found in this study were Fe, Zn, Cu, and Se. In the Philippines, *M. oleifera* is known as “the mother’s best friend” due to its use to increase nursing mothers’ milk production [45]. This effect can be attributed to Cu and Zn, which are essential in increasing the rate of pregnant female milk production [46]. Zn is also important in the healing of wounds and functions as an antioxidant. *M. oleifera* extract has shown significant wound-healing activity against excision, restored incision, and dead-space wounds. This effect could be due to *M. oleifera* high zinc content [47]. Fe has several essential functions in the body, such as its roles in oxygen transport and oxidative metabolism [48]. Se is an essential element in both animal and human nutrition. In addition, the antitumorigenic effects of Se compounds have been described in a variety of *in vitro* and animal models, suggesting that supplemental Se in human diets may reduce the risk of cancer [49]. The anticarcinogenic activity of *M. oleifera* was described [50], and their results suggest that the minerals present in *M. oleifera* leaves may contribute to its therapeutic properties.

The use of herbal medicines is increasing in both developing and developed countries due to their reasonable prices and, in particular, to the assumption that natural products are safe [51]. However, most herbal products are not validated according to the recommended pharmaceutical guidelines and often contain toxic and lethal concentrations of toxic heavy metals [52].

Arsenic is a metalloid that acts on cells through a variety of mechanisms, influencing numerous signal transduction pathways and resulting in cellular effects such as apoptosis induction, growth inhibition, and angiogenesis inhibition [53]. Pb is a metallic element that emanates into the environment from various sources, including the industrial waste, combustion of fossil fuels, and the use of agrochemicals. Higher levels of Pb cause a variety of acute and chronic health problems, including cancer, kidney damage, heart problems, and even death [54].

San Pedro is part of the region known as the “Comarca Lagunera.” It is located in the Coahuila and Durango states in Mexico, a region in which the aquifers are severely contaminated with As [55]. High levels of As have been detected in the groundwater that is used as both drinking water for humans and dairy cattle and for agricultural irrigation [56].

Plants vary widely in their tolerance to toxic metals. *M. oleifera* from San Pedro can grow normally while accumulating arsenic in its roots (data not shown). Among the *M. oleifera* samples considered in this study, the lowest As content was observed in the sample grown in Lombardia (0.0055 mg/100 g), whereas the As content in the samples collected from San Pedro was much higher (0.28 mg/100 g), showing obvious signs of environmental contamination.

The suggested concentration of Pb in plant species is 2 to 6 mg/L [57]. The concentration of Pb was minimum at 0.2–0.35 mg/100 g in our samples. These results were similar to those described for plants grown in other parts of the

world. Various studies have found Pb at similar levels (<0.001 to 2.6 μg Pb/g) in medicinal plants in Italy, Egypt, and the United States [58–60], whereas the highest concentration is found in herbal medicine used in Brazil [61]. A recent study reported lower Pb and As levels in *M. oleifera* leaves [62]. *M. oleifera* varieties in this study contain lower Pb level, which further supports their use as food supplement and their medicinal benefits.

Because it is associated with several metabolic diseases and age-related degenerative disorders are closely related to the body’s oxidative processes, the use of *M. oleifera* as a source of antioxidants to combat oxidation warrants further attention. Some authors reported that methanolic extract of *M. oleifera* leaves had a high antioxidant activity, which may be attributed to the presence of polyphenolics and other antioxidant substances. In addition, the data obtained emphasize the free-radical scavenging effect of an aqueous extract of *M. oleifera* over DPPH free radical, superoxide, and nitric oxide radicals and the inhibition of lipid peroxidation [63, 64].

To better understand the association of flavonoids intake and health outcomes, analyses of flavonoids in plant foods, an intense area of research, and clinical and epidemiological studies are required [65, 66]. A phytochemical analysis was performed to determine the major class of compounds present in the leaf extracts. The quantitative estimations of the polyphenols have shown that the Lombardia samples contained a higher amount of chlorogenic acid ($479.53 \pm 16.24 \mu\text{g/g}$), rutin ($845.25 \pm 18.83 \mu\text{g/g}$), and luteolin ($94.27 \pm 7.6 \mu\text{g/g}$), whereas the San Pedro samples had higher amounts of apigenin ($24.41 \pm 2.16 \mu\text{g/g}$). Our results are consistent with those from previous studies, which strongly suggest that *M. oleifera* may be an important source of natural antioxidants [67, 68]. Previous phytochemical investigations have identified five flavonols in *M. oleifera*, including gallic acid, chlorogenic acid, rutin, ellagic acid, ferulic acid, quercetin, and kaempferol [13, 36]; however, only quercetin, kaempferol, gallic acid, and chlorogenic acid were found in all the studies. However, our values of quercetin and kaempferol are out of the range of those reported by different authors for methanolic extract of *M. oleifera* leaves [13, 68–70]; these two compounds have been reported as the most abundant in the leaves of *M. oleifera* from India, Pakistan, and African nations. It has been reported that the leaves of this plant have very low levels of luteolin ($6.2 \mu\text{g/g}$) [71]. This value corresponds to 15 times less than the obtained in this study (see Table 4). A possible explanation for these results is that the flavonols are the most widespread of the flavonoids in plant food and, unlike flavonols, apigenin and luteolin are not widely distributed with significant concentrations. In addition, the extraction yield of antioxidant compounds from plant materials is influenced primarily by the conditions under which the process of liquid-solid extraction is achieved, the type of solvent used to separate the soluble fraction from the permeable solid, and the degree of polymerization of the phenolics and their interactions with the other components [72, 73].

As mentioned earlier, the abundance and diversity of flavonoids present in *M. oleifera* may be responsible for their

therapeutic effectiveness against various diseases [11]. Though *M. oleifera* is known to contain quercetin and kaempferol, traceable amounts of chlorogenic acid and derivatives have been detected within the leaves from Ghana, Senegal, and Zambia [74]. Chlorogenic acid and its isomers are esters of quinic and caffeic acids that have abilities to inhibit oxidation and also promote various pharmacological activities such as antiobesity, reduction of plasma and liver lipids, and inhibition of acute lung injury [75–77]. On the other hand, rutin is present in substantial amounts in our *M. oleifera* leaves and some investigations showed that this compound has a broad range of physiological activities [78].

It is interesting to mention that when flavones are methoxylated, metabolic stability and membrane transport in the intestine/liver dramatically increase, thus improving oral bioavailability. In addition, methoxyflavones also show increased cancer chemopreventive properties when compared to more common unmethylated flavones [79].

Although the bioactivity of the individual compounds may be known, their effects on health may not be as significant as the combination of the entire class of bioactives working through multiple mechanisms of action. The use of dried leaf powder is recommended to achieve the health benefits of the additive and synergistic effects of the constituents present in the whole leaves. Obviously, it is not yet clear how polyphenolic compounds and minerals are associated with the reduction of human diseases. Therefore, it is necessary to conduct further studies on their *in vivo* activity, bioavailability, and toxicity. Actually, we are conducting studies on the therapeutic properties of our *M. oleifera* leaves extracts.

5. Conclusion

Our results showed that the Mexican species of *M. oleifera* have nutritional potential because their leaves contain a high concentration of energy, nutrients, minerals, and phenolic constituents, mainly flavonoids and phenolic acids, which represent a good source of natural antioxidants. Therefore, the therapeutic potential of *M. oleifera* may be due to the presence of these constituents. The presence of harmful elements such as arsenic and lead in Lombardia *M. oleifera* leaf powder appears to be within permissible limits, but we discovered that a maximum permissible level of As is exceeded in San Pedro samples. Our results suggest that quality assurance and monitoring of toxic metals are needed for plants intended for human consumption.

Conflict of Interests

The authors declare no conflict of interests.

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