

Nutritional and Phytochemical Characterization of *Moringa oleifera* Leaves from an Arid Region: LC-MS and AAS-based Profiling for Potential Dietary Applications

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ABSTRACT

Background: *Moringa oleifera* (MO), widely recognized for its exceptional nutritional value and therapeutic properties, has attracted considerable scientific interest. However, the specific bioactive phytochemicals associated with its potential effects in the treatment of hyperlipidemia and obesity remain insufficiently elucidated. **Objective:** This study aimed to evaluate the nutritional, therapeutic, and antioxidant potential of *Moringa oleifera* leaves (MOL) from the Ghardaïa region (Southern Algeria) using hydromethanolic (HME) and aqueous (AE) extracts. **Materials and methods:** Proteins and carbohydrates contents were quantified using Bradford and DuBois et al. methods respectively. Mineral content was determined using atomic absorption spectrometry (AAS). Bioactive profiles were identified by Ultra-performance liquid chromatography (UPLC-ESI-MS/MS), alongside antioxidant capacity using Ferric Reducing Antioxidant Power (FRAP) assay. **Results:** HME exhibited higher protein (19.34 ± 1.48 g/100 g), while AE contained elevated total sugars (32.24 ± 1.11 g/100 g). Mineral analysis revealed significant iron (59.07 mg/100 g), magnesium (250.72 mg/100 g), and manganese (7.08 mg/100 g). UPLC-ESI-MS/MS identified 28 bioactive compounds, with Quercetin-3-glucoside (47.91%) and myricetin (17.47%) as dominant flavonoids, alongside curcumin (11.21%) and β -carotene (4.2%). Furthermore, AE exhibited significantly higher FRAP values than HME, with IC_{50} values of 207.09 ± 2.31 μ g/mL and 266.23 ± 3.63 μ g/mL, respectively. **Conclusions:** This initial report highlights MOL extracts as a sustainable and cost-effective dietary supplement for managing hyperlipidemia and obesity, due to their rich nutrient content, diverse bioactive compounds, and adaptability to arid climates.

Keywords: *Moringa oleifera*, Dietary supplement, Hyperlipidemia, Obesity, Ghardaïa.

INTRODUCTION

Malnutrition includes undernutrition, overnutrition, and micronutrient imbalances, driving nearly half of under-five deaths globally, especially in low-income countries. Without intervention, stunting may persist or affect over 149 million children under five by 2030. Beyond macronutrient totals, diet quality such as complex carbs with fiber, unsaturated fats, and diverse proteins prevents health risks, while processed high-calorie foods and inactivity fuel obesity^{1,2}. Obesity, marked by excessive adiposity, and diabetes mellitus, driven by insulin deficiency or resistance causing hyperglycemia. Hyperlipidemia, characterized by a disorder in lipid metabolism with elevated plasma triglycerides and cholesterol³. These interconnected metabolic disorders share pathophysiological pathways, including insulin resistance, systemic inflammation, and oxidative stress, which collectively promote atherosclerosis, hepatic dysfunction, and cardiovascular complications⁴. Hypolipidemic and hypoglycemic drugs reduce cardiovascular disease (CVD) mortality but have adverse side effects with long-term use⁵.

Herbal plants play a crucial role in disease prevention and treatment by providing bioactive compounds like polyphenols and flavonoids that

offer antioxidant and anti-inflammatory benefits⁶. These natural remedies align with industry trends to replace synthetic additives with functional foods, which help reduce CVD risk and promote overall health⁷. *Moringa oleifera*, native to the sub-Himalayan regions and cultivated globally, is a highly valued plant known for its exceptional nutritional and medicinal properties⁸. Adaptable to arid climates, MO is rich in essential vitamins (A, C, B-complex), minerals (potassium, magnesium, and zinc), proteins, and antioxidants. It surpasses common foods like carrots, milk, and bananas in vitamin A, calcium, and potassium content⁹.

Moringa oleifera's bioactive compounds, including polyphenols and flavonoids, exhibit antioxidant and anti-inflammatory effects while supporting cardiovascular health and regulating blood glucose levels. Its multifunctionality makes it a sustainable and cost-effective candidate for dietary supplementation and therapeutic applications¹⁰.

Given the overwhelming lack of exhaustive studies characterizing the MO growing in the arid regions of Algeria, our study represents the first detailed biochemical and phytochemical characterization of MOL from the Ghardaïa region (southern Algeria) to assess their potential as a natural alternative for managing metabolic disorders. Furthermore, the study explores the incorporation of MOL into functional foods, leveraging its bioactive components

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to enhance the nutritional and health-promoting properties of dietary supplements with hypolipidemic and slimming effects.

MATERIALS AND METHODS

Preparation of *Moringa oleifera* leaves extracts

Fresh MOL were collected from the Ghardaia region between September and December 2023. The leaves were air-dried in the shade at room temperature for seven days, ground into a fine powder, and stored for further analysis. For HME, 50 g of MOLs powder was macerated with 200 mL of 80% methanol for three days, while AE involved infusing 50 g of powder with 500 mL of distilled water for 24 hours using a magnetic stirrer. Both extracts were filtered through Whatman No.1 filter paper, concentrated using a rotary evaporator, and freeze-dried¹¹. *Moringa oleifera* leaves are versatile and can be consumed fresh, cooked, or as dried powder while retaining their nutritional value for extended periods without refrigeration¹².

Nutritional Value

The nutritional analysis of MOL was conducted in triplicate. The extraction yield was determined using the method described by Garg and Garg¹³. Moisture content was assessed through air drying, while ash content was measured via combustion, following the procedure outlined by Rébufa *et al.*¹⁴. Protein content was quantified using the Bradford method¹⁵, and total available carbohydrates were analyzed according to DuBois *et al.*¹⁶. Energy value calculations were performed based on the methodology of Eid *et al.*¹⁷. While, mineral content was determined using the method according to AOAC¹⁸ and analyzed with atomic absorption spectroscopy (AAS) and flame detection (ICE 3300 FL AA System, Thermo Scientific, China) after acid digestion of the samples.

UPLC-ESI-MS/MS analysis

Ultra-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC-ESI-MS/MS) was employed to identify the compounds present in the HME of MOL. The analysis was performed using a Shimadzu 8040 UPLC-ESI-MS/MS system with ultra-high sensitivity, equipped with UFMS technology and a binary pump (Nexera XR LC-20AD). The electrospray ionization (ESI) parameters were set as follows: collision-induced dissociation (CID) gas pressure, 230 KPa; conversion dynode voltage, -6.00 kV; desolvation line (DL) temperature, 250 °C; nebulizing gas flow rate, 3.00 L/min; heat block temperature, 400 °C; and drying gas flow rate, 10 L/min. The chromatographic separation was carried out on a Restek Ultra C18 column (3 µm, 150 × 4.6 mm) using a mobile phase consisting of solvent A (water with 0.1% formic acid) and solvent B (methanol). The flow rate was set at 0.2 mL/min, with an injection volume of 5 µL. The following gradient elution program was applied: 0–0.2 min, 98% A; 0.2–2.5 min, 25% A; 2.5–4 min, 0% A; 4–7 min, 0% A; 7–7.1 min, 98% A; and 7.1–12 min, 98% A.

FRAP assay

To assess the antioxidant property of MOL, the FRAP assay was employed. The FRAP reagent was freshly prepared by mixing 300 mM acetate buffer, 10 mM TPTZ solution, and 20 mM FeCl₃·6H₂O solution in a 10:1:1 (v/v/v) ratio and warmed to 37 °C prior to use. A total of 100 µL of diluted extracts and standard solutions were combined with 3.0 mL of the freshly prepared FRAP reagent, vortexed, and incubated at room temperature (25 °C) for 10 minutes. The absorbance of the reaction mixtures and blank was measured at 593 nm using a UV-Vis spectrophotometer. Trolox and ascorbic acid served as positive controls. All samples were analyzed in triplicate to ensure reproducibility¹⁹.

Statistical Analysis

For each test carried out, three repetitions were made. Mean ± SD (standard deviation) using Excel 2016 software. Statistical evaluation of data was done following Students't-test. A difference was considered significant at $p \leq 0.05$.

RESULTS AND DISCUSSION

Nutritional value

The chemical composition of plant extracts is a critical determinant of their efficacy, with environmental factors such as precipitation, temperature, altitude, and soil type significantly influencing these values²⁰. The extraction yields of MOL were found to be 14.586 ± 0.530% for HME and 10.22 ± 0.170% for AE. The moisture content was measured at 80.183 ± 0.063%, while the dry matter content was 19.816 ± 0.063%, both exhibiting low standard deviations, indicating high water content and consistent results. The ash content of fresh MOL was determined to be 26.588 ± 0.102%, representing the inorganic residue after complete combustion, while the organic matter content was 73.411 ± 0.078%, reflecting the proportion of carbon-based compounds (Table 1). The low standard deviations in these measurements confirm their reliability. These findings surpass those reported by Oduro *et al.*²¹, who recorded an ash content of 7.13% and a moisture content of 76.53%. In contrast, Tete *et al.*²², reported that minerals constitute a smaller proportion of the dry matter in MOL, ranging from 0.6% to 11.42%. The high ash content observed in this study suggests a substantial mineral presence, which is crucial for various nutritional applications²³.

Minerals

There is a well-established connection between nutrition and health. The development of functional food products can be facilitated through fortification with natural, unmodified ingredients²⁴. *Moringa oleifera* leaves are rich in essential minerals such as iron, phosphorus, potassium, and calcium, making them suitable for use as natural food additives²⁵. In this study, the ash content analysis of MOL powder revealed significant mineral concentrations, including iron (59.066 mg/100g), magnesium (250.719 mg/100g), and manganese (7.084 mg/100g) (Table 2). For comparison, Oduro *et al.*²¹ reported an iron content of 28.29 mg/100g dry matter, while Valdez-Solana *et al.*²⁶

Table 1. Nutritional composition of dried *Moringa oleifera* leaves powder.

Parameters	MOL	AE	HME
Ash content (AC)%	26.588± 0.102	—	—
Organic matter (OM)%	73.411± 0.078	—	—
Moisture content (MC)%	80.183± 0.063	—	—
Dry matter (DM) %	19.816± 0.063	—	—
Yield extraction	—	10.233±0.170	14.586±0.530
Proteins (g/100g)	—	18.676 ± 2.402	19.343 ± 1.484
Energy value (kcal/100g)	—	74.704 ± 9.608	77.372 ± 5.936
Carbohydrates (g/100g)	—	28.151 ± 0.667	32.24 ± 1.113
Energy value (kcal/100g)	—	112.604 ± 2.668	128.96 ± 4.452

Values are mean ±SD, analysed individually in triplicate, and expressed as g/100g of leaf powder; — : non determined.

Table 2. Measured concentration in (mg/100g) of trace element in *Moringa oleifera* leaves powder.

Element	Concentration(mg/100g)
Iron	59.066
Magnesium	250.719
Manganese	7.084

found magnesium values ranging between 322 and 340.6 mg/100g. Millogo-Koné *et al.*²⁷ documented higher values of iron (677.77 mg/kg), magnesium (6.88 g/kg), and manganese (62.93 mg/kg).

The differences in mineral content can be attributed to variations in climatic conditions and leaf maturity stage, as noted by Mashamaite *et al.*²⁸. *Moringa oleifera* leaves also contain high levels of calcium, with values of 15.08 g/kg DM in this study. In contrast, Millogo-Koné *et al.*²⁷, Oduro *et al.*²¹ and Chhikara *et al.*²⁹ reported calcium values of 2,009 mg/100g and 2,079 mg/100g, respectively. Valdez-Solana *et al.*²⁶ found calcium concentrations ranging from 2,016 to 2,620 mg/100g. These minerals contribute to antioxidant activity³⁰. Magnesium plays a crucial role in maintaining cellular stability, energy metabolism, calcium and phosphorus balance, and antioxidant defense³¹. Deficiencies or imbalances in iron, magnesium, and manganese have been linked to cardiovascular and metabolic diseases³².

Total protein content

Proteins play fundamental roles in metabolic processes, fluid and acid-base balance maintenance, and antibody synthesis, with dietary sources significantly influencing cardiovascular health outcomes⁷. *Moringa oleifera* leaves are notable for their high essential amino acid content and protein levels³³. In this study, protein content comparisons between AE and HME revealed no statistically significant difference ($p = 0.71$, Figure 1), with values of 19.343 ± 1.484 g/100g (HME) and 18.676 ± 2.402 g/100g (AE).

These findings align with reported ranges of 19–35% protein content in MOL on a dry matter basis³⁴, though slightly lower than values reported by Oduro *et al.*²¹ (27.51%), Cattan *et al.*³⁵ (27.4%) and Iwara *et al.*³⁶ (27.1 g/100g). Such discrepancies may arise from some variations in agroclimatic conditions, tree age, and leaf maturity stages³⁷. The leaves represent an excellent protein source, with an average content ranging from 19% to 35% for dry matter³⁴. They may also present a rich source of essential amino acids. These attributes position the MO as a viable plant-based protein alternative, particularly when replacing unprocessed meats in diets to mitigate cardiovascular risks⁷. The leaves' dual role as both a nutrient-dense food and a functional ingredient underscores their potential in addressing global nutritional challenges³⁴.

Total sugar content

Carbohydrates serve as the primary energy source for the human body, supporting essential physiological functions such as energy production, nutrient transport, metabolic regulation, and overall growth²⁶. In this study, a box plot (Figure 2) comparing carbohydrate content (g/100g dry weight) between AE and HME, revealed a statistically significant difference ($p = 0.0096$), with an AE exhibiting higher levels of total soluble sugars. This finding highlights the impact of extraction methods on the concentration of soluble compounds, which may influence the functional and bioactive properties of the final product. The carbohydrate content was measured at 28.151 ± 0.667 g/100g for HME and 32.24 ± 1.113 g/100g for AE. These results align with previous studies, such as González-Burgos *et al.*³⁴ who reported a carbohydrate content of 28.5 g/100g in methanolic extracts, while Pari *et al.*³⁸ observed a higher value reaching 38.2 g/100g in AE. Similarly, Oduro *et al.*²¹ and Tete *et al.*²² reported carbohydrate contents of 40 g/100g and 43.88%, respectively, with the latter corresponding to a calorific value of 305.62 cal/g. The variation in carbohydrate content observed across studies underscores the importance of selecting appropriate extraction methods to optimize the nutritional and functional properties of MOL extracts. Carbohydrates not only provide energy but also play a pivotal role in maintaining metabolic balance and supporting cellular functions, making them integral to human nutrition³⁹.

Energy value

The nutritional value of food includes its energy value, which represents the amount of energy released during the biological oxidation of macronutrients⁴⁰. In this study, the HME of MOL demonstrated a higher energy value compared to the AE, primarily due to its greater protein content (19.343 g vs. 18.676 g). Proteins significantly contribute to the total energy value, as their caloric content is equivalent to that of carbohydrates. Although the carbohydrate content was slightly higher in AE (32.24 g vs. 28.151 g in HME), this difference only marginally increased the energy derived from carbohydrates. From a nutritional perspective, the higher protein density in HME may enhance its potential health benefits, particularly in addressing malnutrition. However, both extracts provide a valuable energy source, with proteins and carbohydrates playing complementary roles in supporting physiological functions and overall health⁴¹. The observed differences highlight the importance of selecting appropriate extraction methods to optimize the nutritional properties of MOL extracts for specific dietary or therapeutic applications.

UPLC-ESI-MS/MS examination of *Moringa oleifera* hydromethanolic leaves extract

The HME of MOL was analyzed using UPLC-ESI-MS/MS. Our findings reveal a diverse array of bioactive compounds in MOL, primarily consisting of flavonoids, phenolic acids, triterpenoids, and carotenoids (Table 3). The chromatogram depicted in Figure 3 further highlights the efficacy of the HME method in preserving and enriching these bioactive compounds. Flavonoids can manage metabolic disorders⁴² and modulate lipid metabolism such as inhibition of low density lipoprotein (LDL) oxidation⁴³. Quercetin 3-glucoside has been shown to reduce serum lipid levels and protect against atherosclerosis-related complications⁴⁴ and may also improve glucose metabolism⁴⁵. Myricetin is well known to improve glucose metabolism and lipid profiles⁴⁶, while curcumin reduces LDL and total cholesterol and can diminish the development of atherosclerosis⁴⁷. Salicylic acid, as a naturally occurring phenolic compound, has been shown to induce the biosynthesis of health-promoting metabolites, suggesting its potential as a natural antidiabetic agent⁴⁸. A significant decrease in serum lipid levels can be correlated with increased insulin sensitivity³. Ferulic and p-coumaric acids have demonstrated significant pharmacological effects in treating insulin resistance, obesity, diabetes, hypertension, and other metabolic disorders⁴⁹. Additionally, gallic and chlorogenic acids have been detected in MOL²⁶. Resveratrol, has been validated in animal studies for improving hyperlipidemia and exhibits anti-inflammatory, anti-cancer, and cardioprotective effects⁵⁰. Phenols and flavonoids are notable for their antioxidant properties, neutralizing free radicals and quenching oxygen species³⁰. In the other hand, beta-carotene, contributes to provitamin A activity and offers protection against metabolic and cardiovascular diseases⁵¹. Riboflavin plays a crucial role in amino acid metabolism⁵², while terpenoids, such as oleanolic acid, improve lipid metabolism, reduce oxidative stress and has beneficial effects on vessel walls⁵³. *Moringa oleifera* represents a rich natural source of antioxidants, including flavonoids, phenolics, and carotenoids, which not only enhance the shelf life of fat-containing foods but also exhibit protective effects against hypercholesterolemia and diabetes⁵⁴. These findings support MO's potential in functional food development for cardiovascular and metabolic disease prevention, aligning with recent advances in plant-derived therapeutics^{55,56}. Pharmaceutical activity is often the result of the synergistic interaction among multiple active compounds within a mixture, rather than the effect of a single constituent⁵⁷. The findings underscore the exceptional nutritional and medicinal potential of MOL from Ghardaia region.

Table 3. Phytochemical composition of *Moringa oleifera* hydromethanolic leaves extract revealed by UPLC-ESI-MS/MS.

Name	Formula	Classes of compound	R _t time	Area	Area (%)
Catéchine(-)	C15H14O6	Flavonoid	8.166	4467962	5.92
Chrysin-6-C-glucoside	C21H20O9	Flavonoid	0.000	148219	0.19
Myricetin 3-arabinoside	C20H18O12	Flavonoid	7.742	439248	0.58
myricetin	C15H10O8	Flavonoid	7.141	13176526	17.47
Myricetine-3-rhamnose	C27H30O17	Flavonoid	6.986	391504	0.51
Orxyline A	C16H12O5	Flavonoid	7.404	10404	0.014
Quercetine-3-arabinose	C20H18O11	Flavonoid	6.788	76167	50.1%
Quercetine-3-glucoside	C21H19O12	Flavonoid	6.707	36138510	47.91
Tiliroside	C30H26O13	Flavonoid	6.907	60666	0.08
Apigénine	C15H10O5	Flavonoid	8.140	101069	0.14
Hispiduline	C16H12O6	Flavonoid	7.259	105727	0.14
2-mythoxybenzoic Acid	C8H8O3	Phenolic acid	0.000	739560	0.98
Curcumin	C21H20O6	Phenolic acid	6.465	8458353	11.21
Epicatechin	C15H14O6	Flavonoid	0.000	283538	0.37
Ferulic Acid	C10H10O4	Phenolic acid	5.673	196497	0.27
Luteolin	C15H10O6	Flavonoid	7.044	705904	0.94
Oleanolic Acid	C30H48O3	Triterpenoid	8.106	27183	0.04
Quercetine	C15H10O7	Flavonoid	6.686	1186310	1.57
Resveratol	C14H12O3	Phenolic acid	6.821	432133	0.57
Riboflavin	C27H30O16	Flavonoid	8.283	3075670	4.08
Rutin	C27H30O16	Flavonoid	6.681	105716	0.14
Sinapic Acid	C11H12O5	Phenolic acid	6.673	112758	0.16
beta carotene	C40H56	Carotenoid	8.384	3163695	4.19
naringenin	C15H12O5	Flavonoid	7.057	232966	0.3
tymol	C10H14O	Phenolic acid	6.877	283644	0.4
vannilin	C8H8O3	Phenolic acid	6.192	694703	0.92
p-Coumaric Acid	C8H8O4	Phenolic acid	0.000	80423	0.10
salicilic acid	C7H6O3	Phenolic acid	7.093	537464	0.71

Table 4. FRAP test results of both aqueous and hydromethanolic extracts from *Moringa oleifera* leaves against standard compounds

Extracts	FRAP EC ₅₀ (µg/mL)	Standards	FRAP EC ₅₀ (µg/mL)
AE	266.21 ± 0.95	ASC	48.74 ± 0.95
HME	207.09 ± 1.35	TRX	126.49 ± 1.35

ASC: Ascorbic acid; TRX: Trolox. Each value represents the means ± SD of the three experiments (Students 'test at p < 0.05).

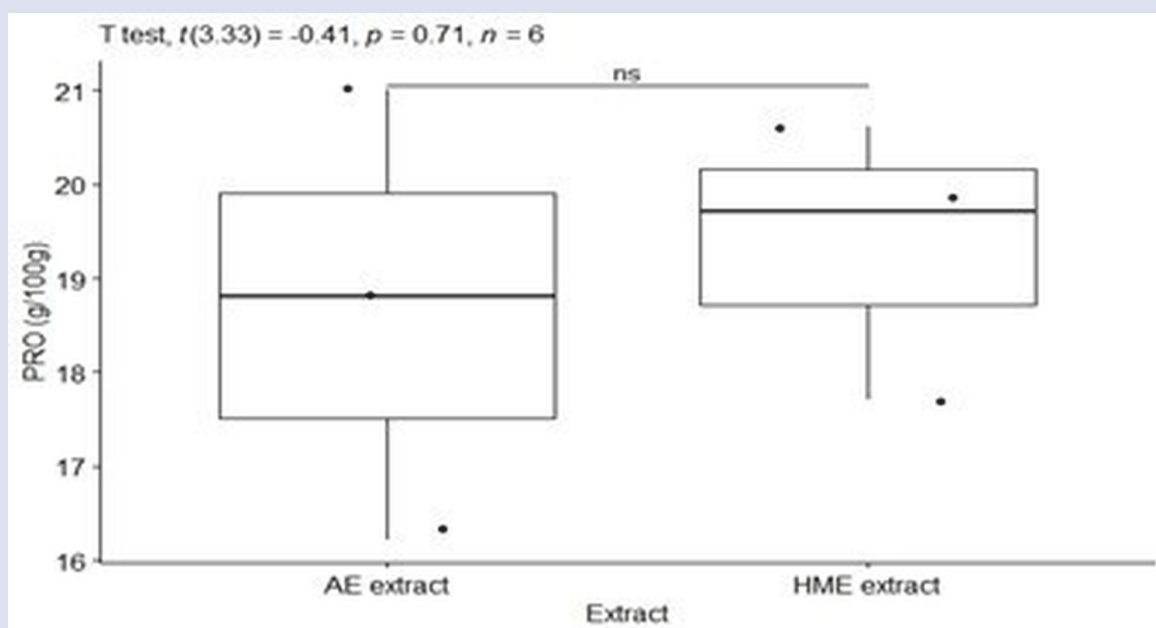


Figure 1. Total protein content from *Moringa oleifera* aqueous and hydro-methanolic leaves extracts

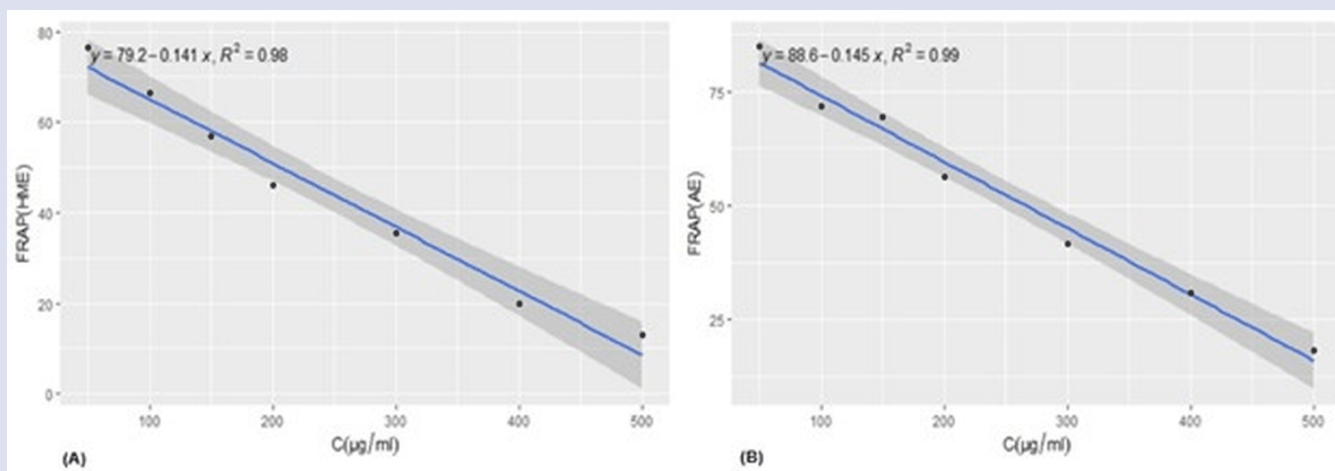


Figure 4. Comparison of FRAP inhibitory effect of *Moringa oleifera* hydromethanolic (A) and aqueous (B) leaves extracts.

Evaluation of Antioxidant Activity

Antioxidants are vital compounds that protect the human body from oxidative stress and damage induced by free radicals, thereby maintaining overall health⁵⁸. In this study, the antioxidant activity of AE and HME of MOL was evaluated using the FRAP assay across various concentrations ($\mu\text{g}/\text{mL}$). The antioxidant potential of both extracts was compared, with linear regression analyses presented in Figure 4. Ascorbic acid (ASC) and Trolox (TRX) were used as standards. A strong positive correlation was observed between AE and HME concentrations, with high coefficients of determination ($R^2 = 0.99$ for AE and $R^2 = 0.98$ for HME), indicating consistent antioxidant performance.

The results revealed that our AE exhibited significantly higher FRAP values than HME, with EC_{50} values of $207.09 \pm 2.31 \mu\text{g}/\text{mL}$ and $266.23 \pm 3.63 \mu\text{g}/\text{mL}$, respectively. However, these values were higher than those of the standards ASC ($EC_{50} = 48.74 \pm 3.63 \mu\text{g}/\text{mL}$) and TRX ($EC_{50} = 126.49 \pm 2.63 \mu\text{g}/\text{mL}$) (Table 4). The antioxidant potential observed in this study was lower than the values obtained by Peñalver *et al.*⁵⁹ ($396.43 \pm 17.12 \mu\text{mol TE}/\text{g}$). Xu *et al.*⁶⁰ reported values ranging from 0.48 to 1.08 mmol FeSO_4 equivalents per gram, highlighting variability due to differences in the type of solvents and method used for extraction, plant maturity, or environmental factors⁶¹.

Moringa oleifera is recognized as a rich source of natural antioxidants, including polyphenols and flavonoids, which contribute to its therapeutic potential in food and health industries⁶². These bioactive compounds have been associated with protective effects against oxidative stress-related diseases such as cardiovascular disorders, cancer, neurodegenerative conditions and Type 2 diabetes mellitus⁶³. Flavonoids, in particular, exhibit diverse pharmacological properties, including antioxidant, anti-inflammatory, and anticancer activities^{58,64}. In their study, Edinilze *et al.*⁶⁵ concluded that catechin, myricitrin, and quercitrin exhibited free radical scavenging activity and hypoglycemic effects, without demonstrating any cytotoxicity. The growing interest in natural antioxidants stems from their safety and efficacy compared to synthetic alternatives, making MOL a promising candidate for developing functional foods and nutraceuticals³⁹. This study underscores the importance of optimizing extraction methods to maximize the antioxidant potential of MOL extracts while contributing to their application in health-promoting products.

CONCLUSION

This study provides initial biochemical characterization of MOL from the Ghardaïa region, showing promising nutritional content and bioactive compounds. While *in vitro* antioxidant activity was demonstrated, the therapeutic potential for managing metabolic disorders requires validation through; *In vivo* studies in appropriate animal models, toxicology and safety assessments, human clinical trials to establish efficacy and optimal dosing and bioavailability studies of key bioactive compounds. The adaptability of MO to arid climates makes it a promising candidate for further investigation as a functional food ingredient. However, claims regarding management of hyperlipidemia, obesity, and hyperglycemia remain speculative pending rigorous clinical evaluation.

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