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Moringa peregrina (Forssk.) Fiori Leaf Extract: Proximate Analysis, Nutritional Potential, Phytochemical Study, and *In Vivo* Assessment of Anti-hyperlipidemia and Anti-obesity

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ABSTRACT

Objectives: Globally, obesity and hyperlipidemia are substantial public health challenges and risk factors for morbidity and mortality. The monotype genus *Moringa* (Family Moringaceae) comprises nearly 13 species of herbs and trees with considerable nutritional and therapeutic values. The current investigation, aimed to explore the nutritional and phytochemical composition of *Moringa peregrina* (Forssk.) Fiori leaves cultivated in Egypt and screen its anti-hyperlipidemic, and anti-obesity activities in an appropriate *in vivo* model. **Methods**: Proximate analysis was conducted based on the Association of Analytical Chemists' standard procedure. The Folin-Ciocalteu method determined the total phenolic content (TFC) in the 70% aqueous ethanolic extract (AEE), while the aluminum chloride colorimetric method was adopted for the total flavonoid content (TFC). For the *in vivo* study, Swiss albino mice (25-30 g) were used to determine the LD_{50} of the AEE, while albino rats (120-150 g) were adopted for the hyperlipidemic and obesity-induced models. **Results**: *M. peregrina* leaves showed high nutritional value deduced from their high carbohydrate, fiber, and protein content. The total identified non-essential amino acids (5.50%) exceeded the essential amino acids (3.86%). Glutamic acid (1.21%) and aspartic acid (0.95%) were the major identified non-essential amino acids, while leucine (0.79%) and phenylalanine (0.70%) were the chief detected essential amino acids. The leaves are a good supply of calcium, magnesium, iron, copper, manganese, and vitamins A and C. The extract was rich in phenolic content calculated as 91.97±2.53 mg of GAE/g of dry extract. The fractionation and purification of the aqueous ethanol extract (AEE) afforded *β*-amyrin (**1**), *β*-sitosterol (**2**), corosolic acid **(3),** caffeic acid (**4**), rutin (**5**)**,** astragalin (**6**), salvigenin (**7**), catechin (**8**), and quercetin (**9**). Compounds **3** and **7** were isolated from the leaves of the Egyptian *M. peregrina* for the first time. The total AEE exerted significantly potent antihyperlipidemic, hepatoprotective, and reduced body weight of obese rats. The observed activity could be, at least in part, due to the synergistic effect of the phenolic components and to a lesser extent non-polar metabolites that are concurrently present in the extract. **Conclusion:** *M. peregrina* is a promising natural alternative for managing high-diet related disorders, yet deep investigation is required.

Keywords: *Hyperlipidemia, Moringa peregrina, Nutritional potential, Obesity, Phenolics*

INTRODUCTION

Globally, obesity is one of the most significant public health challenges[1]. According to the World Health Organization (WHO), 2.5 billion adults were categorized in 2022 as overweight, with a prevalence of 31% in Asia and Africa, and 67% in the Americas. Obesity is a chronic, complex ailment characterized by excessive fat accumulation with an elevated risk of acquiring chronic conditions such as diabetes mellitus, hypertension, coronary heart disease, and cancer[2]. Consequently, recent studies[3] have correlated obesity to metabolic problems such as regulating glucose and lipid metabolism, insulin sensitivity, and inflammatory response, escalating global attention to the alerting hazard of this disease. As current synthetic drugs adopted for the management of obesity are associated with adverse side effects hence, there is a great trend in shifting treatments to natural resources in a quest for compounds with no or fewer side effects.

Hyperlipidemia (Hypercholesterolemia) is one of the substantial risk factors for morbidity and mortality worldwide. It is an unhealthy condition where cholesterol level exceeds the standard limit of 200 mg/mL in the blood. Concurrently, the case is noticeable by rising total cholesterol (TC) and low-density lipoprotein (LDL) and reducing high-density lipoprotein (HDL)[4]. Increased LDL is a critical feature for the development and progression of atherosclerosis[5], which is the main cause of cardiovascular diseases, including stroke and heart attack. Cholesterol-lowering medication is the first treatment line for the control of high cholesterol levels, but it possesses one drawback. These drugs are not suitable for long-term consumption due to their noticeable side effects on the liver[6]. On the contrary, plant-based supplements can lower serum cholesterol and triglyceride concentrations, thus reducing the risk of cardiovascular diseases with negligible drawbacks. The prevalence of the disease highlights the paramount demand for alternative natural-based remedies for the control of hyperlipidemia.

In the tropical world, *Moringa* is the only genus in the monotype family Moringaceae[7]. The genus encompasses nearly 13 species that range from herbs to trees. Moringa species are considered as valuable food product[8], while it is applied traditionally to manage an array of ailments[9]. A recent study[10] reported certain species of *Moringa* (*M. oleifera* Lam) as anti-obesity herbal medicine and to have hyperlipidemic rat model. Moreover *M. oleifera* leaf extract-loaded nanoparticles showed significant antifungal efficacy so as could be employed against invasive and drug resistant otomycosis infarction[11].

Moringa peregrina (Forssk.) Fiori, known as drum-stick tree and horseradish tree, is a small, deciduous, flowering tree native to Egypt and the Arabian Peninsula[12]. It is also well-adapted to harsh conditions and cultivated across regions from tropical Africa to East India[13]. Recently, *M. peregrina* has been gaining consideration due to its traditional, dietary, industrial, and therapeutic values[9]. The young leaves are traditionally consumed as vegetables, while the mature leaves are used as flavoring agents[14]. The leaf extract is applied topically for skin rashes[15], and the bark juice promotes wound healing. The seeds of *M. peregrina* have economic and medicinal value due to their unique micro- and macro-nutrient content[16-19]. In Egypt, the plant is traditionally used to treat conditions such as slimness, constipation, headache, fever, burns, and muscle pain[20]. According to the available review of the literature, *M. peregrina* leaves possess various pharmacological properties as analgesic, antibacterial, and anti-inflammatory properties[17], as antioxidant[21] and as anticancer[22,23]. These activities are due to various phytochemical classes such as flavonoids, steroids, phenolic acids, and glycosides of different plant extracts[23]. Despite its indigenous status in Egypt, few studies have explored its chemical profile and pharmacological properties; therefore, this study aims to investigate the phytochemical profile and evaluate the anti-hyperlipidemia and anti-obesity potential of *M. peregrina* leaf extract and fractions.

MATERIALS AND METHODS

General experimental material

Spectroscopic analysis was conducted on Bruker spectrometer at 400 and 500 MHz for ^{1}H NMR; while 100 and 125 MHz for ^{13}C NMR. Samples were prepared in an appropriate deuterated solvent, and the results were reported as δ [ppm] values relative to TMS as an internal reference. UPLC-Ion Trap-MS system consisting of 6420 series UPLC Triple Quadrupole (Agilent Technologies, Germany) coupled to an Esquire 6000 ESI-Ion Trap mass spectrometer and equipped with BEH C18 column (2.1x150 mm, i.d.1.7 µm). Masses ranging from 60 to 1500 m/z were recorded in negative and positive ion modes. Diaion HP-20 (Supelco, Bellefonte, PA), Sephadex LH-20 (Sigma-Aldrich, Sweden), and silica gel 60 (Fluka, Germany) were used for column chromatography (CC). Fractions were screened for the presence of metabolites using silica gel 60 precoated thin layer chromatography (TLC) plates (Merck, Germany) eluted with EtOAc: MeOH: H_2O: Formic acid 100:13.5:16.5:0.5 v/v/v/v. Eluted spots were visualized under UV light (λ_{254} and λ_{356}), after exposure to ammonia vapor, sprayed with $FeCl_3$, or vanillin/sulphuric acid.

Plant Material

Moringa peregrina (Forssk.) Fiori leaves were collected from El Orman Botanical Garden, Cairo, Egypt, in April 2004 following the local

garden`s guidelines and Egypt's collection regulations. The plant was identified by Professor Salama M. Azza, Flora Botany Department, Faculty of Agriculture, Cairo University, A sample was deposited in the Helwan University Herbarium, Faculty of Pharmacy, and assigned the code MP, 2013.

Proximate analysis[24]

Association of Analytical Chemists (AOAC), standard methods were implemented to estimate the quantitative percentage of ash, moisture, total carbohydrates, crude fats, crude fibers, and crude proteins.

Quantitative analysis of nutritional components

Amino acid content[25]

About 100 mg of defatted powdered leaves were added to 5 mL of formic acid in a conical flask in an ice water bath for 16 h. Afterward, 25 mL of sodium bisulfate and 25 mL of 6N HCl were added and stored at 110°C for 24 h, the solution was evaporated under vacuum (40°C), the residue was dissolved in sodium citrate buffer (pH 2.2). The amino acids mixture was eluted by a programmed buffer system and interacted with a stream of ninhydrin reagent (flow rate of 25 mL/h). The absorbance of the developed color was measured photometrically at 570 and 440 nm. Results obtained were compared with those of a standard solution of amino acids.

Minerals content[26]

One gram of fresh leaves was ash-dried at 550°C. The resultant ash was dissolved in 5 mL of HNO_3/ HCl/ H_2O (1:2:3) and gently heated until brown fumes disappeared. Distilled water (5 mL) was added. The mineral solution was filtered into a volumetric flask and the volume was completed to 100 mL by distilled water. The solution was analyzed in triplicate for its elemental composition using the Parking Elmer 403 model of atomic absorption spectrophotometer.

Vitamin B_1 (Thiamin) content[27]

A thiochrome fluorometric procedure was adopted to determine the content of vitamin B_1. In brief, a sample from the leaves was weighed and digested by autoclaving with 0.1 N HCl for 15 min at 121°C. Then, the thiamin content was oxidized to thiochrome using a solution of potassium ferricyanide. The thiochrome is then extracted by isobutanol and the fluorescence is measured. The thiamin content (mg of thiamin/g sample) was calculated as follows:

$[S - S_b / Std - Std_b] \times C/A \times 1/WT$

Where: S and S_b = fluorescence of sample and sample blank, respectively. Std and Stdb = fluorescence of standard and standard blank, respectively. C = concentration of standard, mg/mL

A = volume of standard for fluorescence measurement in mL. WT = sample weight in g.

Vitamin B2 (Riboflavin) content[27]

Vitamin B_2 content was estimated using the fluorometric method as mentioned before, the leaves sample was digested by acid followed by precipitation of interfering substances and oxidation by 3% $KMnO_4$ and 3% H_2O_2. The developed fluorescence was measured, and the riboflavin content as mg of riboflavin/g of sample was calculated as follows: $[(A-C) / (B-A)] \times (C_{Std} / V) \times (F/ Wt)$

A and C = fluorescence of sample containing water and sodium hydrosulfite, respectively; B = fluorescence of sample containing riboflavin standard; C_{std} = concentration of the standard in mg/mL; V = volume of sample in mL; F = dilution factor; Wt = sample weight in g.

Vitamin A (β-carotene) content[28]

Vitamin A content was determined spectrophotometrically, (1 gm) fresh leaves was extracted by petroleum ether (3x 4 mL). The combined extracts were subjected to active alumina column chromatography and eluted with petroleum ether until the eluate became colorless. The eluate was made up to 25 mL and the absorbance was determined by a spectrophotometer at 450 nm. Vitamin A content (µg /g of sample) was calculated according to the following equation:

$= (A/A_{std}) \times (C_{std} / V) \times (F/ Wt)$ where: A= Absorbance of sample; A_{std}= Absorbance of standard.

C_{std} = concentration of the standard in µg/mL; V = volume of standard for absorbance measurement in mL; F = dilution factor; Wt = weight of sample in g

Thus, the content of β-carotene (µg/g) =

$$\frac{A_{std} \times 100 \times 25}{25 \times 10} = A_{std} \times 10$$

Vitamin E content[29]

A normal-phase high-performance liquid chromatography (HPLC) protocol was implemented to estimate the content of vitamin E (tocopherols and tocotrienols) content. The leaves samples were extracted and then chromatographed using an Intersil 5 SI column chromatography eluted with hexane:1,4-dioxane (95.5:4.5 v/v) at 0.7 mL/min flow rate. The effluent was monitored by a series arrangement of a diode array followed by a fluorescence detector.

Vitamin C (ascorbic acid) content[30]

The standard method was adopted with a little modification. One gram of leaves was extracted with 25% aqueous phosphoric acid. Ascorbic acid (50 mg) was dissolved in 100 mL of metaphosphoric acid to make a stock solution. On the other side, 10 g of 2,4-

dinitrohydrazin was added to 500 mL of H_2SO_4 (4.5 M), while 5 g of Tioreh was dissolved in H_2O (100 mL). Also, anhydrous copper sulfate (0.6 g) was solubilized in 100 mL H_2O. Then, equal volume (5 mL) of Tiore solution and $CuSO_4$ solution was added to 100 mL of the 2,4-dinitro hydrazine solution. The absorbance of the tested samples was recorded at 520 nm. against the standard stock solution of ascorbic acid, a calibration curve was established of three dilutions across a range of concentrations of 0.1–2 g/mL, using the standard curve equation:

$$y = 0.02356 \times -0.009 \ (r2 = 0.9988).$$

Determination of total phenolic content (TPC)[31]

Folin-Ciocalteu reagent was used to determine the total phenolic content (TPC) of the 70% aqueous ethanolic extract adopting the procedure stated earlier. Gallic acid was used as a reference standard (20-100 µg/mL) for plotting the calibration curve. A volume of 0.5 mL of the extract (100 µg/mL) was mixed with 1.5 mL of Folin-Ciocalteu reagent (diluted 1:10 with deionized water) and neutralized with 3 mL Na_2CO_3 solution (7.5%w/v). The reaction mixture was kept in the dark at room temperature for 30 min with intermittent shaking for color development. The absorbance of the resulting color was measured using a double-beam UV-Vis spectrophotometer (UV Analyst-CT 8200) at λ_{765} nm. The TPC was determined using a linear regression equation obtained from the standard plot of gallic acid. The content of total phenolic compounds was calculated as mean ± SD (n=3) and expressed as mg/g gallic acid equivalent (GAE) of dry extract. Total phenolic content (% w/w) = $GAE \times V \times D \times 10^{-6} \times 100/W$

GAE: Gallic acid equivalent (µg/ml), V: total volume of sample (mL), D: Dilution factor, W: Sample weight (g).

Estimation of total flavonoid content (TFC)[32]

TFC in the 70% aqueous ethanolic extract was determined using the aluminum chloride colorimetric method reported. Different concentrations of the standard flavonoid quercetin (20-100 µg/mL) were used to construct the calibration curve. The diluted extract (0.5 mL) was separately mixed with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1 mol/L potassium acetate, and 2.8 mL of distilled water in a test tube. The tubes were incubated for 30 min at room temperature to complete the reaction. The absorbance of the reaction mixture was measured at λ_{415} nm with a double-beam UV-Vis spectrophotometer against blank. The amount of flavonoid was calculated from a linear regression equation obtained from the quercetin calibration curve. The flavonoid content was calculated as mean±SD (n=3) and expressed as mg/g of dry extract's quercetin equivalent (QE). TFC (% w/w) = $QE \times V \times D \times 10^{-6} \times 100/W$

QE: quercetin equivalent (µg/mL), V: total volume of sample (mL), D: dilution factor, W: sample weight (g).

Extraction, fractionation, and isolation of metabolites

Air-dried powdered leaves (1,500 g) of *M. peregrina* were percolated with 70% aqueous ethanol (AE) (7 × 5 L). The extracts were filtered and dried to yield 250 g of viscous, brown AE extract (AEE). About 200 g of the extract was transferred to a separating funnel, suspended in distilled water, and partitioned between chloroform and *n*-butanol (saturated with water). The solvents were evaporated under reduced pressure to yield 85 and 128 g dry chloroform and *n*-butanol fractions, respectively. About 10 g of the chloroform fraction was chromatographed on Si gel 60 (100 g) using vacuum liquid chromatography eluted gradually with *n*-hexane: CH_2Cl_2 mixtures followed by CH_2Cl_2: EtOAc mixtures. Eluted fractions were pooled together based on their TLC similarity pattern yielding three collective fractions (**A-C**). Fraction **A** (0.8 g) eluted by 20-25% *n*-hexane: CH_2Cl_2 was purified using Si gel 60 CC using *n*-hexane: EtOAc (9.9:0.1 v/v) to give compound **1** (27 mg). Fraction **B** (35-45% *n*-hexane: CH_2Cl_2, 0.6 g) was purified over Si gel 60 CC using *n*-hexane: EtOAc (9.8:0.2 v/v) affording compound **2** (18 mg). Ultimately, **Fraction C** (50-65% *n*-hexane: CH_2Cl_2, 0.9 g) was fractionated over a Si gel 60 CC using *n*-hexane: EtOAc (9.5:0.5 v/v) affording compound **3** (7 mg). On the other side, 10 g of *the n*-butanol fraction was fractionated using Diaion HP-20 CC eluted by 100% H_2O followed by H_2O: MeOH mixtures until 100% MeOH to yield three collective fractions **I-III**. Fraction, **I** eluted by 100% H_2O (1.9 g) was successively fractionated on sephadex LH-20 CC using 10% MeOH followed by increasing MeOH resulting in the isolation of compound **4** (8 mg). Fraction **II** (eluted by 50% MeOH/H_2O, 4 g) was purified using successive sephadex LH-20 CC and eluted gradually with 20% MeOH to 100% MeOH affording compounds **5** (28 mg), **6** (20 mg) and **7** (9 mg). Fraction **III** (eluted by 100% MeOH, 2 g) was subjected to successive purification using sephadex LH-20 eluted by 50% MeOH to 100% MeOH to give compounds **8** (11 mg) and **9** (8 mg).

***In vivo* investigation**

Animal housing and chemicals

Swiss albino male mice (25-30 g) were used for the acute toxicity study. Sprague-Dawley healthy adult male rats (120-150 g) were adopted for the hyperlipidemia- and obesity-induced *in vivo* models, respectively. All experimental animals were obtained from the animal house at the National Research Center (Dokki, Giza, Egypt). Animals were accommodated (3 /cage) and maintained at 23ºC ± 2ºC with a 12 h light/dark cycle with free access to food pellet and tap water *ad libitum*. The study was conducted according to the National Research Council's Guide to the Care and Use of Laboratory Animals. Positive control drugs: Ator (Atorvastatin, 10 mg) and Silymarin were purchased

from the Egyptian International Pharmaceutical Industries Co. (EIPICO) (10th of Ramadan City, Cairo, Egypt).

Determination of median lethal dose (LD_{50})[33]

The protocol stated to determine the LD_{50}, as follows 70% aqueous ethanol extract (AEE) of *M. peregrina* was given orally to Swiss albino mice (25-30 g) at different concentrations according to. Doses were freshly prepared and given daily to the animals following the guidelines on dosage calculation and stock solution preparation in experimental animal studies. Mice were observed for one day for any acute signs of toxicity.

High-cholesterol-induced hyperlipidemia and liver inflammation in rats[34]

Forty-two experimental male albino rats (120-150 g) were adopted for this experiment. **Group 1** (n=6) was kept on a normal diet, received the vehicle orally for 60 days, and was assigned as a negative control. On the other side, hyperlipidemia was induced in thirty-six rats by the oral administration of cholesterol (400 mg/kg) and cholic acid (50 mg/kg) in coconut oil once daily and randomly allocated into seven groups (n=6). **Group 2**: placed on a high-cholesterol diet and administered the vehicle orally (positive control) for 60 days. **Group 3**: administered the 70% aqueous ethanol extract (AEE) of *M. peregrina* orally (100 mg/kg b.wt.) for 60 days. **Group 4**: received the *n*-butanol fraction (BF) of *M. peregrina* orally (100 mg/kg b.wt) for 60 days. **Group 5**: administered the chloroform fraction (CF) of *M. peregrina* orally (100 mg/kg b.wt) for 60 days. **Group 6**: administered the standard anti-hyperlipidemic drug, Atorvastatin orally (10 mg/kg b.wt) for 60 days. **Group 7**: administered the standard hepatoprotective drug, silymarin orally (100 mg/kg b.wt) for 60 days. While animals were fasting for 8 h, blood samples were collected before and after treatment on the 30th and 60th day, by retro-orbital sinus puncture under mild anesthesia. Samples were allowed to clot for 30 min at room temperature, centrifuged at 3000 rpm for 20 min, then serum was separated and stored at -20°C until biochemical analysis.

High-fat diet-induced obesity in rats[34,35]

Thirty experimental female rats were used for this experiment. Six animals were assigned as **Group 1** and were fed a normal diet and received the vehicle orally for 60 days (negative control). The remaining twenty-four animals were given a cafeteria diet (hypercaloric diet) composed of a basal diet supplemented with 0.2% folic acid, 1% cholesterol, and 10% fat for 60 days. Animals were randomly allocated into six groups (n=6). **Group 2:** administered the vehicle for 60 days (positive control). **Group 3**: administered the 70% aqueous ethanol extract (AEE) of *M. peregrina* orally (100 mg/kg b.wt., p.o.) for 60 days orally. **Group 4**: received the *n*-butanol fraction (BF) of *M. peregrina* orally (100 mg/kg b.wt., p.o.) for 60 days. **Group 5**: administered the chloroform fraction (CF) of *M. peregrina* (100 mg/kg b.wt., p.o.) for 60 days. Body weight gain was measured every four weeks before and after administration of the treatments.

Statistical analysis

The *in vivo* study results were expressed as mean ± SEM. ANOVA was used to verify the statistical significance between the tested groups using GraphPad version 8 (San Diego, CA, United States). A significant difference of $p < 0.05$ was applied.

RESULTS AND DISCUSSION

Proximate analysis is a conventional analytical protocol that quantitatively and qualitatively estimates the nutritional values of a plant sample including its total carbohydrate, crude fat, protein, fiber, ash, and moisture content[36,37]. In this study, the proximate analysis results (**Table 1)** revealed that *M. peregrina* leaves contain a high carbohydrate percentage followed by fiber and protein content. Conversely, crude fat, total ash, and moisture are presented in the lowest percentages. By comparing our values with previously reported data of the leaves and seeds of *M. peregrina* cultivated in Saudi Arabia[38] and with data of the seed oil[39], it was noticed that it showed lower carbohydrate (34.3 ± 0.57) and ash (5.6 ± 1.2) contents, while the total protein was three-fold (35.3± 3.5) higher than the Egyptian species. The observed deviation may be due to the variability in soil composition and cultural conditions.

Table 1. Proximate analysis of *M. peregrina leaves*

Content	% on dry weight basis
Moisture	7.4±2.3
Crude protein	11.35±0.39
Crude fat	7.95±0.47
Crude fiber	16.15±0.31
Total ash	9.79±0.25
Carbohydrate	47.37±0.31

As per the promising crude protein yield deduced from the proximate analysis, we pursue a deep insight into its compositional amino acid building blocks. **Table (2)** shows that amino acids constitute 9.36% calculated on the dry-weight basis of *M. peregrina*. The total identified non-essential amino acids (5.50%) exceeded that of the essential amino acids (3.86%). Glutamic acid (1.21%) and aspartic acid (0.95%) were the major identified non-essential amino acids, while leucine (0.79%) and phenylalanine (0.70%) were the chief detected essential amino acids. Plant-derived

amino acids are indispensable for boosting the human's body health and physical function[40]. Hence, ***M. peregrina*** could be considered a good supply of essential and non-essential amino acids. It is noteworthy that this study is the first report on the amino acid content of *M. peregrina* leaves cultivated in Egypt.

Table 2. Percentage of content of amino acids in *M. peregrina* leaves

Amino acid	Conc. (g/100 g dry wt.)
Arginine (n)	0.62
Aspartic acid(n)	0.95
Alanine(n)	0.60
Isoleucine(e)	0.42
Proline(n)	0.53
Therionine(e)	0.44
Glutamic acid(n)	1.21
Glycine(n)	0.52
Serine(n)	0.47
Cystine(n)	0.16
Valine(e)	0.52
Phenylalanine(e)	0.70
Lysine(e)	0.57
Leucine(e)	0.79
Methionine(e)	0.15
Histidine(e)	0.27
Tyrosine(n)	0.44
Total essential amino acid (e)	3.86
Total non-essential amino acid (n)	5.50
Total determined amino acid	9.36

(e) essential amino acid; (n) non-essential amino acid

Minerals are categorized into macro- and micro-elements in which macro-elements are nutritionally important components and required in daily oral intake, while microelements have a well-defined role as metalloenzymes, and their recommended daily allowances are below 100 mg. On the other side, vitamins are a group of organic-related molecules that are essential to an organism in small quantities for proper metabolic function. They cannot be synthesized in the human body in sufficient quantities for survival and therefore must be obtained through the diet.

Inadequacy of minerals and vitamins in the human diet may have long-term negative effects on well-being and lead to micronutrient deficiency diseases[41]. Herein, the mineral and vitamin contents of *M. peregrina* leaves were delineated in **Table 3.** The results revealed that *M. peregrina* encompasses high levels of macro-elements just as calcium and magnesium in which their values exceed the recommended daily allowance. Additionally, *M. peregrina* leaves were rich in micro-elements such as iron, copper, and manganese to a level higher than their recommended daily allowance. Meanwhile, among all screened vitamins, *M. peregrina* leaves showed their wealth with vitamins A and C (53.1 mg/100 and 100 mg/100 dry leaves, respectively) and as compared with reported data[42].

Table 3. Macro-elements, micro-elements, and vitamins estimated in *M. peregrina* leaves

	Conc. mg/ 100 g dry wt.	Recommended dietary allowances* (mg/day)
Macro-elements		
Calcium	2010	800-1200
Phosphorus	182	700
Sodium	422.65	500
Potassium	839.1	2000
Magnesium	452.4	280-400
Micro-elements		
Iron	232.25	10-15
Zinc	16.47	12-15
Copper	10	2
Manganese	170	5
Vitamins		
B1	0.3	1.1-1.4
B2	0.5	1.1-1.6
E	0.8	10
A	53.1	0.9
C	100	75

**National Research Council (NRC) has Recommended dietary allowances for adults in 1989*

M. peregrine's aqueous ethanol extract (AEE) was screened for its total phenolic content (TPC) and total flavonoid content (TFC). The results showed that the extract was enriched with phenolics and the calculated TPC (91.97±2.53 mg of GAE/g of dry extract) appeared to surpass the TFC (25.23± 3.4 mg of QE/g of dry extract) by approximately 3.6-fold. Accordingly, The AEE was fractionated to unravel its phenolic constitution using liquid/liquid extraction to afford chloroform-soluble fraction (CF) and butanol-soluble fraction (BF). The preliminary chromatographic screening of each fraction revealed the presence of a mixture of polyphenolic and terpenoids/sterols compounds based on their behavior under UV lamb and spraying reagents. The fractionation and purification of the CF afforded three compounds (**1-3)** that were elucidated based on their NMR spectroscopic data compared to the literature and assigned as *β*-amyrin (**1**), *β*-sitosterol (**2**), and corosolic acid **(3).** Meanwhile, the fractionation and purification of the BF afforded six compounds (**4-9)** that were identified based on their NMR spectroscopic data compared to the literature and elucidated as caffeic acid (**4**), rutin (**5),** astragalin (**6**), salvigenin (**7**), catechin (**8**), and quercetin (**9**). Compounds **1, 2, 4, 5,** and **9** were previously isolated from the leaves[9,22,43] compound **6** was reported in the

leaves by HPLC[44], while compounds **3**, and **7** were isolated from the leaves of the Egyptian *M. peregrina* for the first time, (their ^{1}H and ^{13}C-NMR data is included as supplementary material).

Compound 3: White amorphous powder (7mg) Chromatographic properties : R_f value:0.35 on (n hexan-ethylacetate 9:1) mp: 251-254°C, and gave blue spot with ρ-anisaldhyde spray.^{1}H-NMR (Fig. 1) (pyridine-d5, 500 MHz): δ 4.07 (1H, ddd, J = 3.8, 9.4, 11.0 Hz, H-2), 3.41 (1H, d, J = 9.5 Hz, H-3), 5.42 (1H, br s, H-12), 2.6 (1H, d, J = 10.9 Hz, H-18), 1.26 (3H, s, H-23), 1.19 (3H, s, H-24), 1.06 (3H, s, H-25), 1.04 (3H, s, H-26), 1.26 (3H, s, H-27), 1.03 (3H, d, J =6 Hz, H-29), 0.97 (3H, d, J = 6.0 Hz, H 30). ^{13}C-NMR, (Fig. 2) (pyridine-d5) δ 48.58 (C-1), 69.14 (C-2), 84.35 (C-3), 40.39 (C-4), 56.46 (C-5), 19.35 (C-6), 34.9 (C-7), 41.2 (C-8), 49.5 (C-9), 39.2 (C-10), 23.7 (C 991 11), 126.28 (C-12), 139.85 (C-13), 43.8 (C-14), 29.5 (C-15), 25.9 (C-16), 48.65 (C 17), 54.28 (C-18), 40.22 (C-19), 40.45 (C-20), 31.58 (C-21), 38.4 (C-22), 29.7 (C 23), 19.1 (C-24), 18.6 (C-25), 17.95 (C-26), 25.2 (C-27), 180 (C-28), 18.24 (C-29), 21.95 (C-30). ^{1}H-NMR spectral data of compound 3 showed two oxymethine protons resonating at δ 3.38 and 4.07; and a trisubstituted olefinic proton at δ 5.42. The presence of five methyl singlets and two methyl doublets suggested that compound 3 belongs to ursane type triterpenoid having two secondary hydroxyl groups and a trisubstituted double bond between C-12/C-13. The appearance of a carbonyl group resonating at δ 180 in the ^{13}C-NMR spectral data suggested the presence of an acid functional group and its location was identified at C-28. According to the chromatographic properties, compound 3 was confirmed to be corosolic acid

Compound 7: White needle (8 mg) Chromatographic properties: R_f value: 0.89 on (ethylacetate: methanol: water100:13.5:16.5 2 drops of formic acid); having violet color under UV-light turned to bright yellow on exposure to NH_3 vapor and gives blue with $FeCl_3$ spray reagent. ^{1}H-NMR (600 MHz, C_5D_5N) (Fig. 3): δ 12.78 (s, OH), 7.83 (d, 2H, J = 8.8 Hz, H 2´,6´), 6.98 (d, 2H, J = 8.8 Hz, H-3´,5´), 6.56 (s, 1H, H-3), 6.52 (s, 1H, H-8), 3.94 (s, -OCH_3), 3.90 (s, - OCH_3), 3.67 (s, -OCH_3); ^{13}C-NMR (125 Hz, C_5D_5N) (Fig. 4): δ 182.6 (C-4), 164.2 (C-2), 162.8 (C-4´), 158.7 (C-8), 153.08 (C-9), 153.2 (C-6), 132.6 (C-7), 128.0 (C-2´,6´), 123.5 (C-1´), 114.8 (C-3´,5´), 106.1 (C-5), 104.1 (C-3), 90.6 (C-8), 60.8 (-OCH_3), 56.3 (-OCH_3), 55.5 (-OCH_3). ^{1}H-NMR spectral data of compound 7 showed the presence of three singlets at δ 3.67, 3.90, and 3.94 corresponding to three methoxy groups and another singlet at δ 12.74, of a hydroxyl group. ^{13}C-NMR spectral data also showed the presence of twelve aromatic carbons; comprises of eight quaternary carbons and four methine carbons, and an unsaturated carbonyl carbon. The above ^{1}H- and ^{13}C-NMR data confirming the structure to be a trimethoxy substituted flavone having an additional phenolic hydroxyl group. The placement of the three methoxy groups were identified at C-4´, C-6, and C-7 positions, and that of the phenolic hydroxyl at C-5 position spectral data, structure of compound 7 was assigned as 5-hydroxy-4´, 6, 7 trimethoxy flavone, which is consistent to the reported literature values, thus, compound **7** was identified as Salvigenin.

The acute toxicity study revealed that the median lethal dose LD_{50} of the 70% (aqueous ethanol extract (AEE) of *M. peregrina* is 8.2 gm/kg. Hence, we selected to test the AEE, the butanol soluble fraction (BF), and the chloroform soluble fraction (CF) at 100 mg/Kg as a preliminary non-toxic single screening dose. Firstly, we investigated the effect of the three samples in a high cholesterol/cholic acid-induced hyperlipidemia in vivo model. Cholesterol is an essential building block for cell membrane formation, sexual hormones, and bile salt formation, while it could be consumed through diet as food products and animal fat. Upon ingestion, cholesterol is disseminated in the bloodstream in the form of lipoproteins that are categorized according to their constitution into low-density lipoproteins (LDL) that are rich in cholesterol and high-density lipoproteins (HDL) that are rich in cholesterol and proteins[45]. Feeding rats with cholesterol/cholic acid in coconut oil resulted in the successful development of hyperlipidemia deduced from the significantly elevated values of total cholesterol (TC), triglycerides (TG), and LDL in addition to reduced levels of HDL. Meanwhile, cholic acid (bile acid) significantly increased cholesterol absorption and supported the prompt induction of hyperlipidemia[46]. **Table 5** shows the variable capability of the tested samples in counteracting hyperlipidemia. It is worth mentioning that the AEE escalates the beneficial HDL better than the standard antihyperlipidemic drug, Atorvastatin, while significantly lessening the TC, TG, and LDL levels ($p< 0.01$). BF showed moderate antihyperlipidemic activity followed by CF.

Induction of hyperlipidemia provokes an increase in liver transaminase levels due to the injured hepatic cells and intracellular accumulation of lipids[3,47]. In almost all liver diseases, ALT values are higher than AST values because ALT has a longer circulating half-life[48]. The current investigation showed that feeding rats with high cholesterol/cholic acid in coconut oil resulted in significantly higher serum levels of ALT and AST suggesting liver damage (**Table 6**). The AEE and BF exerted potent hepatoprotective and ameliorated liver enzymes probably due to their phenolic content's antioxidant and anti-inflammatory effect[49]. Interestingly, the AEE effect was comparable to silymarin by the 4th week, yet the AEE surpassed the hepatoprotective effect of silymarin in the 8th week. Additionally, the BF in the 8th week reduced the AST and ALT levels by 55% and

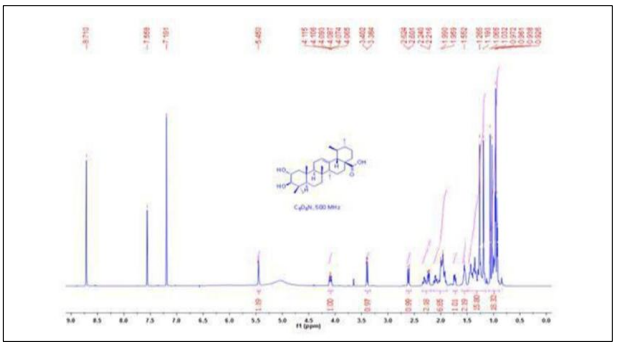

Fig. 1. ^{1}H-NMR spectrum of compound 3

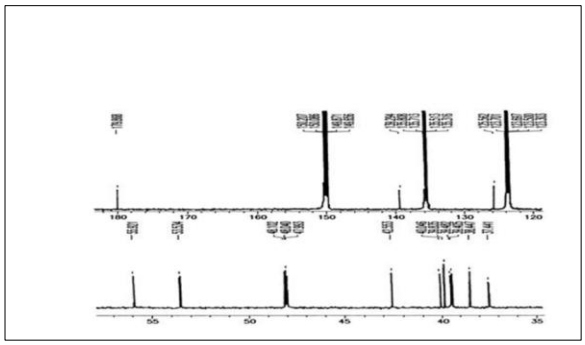

Fig. 2. ^{13}C-NMR spectrum of compound 3

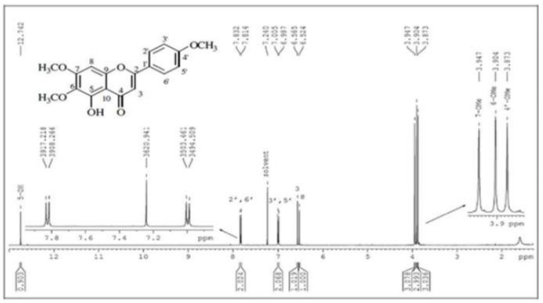

Fig. 3. ^{1}H-NMR spectrum of compound 7

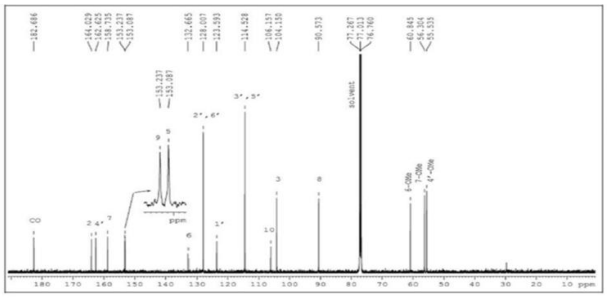

Fig. 4. ^{13}C-NMR spectrum of compound 7

Table 5. Effect of the aqueous ethanol extract (AEE), *n*-butanol fraction (BF), and chloroform fraction (CF) of *M. peregrina* leaves treatments for two months on cholesterol, triglycerides, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) levels of female albino rats (n=6) in obesity-induced *in vivo* model.

Time	AEE (100 mg/Kg/PO)				BF (100 mg/Kg/PO)				CF (100 mg/Kg/PO)				Atorvastatin (10mg/kg)			
	Chol.	Trigly.	HDL	LDL	Chol.	Trigly.	HDL	LDL	Chol.	Trigly.	HDL	LDL	Chol.	Trigly.	HDL	LDL
Zero	179.1 ±6.7	141.2 ±5.2	24.2 ±0.3	149.2 ±6.2	175.7 ±9.3	151.5 ±5.3	25.9 ±0.6	143.7 ±5.9	166.9 ±5.6	157.3 ±4.8	26.1 ±1.5	146.8 ±6.4	170.5 ±6.5	148.7 ±3.4	21.1 ±0.6	144.3 ±6.2
4 weeks	136.2 ±4.7*	92.3 ±3.4*	33.8 ±1.3*	103.6 ±4.2*	146.9 ±7.2*	102.9 ±4.8*	31.7 ±0.9*	113.6 ±6.7*	141.6 ±5.1*	123.9 ±4.5*	33.7 ±1.2*	118.2 ±4.8*	140.3 ±3.1*	90 ±1.5*	24.6 ±2.9*	110 ±1.6
8 weeks	112.3 ±4.6*	73.8 ±2.2*	43.2 ±1.5*	32.7 ±1.1*	124.6 ±4.7*	86.1 ±3.8*	39.4 ±1.3*	61.7 ±2.6*	116.4 ±4.7*	112.7 ±4.2*	39.2 ±0.9*	91.7 ±2.6*	65.4 ±2.5*	74.6 ±9.65*	27.5 ±3.78*	37.45 ±3.5 *
Change	37.3	47.7	78.5	78.1	29.1	43.2	52.1	57.1	30.3	28.4	50.2	37.5	61.6	49.8	30.3	74

**Statistically significant (p <0.01) from zero time.*

Table 6. Effect of the aqueous ethanol extract (AEE), *n*-butanol fraction (BF), and chloroform fraction (CF) of *M. peregrina* leaves treatments for two months on aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels of female albino rats (n=6) in obesity-induced *in vivo* model.

Time	AEE (100 mg/Kg/PO)		BF (100 mg/Kg/PO)		CF (100 mg/Kg/PO)		Silymarin (10mg/kg)	
	AST	ALT	AST	ALT	AST	ALT	AST	ALT
Zero	168.9±6.8	169.2±6.3	147.3±5.2	168.7±6.7	162.1±4.3	172.5±7.4	174±3.1	180±2.4
4 weeks	133.1±6.5*	121.6±6.1*	91.2±2.7*	121.3±4.8*	141.7±6.5*	150.2±7.2*	130±2.9*	139 ±4.2*
8 weeks	51.4±3.6*	68.2±4.1*	65.3±2.9*	82.4±3.8*	122.9±4.8*	143.1±4.6*	80.1±6*	75±5.7*
Change	69.6	59.5	55.7	51.2	24.2	17	54	58.3

**Statistically significant (p <0.01) from zero time.*

Table 7. Effect of the aqueous ethanol extract (AEE), *n*-butanol fraction (BF), and chloroform fraction (CF) of *M. peregrina* leaves treatments for two months on the body weight of female albino rats (n=6) in obesity-induced *in vivo* model.

Group/Treatment	Zero Time	4 weeks	8 weeks	Percentage of change
Negative control	131.6±3.4	149.6 ±3.5 *	151.9±4.2*	23
Positive control	132.9± 3.1	169.3±4.6*	198.3±5.8 *	33
AEE	193.2± 3.2	170.5±5.5*	164.9±4.6 *	14.6
BF	194.8±3.4	184.1±5.4*	170.1±3.7*	12.8
CF	192.8± 3.0	188±6.3*	180.9 ±5.1*	6.3

**Statistically significant (p <0.01) from zero time.*

51%, respectively compared to 54% and 58% for silymarin (**Table 6)**. The CF showed the least amount of activity deduced from dropping the enzymes' level by only 17%-24.2%.

Lastly, the anti-obesity effect of the tested samples was deduced from monitoring the reduction in the animal's body weight at the end of the experiment. The body weights of the negative and positive control groups measured on Days 0, 30, and 60 are delineated in **Table 7**. An increase in body weight of 33% was observed in the positive control group (maintained on a high-caloric cafeteria diet) after two months compared to the negative control group (normal diet group). Treating obese rats with the AEE or its fractions revealed that the AEE significantly reduced the animal's body weight by 14.6% (**Table 7)**. On the other hand, the BF reduced the animal's body weight by 12.8% and the CF by 6.3%. Accordingly, the AEE was more efficient in reducing the animal's body weight, at least in part, due to the synergistic effect of the phenolic components and to a lesser extent non-polar metabolites that are concurrently present in the extract. To the best of our knowledge, this is the first report of these activities on the AEE, and its fraction is derived from *M. peregrina* leaves cultivated in Egypt, however, these activities have been reported on the same species using different organ[44] or from different countries[18,20,42,50]

CONCLUSION

M. peregrina leaves showed high nutritional value deduced from their high carbohydrate, fiber, and protein content. The leaves are a good supply of calcium, magnesium, iron, copper, manganese, and vitamins A and C. The total identified non-essential amino acids (5.50%) exceeded the essential amino acids (3.86%). Glutamic acid (1.21%) and aspartic acid (0.95%) were the major identified non-essential amino acids, while leucine (0.79%) and phenylalanine (0.70%) were the chief detected essential amino acids. The extract was enriched with phenolic content calculated as 91.97±2.53 mg of GAE/g of dry extract. The fractionation and purification of the aqueous ethanol extract (AEE) afforded *β*-amyrin (**1**), *β*-sitosterol (**2**), corosolic acid **(3),** caffeic acid (**4**), rutin (**5),** astragalin (**6)**, salvigenin (**7**), catechin (**8**), and quercetin (**9**). Compounds **3** and **7** were isolated from the leaves of the Egyptian *M. peregrina* for the first time. The total AEE exerted significantly more potent antihyperlipidemic, hepatoprotective, and reduced body weight of obese rats than its subfractions. The observed activity could be, at least in part, due to the synergistic effect of the phenolic components and to a lesser extent non-polar metabolites that are concurrently present in the extract. *M. peregrina* is a promising natural alternative for managing high diet-related disorders. However, consuming moringa leaves in high doses can lead to digestive issues like diarrhea, bloating, stomach pain. In conclusion, propagation of *M. peregrina* in Egypt should be taken into consideration for its promising wide range of application in cosmetic and pharmaceutical industries, yet deep investigation is among our future directions.

Conflicts of interest

The authors declare that they have no conflicts of interest regarding the publication of this paper.

Author contributions

Eman Haggag, Shahira Ezzat: Conceptualization, Supervision, Investigation, Resources, Methodology, Writing - review and editing. Reham Hassan: Methodology, Data curation, Writing - original draft.

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Data availability statement

The original data are included in the manuscript, and further queries can be directed to the corresponding author.

REFERENCES

1. Lin, X., and Li, H. Obesity: Epidemiology, Pathophysiology, and Therapeutics. *Frontiers in Endocrinology*, **2021**, *12*, 706978. https://doi.org/10.3389/fendo.2021.706978
2. World health organization (WHO) 2021 accessed on 11-9-**2024.**
3. Kakimoto, P. A.; Kowaltowski, A. J.; Effects of high fat diets on rodent liver bioenergetics and oxidative imbalance. *Redox Biology*, **2016**, *8*, 216–225. https//doi:10.1016/j.redox.2016.01.009
4. Mach, F.; Baigent, C.; Catapano, A. L.; Koskinas, K. C.; Casula, M.; Lina Badimon, L.; Chapman, M J.; De-Backer, G. G; Delgado, V.; Brian, A. F.; Graham, I. M.; Halliday, A.; Landmesser, U.; Mihaylova, B.; Pedersen, T. R.; Riccardi, G.; Richter, D. J.; Sabatine, M. S.; Taskinen, M-R.; Tokgozoglu, L.; Wiklund, O. 2019 ESC/EAS guidelines for the management of dyslipidaemias: lipid modification to reduce cardiovascular risk. *European Heart J*., **2020**, *41* (1),111–188. https//doi:10.1093/eurheartj/ehz455
5. Deepa, P. R.; Varalakshmi, P. Athero-protective effect of exogenous heparin-derivative treatment on the aortic disturbances and lipoprotein oxidation in hypercholesterolemic diet fed rats *Clin. Chim. Acta*, **2005,** 355 (1-2), 119-130. https://doi.org/10.1016/j.cccn.2004.12.007
6. Alsheikh-Ali, A. A.; Kuvin, J. T.; Karas, R. H. Risk of adverse events with fibrates. American Journal of Cardiology, **2004**, *94* (7), 935-938.
7. Dhakar, R. C.; Maurya, S. D.; Pooniya, B. K.; Bairwa, N.; Gupta, M.; Sanwarmal Moringa: the herbal gold to combat malnutrition. *Chron. Young Sci.*, **2011**, *2* (3), 119-125.
8. Natsir, H.; Wahab, A. W.; Budi, P.; Dali, S.; Arif, A.R. Amino acid and mineral composition of *Moringa Olivera* leaves extract and its bioactivity as antioxidant. *Journal of Science*, **2019**, 1317. https//doi:1088/1742-6596/1317/1/012030
9. Senthilkumar, A.; Karuvantevida, N.; Rastrelli, L.; Kurup, S. S.; Cheruth, A. J. Traditional Uses, Pharmacological Efficacy, and Phytochemistry of *Moringa peregrina* (Forssk.) Fiori. A Review. *Frontiers in Pharmacology*, **2018**, *9*, 465. https://doi.org/10.3389/fphar.2018.00465
10. Ezzat, S. M.; El Bishbishy, M. H.; Aborehab, N. M.; Salama, M. M.; Hasheeh, A.; Abdel Motaal, A. Rashed, H.; Metwally, F. M. Upregulation of MC4R and PPAR-α expression mediates the antiobesity activity of *Moringa oleifera* Lam in high-fat diet-induced obesity in rats. *Journal of Ethnopharmacology*, **2020**, 251 112541 https://doi.org/10.1016/jep.2020. 112541
11. Zahran, E. M.; Mohamed, S.A.; Yahia, R.; Badawi, A.M.; Sayed, A. M.; Abdelmohsen, U. R. Anti-otomycotic of nanoparticles of *Moringa oleifera* leaf extract: an integrated *in vitro*, *in silico* and phase 0 clinical study. *Food & Function*, **2022** https://doi.org/10.1039/d2fo02382b
12. Bellostas N., Sørensen J. C., Nikiema A., Sørensen H., Pasternak D., Kumar S. Glucosinolates in leaves of *Moringa* species grown and disseminated in Niger. African Journal of Agricultural. Research, **2010**, *5*, 1338–1340.
13. Sengupta A., Gupta M. P. Studies on the seed fat composition of Moringacea family. European Journal of Lipid Science and Technology, **1970**. *72*, 6–10.
14. Al-Dhaheri S. M. *In vitro* Re-Generation and Marker Assisted Evaluation of Genetic Fidelity in Endangered Tree Species *Moringa peregrina* (Forsk) Fiori. Master thesis, **2016**, `Al Ain - Abu Dhabi, United Arab Emirates University.
15. Al-Kahtani, H. A. and Abou-Arab, A. A. Comparison of physical, chemical, and functional properties of *Moringa peregrina* (Al-Yassar or Al-Ban) and soybean proteins. *Cereal Chemistry*, **1993,** *70* (6), 619-626.
16. FAO. Traditional Food Plants - A Resource Book for Promoting the Exploitation and Consumption of Food Plants In Arid, Semi-arid and Sub-Humid Lands of Eastern Africa. Food and Agriculture Organization of the United Nations, **1988**, Rome, Italy.
17. Elbatran, S. A.; Abdel-Salam, O. M.; Abdelshfeek, K. A.; Nazif, N. M.; Ismail, S. I.; Hammouda, F. M. Phytochemical and pharmacological investigations on *Moringa Peregrina* (Forssk) Fiori. *Natural Products Science*, **2005**, *11*, 199–206.
18. Afsharypuor, S.; Asghari, G.; Mohagheghzadeh, A., Dehshahri, S. Volatile constituents of the seed kernel and leaf of *Moringa peregrina* (Forssk.) Fiori, Agricolt. Cultivated in Chabahar (Iran). *Iranian Journal of Pharmaceutical Research*, **2010**, *6*, 141–144.
19. Hanif, M. A.; Al-Maskri, A. Y.; Al-Mahruqi, Z. M.; Al-Sabahi, J. N.; Al-Azkawi, A.; Al-Maskari, M. Y. Analytical evaluation of three wild growing Omani medicinal plants. Natural Products Communication, **2011**, *6* (10), 1451–1454.
20. Marwah, R. G.; Fatope, M. O.; Al Mahrooqi, R.; Varma, G. B.; Al Abadi, H.; Al-Burtamani, S. K. S. Antioxidant capacity of some edible and wound healing. Plants of Oman. *Food Chemistry*, **2007**, *101* (2), 465–470.
21. Dehshahri S.; Wink, M.; Afsharypuor, S.; Asghari, G.; Mohagheghzadeh, A. Antioxidant activity of methanolic leaf extract of *Moringa peregrina*

(Forssk.) Fiori. *Research Pharmaceutical*, **2012**, *7* (2), 111-118.
22. Albaayit, S. F. A. *In-vitro* evaluation of anticancer activity of *Moringa peregrina* seeds on breast cancer cells. *The Eurasia Proceedings of Science, technology, Engineering & Mathematics*, **2020**, *11*, 163-166.
23. Al Kaabi, S. K. S.; Senthilkumar, A.; Kizhakkayil J.; Alyafei, M. A. M.; Kurup S. S.; Al Dhaheri, A. S.; Jaleel, A. Anticancer Activity of *Moringa peregrina* (Forssk.) Fiori.: A Native Plant in Traditional Herbal Medicine of the United Arab Emirates. *Horticulturae*, **2022**, *8*, 37. https://doi.org/10.3390/horticulturae8010037
24. AOAC Official Methods of Analysis 15th edition, Association of Official Analytical Chemists, Washington D.C. **1995**.
25. Japanese Pharmacopoeia, General Notices of Japanese Pharmacopoeia and Other Standard, 15thEdn., *MHLW Ministerial Notification* **2007**, *65*, 1655-1662.
26. Adeyeye, I. A. and Omotayo, F. O. Chemical Composition and functional properties of leaf protein concentrates of *Amaranthus hybridus* and *Teliferia occidentalis*. *Agriculture and Biology Journal of North America*, **2011**, *2* (3), 449–511. https://doi:10.5251/abjna.2011.2.3.499.511
27. Douglas, J. H. Chemical Methods for Determination of Vitamin B1. *Industrial and Engineering Chemistry (Analytical Edition)*, **1941**, *13* (4), 216–218.
28. Arroyave, G.; Mejía, L. A.; Aguilar, J. R. The effect of vitamin A fortification of sugar on the serum vitamin A levels of preschool Guatemalan children: a longitudinal evaluation. *American Journal of Clinical Nutrition*, **1981**, *34* (1), 41-49.
29. Pyka, A.; and Sliwiok, J. Chromatographic separation of tocopherols. *Journal of Chromatography A*, **2001**, *935* (1-2), 71-76.
30. Hussein, S. A.; Barakat, H. H; Merfort, I.; Nawwar, M. A. Tannins from the leaves of *Punica granatum*. *Phytochemistry*, **1997**, *45*, 819–23.
31. Alhakmani, F.; Kumar, S. Khan, S. A. Estimation of total phenolic content, *in vitro* antioxidant and anti-inflammatory activity of flowers of *Moringa oleifera*; *Asian. Pacific Journal of Trop Biomed*, **2013**, *3* (8), 623-627. DOI: 10.1016/S2221-1691(13)60126-4
32. Madaan, R.; Bansal, G.; Kumar, S.; Sharma, A. Estimation of total phenols and flavonoids in extracts of *Actaea spicata* roots and antioxidant activity studies. *Indian Journal of Pharmaceutical Science*, **2011,** *73* (6), 666-669.
33. Karber, G. Determination of LD_{50}. Archives of *Experimental Pathology and Pharmacology*, **1931**, 162, 480-487.
34. Ney, D. M.; Laska, G. P.; Shinnick, E. F. Induction of hyperlipidemia. *Journal of Nutraceutical*, **1988**, 118, 1455.
35. Hamilton, B. S. and Doods, H. N. Chronic application of MTII in a rat model of obesity results in sustained weight loss. *Obesity. Research*, **2002**, *10* (3), 182-187. doi: 10.1038/oby.2002.28
36. Pearson, D. The Chemical Analysis of Foods, 7thEdn., Churchill Livingstone, London, **1976**
37. Onyeike, E. N.; Osuji, J. O. (eds) Histochemical Research Techniques In "Research Techniques in Biological and Chemical Sciences". Springfield Publishers Limitted, Owerri, Nigeria, ISBN 978-8020-84-4, **2003**
38. Abdalla, H. A. M.; Ali, M.; Amar, M. H.; Chen, L.; Wang, Q-F. Characterization of phytochemical and nutrient compounds from the leaves and seeds of *Moringa oleifera* and *Moringa peregrina*. *Horticulturae*, **2022**, *8* (11) 1081. http://doi.org/10.3390/horticuturea8111081
39. Al-Kahtani, H. *Moringa perigrina* (Al-Yassar or Al-Ban) seeds oil from northwest Saudi Arabia. *Journal of King Saud Univ. Agric. Sci.*, **1995**, *7*, 31–45.
40. Hertzler, S. R.; Lieblein-Boff, J. C.; Weiler, M.; Allgeier, C. Plant proteins: assessing their nutritional quality and effects on health and physical function. *Nutrients*, **2020**, *12* (12), 3704. https://doi.org/10.3390/nu12123704
41. Radha, K. M.; Puri, S.; Pundir, A.; Bangar, S. P.; Changan, S.; Choudhary, P.; Parameswari, E.; Alhariri, A.; Samota, M. K.; Damale, R. D.; Singh, S.; Berwal, M. K.; Dhumal, S.; Bhoite, A. G.; Senapathy, M.; Sharma, A.; Bhushan, B.; Mekhemar, M. Evaluation of nutritional, phytochemical, and mineral composition of selected medicinal plants for therapeutic uses from Cold Desert of Western Himalaya. *Plants (Basel, Switzerland)*, **2021**, *10* (7), 1429. https://doi.org/10.3390/plants10071429
42. Asghari, G.; Palizban, A.; Bakhshaei, B. Quantitative analysis of the nutritional components in leaves and seeds of the Persian *Moringa peregrina* (forssk.) Fiori. *Pharmacognosy Research*, **2015**, *7* (3), 242-248. doi: 10.4103/0974-8490.157968
43. El-Alfy, S.; Ezzat, M. S.; Hegazy, K. A.; Amer, M. M. A.; Kamel M. G. Isolation of biologically active constituents from *Moringa peregrina* (Forssk.) Fiori. (Family: Moringaceae) growing in Egypt. *Pharmacognosy Magazine*, **2011**, *7* (26), 109–115.
44. Elarabany, N.; Hamad, A.; AlSobeai, S.M. Evaluating anti-obesity potential, active components, and antioxidant mechanisms of *Moringa peregrina* seeds extract on high-fat diet-

induced obesity. *Journal of food. Biochem*istry **2022**, *46*, e:14265. https://doi.org/10.1111/jfbc.14265

45. Xie, Y.; Zhang, H.; Liu, H.; Xiong, L.; Gao, X.; Jia, H.; Lian, Z.; Tong, N.; Han, T. Hypocholesterolemic effects of *Kluyveromyces marxianus* M3 isolated from Tibetan mushrooms on diet-induced hypercholesterolemia in rat. *Brazelian Journal of Microbiology*, **2015**, *46* (2), 389-395. https://doi.org/10.1590/S1517-838246220131278
46. Woollett, L. A.; Buckley, D. D.; Yao, L.; Jones, P. J.; Granholm, N. A.; Tolley, E. A.; Tso, P.; Heubi, J. E. Cholic acid supplementation enhances cholesterol absorption in humans. *Gastroenterology*, **2004**, *126* (3), 724–731. https://doi.org/10.1053/j.gastro.2003.11.058
47. Hasan, K. M. M.; Nasrin, T.; Anwarul, H. Biochemical and histopathological profiling of Wistar rat treated with Brassica napus as a supplementary feed. *Food Sci. Human Wellness*, **2018**, *7*, 77-82, doi: 10.1016/j.fshw.2017.12.002
48. Vuppalanchi, R.; Chalasani, N. Laboratory Tests in Liver Disease, Editor: Saxena, R. "Practical Hepatic Pathology: A Diagnostic Approach", Elsevier Inc. **2011,** ISBN 978-0-443-06803-4 https://doi.org/10.1016/C2009-0-38374-9
49. Elbouny, H.; Ouahzizi, B.; Bammou, M.; Sellam, K.; Alem, C. Anti-hyperlipidemic and antioxidant potential of phenolic monoterpenes rich *Thymus satureioides* Coss. volatile oil. *Journal of Biologically Active Products from Nature*, **2023**, *13* (5),448–459. https://doi.org/10.1080/22311866.2023.2277899
50. Alkhudhayri, D.A.; Osman, M. A.; Alshammari, G. M.; Al Maiman, S. A.; Yahya, M. A. *Moringa peregrina* leaf extracts produce anti-obesity, hypoglycemic, anti-hyperlipidemic, and hepatoprotective effects on high-fat diet fed rats. *Saudi journal of biological sciences*, **2021**, *28* (6), 3333–3342. https://doi.org/10.1016/j.sjbs.2021.02.078