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Unveiling the Antianemic Activity, Physicochemical Aspects, Antioxidant Properties, and Mineral Profile of *Petroselinum crispum L*

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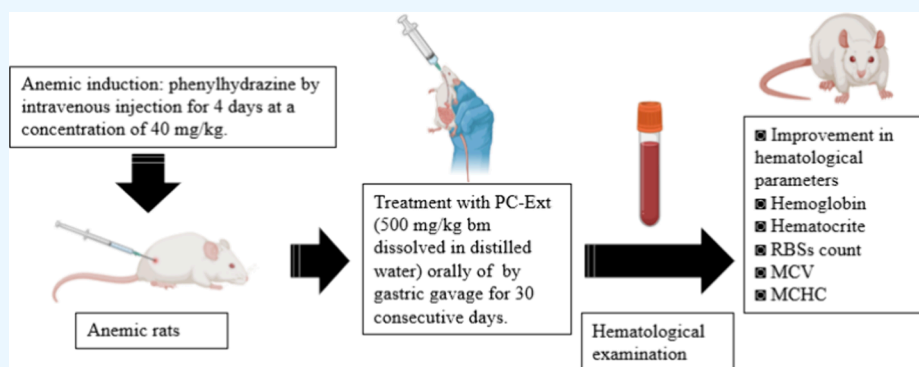
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ABSTRACT: Anemia is a widespread global health concern necessitating effective, accessible, and natural interventions. The potential of medicinal plants to address anemia has garnered significant interest. Among these plants, parsley (*Petroselinum crispum* (*Petroselinum crispum*) L.) stands out as an edible and herbal-based option for combating anemia. Aim of the study: This study investigated the potential of *P. crispum* (PC-Ext) as an emerging antianemic product, focusing on its physicochemical attributes, antioxidant properties, and mineral profile. Both qualitative and quantitative analyses of the phenolic compounds in *P. crispum* were conducted by using high-performance liquid chromatography with a diode array detector (HPLC-DAD). Anemia was induced in rats by intravenous injections of phenylhydrazine, administered at a dose of 40 mg/kg for two consecutive days. The antianemic activity of PC-Ext was assessed at a dose of 500 mg/kg twice daily for 5 weeks by estimating blood parameters, such as serum iron and ferritin. Additionally, the osmotic fragility test measured the capacity of red blood cells to withstand osmotic shock of various concentrations of saline. Aqueous extract of *P. crispum* was rich in phytochemical compounds, including syringic acid, quercetin, catechin, gallic acid, and luteolin. The findings demonstrate the effectiveness of *P. crispum* in ameliorating phenylhydrazine-induced reductions in red blood cell count (RBCs), hemoglobin (Hb), and hematocrit (HCT) levels. Consequently, PC-Ext exhibits significant activity against phenylhydrazine-induced anemia in rats, as demonstrated by its ability to prevent hemolysis. Iron estimation within PC-Ext further confirms its utility in addressing both iron deficiency and ferritin-deficiency anemia. Therefore, PC exhibits a favorable effect against both types of anemia, iron deficiency, and hemolysis. The results of this study provide robust scientific validation for ethnomedicinal use and the potential utility of *P. crispum*, positioning it as a promising source for future pharmaceutical development.

1. INTRODUCTION

Anemia represents a prevalent public health issue on a global scale, with particular significance in developing nations. According to the World Health Organization,[1] about a quarter (1.62 billion) of the world's population suffer from anemia. It occurs at all life cycle stages, but is more common in pregnant women and young children.[2]

Anemia is a pathological condition, in which the number of red blood cells cannot meet the body's physiological needs.[3] It is an indicator of poor nutrition and health and is due, in most

cases, to iron deficiency.[4] However, other causes such as severe carcinoma, genetic abnormalities, infectious diseases, prolonged

use of nonsteroidal drugs, and exposure to toxic substances such as phenylhydrazine can also be associated with anemia.[5]

Several treatments, including oral iron supplementation, vitamin B9 or B12, erythropoietin injection, blood transfusion, and bone marrow transplants, have been used for management of anemia.[5−8]

Food supplements can be a nutritional solution[9] to effectively treat anemia and be an alternative to conventional treatments in hospitals or clinics. To this end, valorizing vegetable resources rich in protein and micronutrients, accessible at the least cost, would be a strategy to effectively mitigate anemia.[10] The potential of medicinal plants to address anemia has been a subject of significant interest.[11]

Among the resources of the floristic heritage, including in Morocco, *Petroselinum crispum* (*P. crispum*) L. is a biennial herbaceous plant of the Apiaceae family.[12] For centuries, they have been cultivated across the globe for their use in food flavoring, essential oils, and traditional medicinal practices. It is a rich source of natural antioxidants.[13] Antioxidant,[14] antibiotic,[15] antifungal,[16] neuroprotective,[17] nephroprotective,[18] antidiabetic,[19] analgesic,[20] spasmolytic,[21] antiplatelet,[22] laxative,[21] diuretic,[23] and aphrodisiac[24] are just a few of the pharmacological effects linked to parsley. Despite the extensive utilization of parsley in traditional medicine, this study was the first attempt to assess the antianemic effects of *P. crispum*. The results of the study presented here suggest the inclusion of its effective use to treat iron deficiency anemia among the various biological activities attributed to parsley. Therefore, the present study was designed to evaluate physicochemical aspects, antioxidant activity, mineral profile, and the protective effect of Moroccan *P. crispum* against phenylhydrazine-induced hemolytic anemia in albino rats with potential future pharmaceutical applications.

2. MATERIALS AND METHODS

2.1. Plant Collection and Authentication. Fresh aerial parts of *P. crispum* (Mill.) Fuss. were collected from the Sefrou area of Morocco during the flowering period, which spans from June to October. After taxonomic identification, a voucher specimen (RAB40104) was deposited at the Laboratory of Natural Substances, Pharmacology, Environment, Modeling, Health, and Quality of Life (SNAMOPEQ) at the Faculty of Sciences, Dhar El-Mehraz, Sidi Mohamed Ben Abdellah University. The collected parsley aerial parts were then mechanically fragmented and dried at ambient temperature for 7 days in a well-ventilated area, protected from light. Once dried, they were stored in sealed containers at 0 °C.

2.2. Preparation of Plant Extract. Extraction was accomplished based on the procedure outlined by the World Health Organization (WHO).[12] The process involved macerating 10 g of powdered material in 100 mL of aqueous solvent for 1 week with continuous agitation at 15,000g and at room temperature. Subsequently, the resulting solution was filtered using Whatman filter paper no. 1 and concentrated in a rotary evaporator (Büchi R-210, Flawil, Switzerland) at 40 °C under reduced pressure to yield approximately 23% (w/w) of the solid residue.[13] This solid residue was later reconstituted in distilled water to achieve the desired concentrations for the *in vitro* and *in vivo* experiments.

2.3. Chemical Analysis of PC-Ext. *2.3.1. Total Carbohydrates.* Carbohydrate analysis was conducted using a modified phenol-sulfuric acid method.[27] In brief, 100 μL of parsley extract (PC-Ext) was mixed with 500 μL of sulfuric acid (96−98% v/v). Subsequently, 120 μL of 5% phenol reagent was added, and the mixture was heated at 90 °C for 5 min. After the solution was allowed to cool to room temperature for 5 min, the absorbance was measured at 490 nm using a PerkinElmer Lambda 40 UV/vis spectrophotometer. Glucose, in the concentration range of 2−300 mg/L, was used as a standard to construct the calibration curve (R^2 = 0.992). The total carbohydrate content was expressed as milligrams of glucose equivalents (mg of Glc/g) per gram of parsley.

2.3.2. Protein Content. The soluble protein content was determined using a modified Bradford assay.[28] In this method, 100 μL of parsley extract (PC-Ext) was mixed with 1 mL of Bradford reagent. The mixture was kept in the dark for 5 min, after which its absorbance was measured at 595 nm using a PerkinElmer Lambda 40 UV/vis spectrophotometer. A standard curve (ranging from 4 to 5000 μg/mL, R^2 = 0.997) was generated using bovine serum albumin (BSA). The protein content was then expressed as milligrams of BSA equivalents per gram of parsley (mg of BSA/g).

2.3.3. Total Phenolic Content. Total phenolic content (TPC) was quantified by use of the Folin Ciocalteu methodology using a UV/vis spectrophotometer.[13] The gallic acid calibration curve (8−2000 mg/L, R^2=0.9992) was used as a standard. The results are expressed in the dry plant's gallic acid equivalent (GAE) g[1].

2.3.4. Total Flavonoid Content. Total flavonoid content (TFC) was determined using a colorimetric method described previously,[1] utilizing a UV/vis spectrophotometer. Quercetin, in the concentration range of 7.81−125 mg/L, was used to create the standard curve (R^2 = 0.996). The results were expressed in milligrams of quercetin equivalents (QE) per gram of dry parsley (mg QE/g dw).

2.3.5. Identification and Quantification of Phenolic Compounds. The aqueous extract of *P. crispum* was characterized by the use with C18 of a Waters e 2695 HPLC analysis with a C18 column that has (5 μm, 250 mm × 4.6 mm) dimensions. Eluents were A: water/acetic acid (0.5%v/v) and B: methanol. The analysis was done by gradient mode, and the flow was maintained to 0.8 mL/min for 26 min. Extracts (20 μL) were injected, and compounds were identified using a diode array detector on 280 and 360 nm interval.[15] The compounds were characterized based on comparing chromatogram retention time and λ max with standards.[16]

2.4. Antioxidant Activity. Five methods to measure the antioxidant activity of PC-Ext were used: 2,2-diphenyl-1-picrylhydrazyl radical (DPPH•), 2,2′-azino-bis (3ethylbenzothiazoline-6-sulfonic acid) radical cation-based (ABTS•+), ß-carotene bleaching tests (BCBT), reducing antioxidant power (RP) assays, and total antioxidant activity (TAC). All assays were determined spectrophotometrically using a UV/vis spectrophotometer.[17−19]

For the DPPH assay, a calibration curve was prepared using BHT with a concentration range of 100−5 mmol/L (R^2 = 0.986) and 434−11 mmol/L (R^2 = 0.992) for the ABTS assay with a Trolox control. For both the RP and TAC assays, ascorbic acid was standard with a reference (1000−100 μM, R^2 = 0.996). RP values are expressed as μg/g of Asc ac equivalent per g of PC-Ext (μg AAE/g), and (mg AAE/g Dw) for the TAC test.

Radical-scavenging activity for the DPPH and ABTS methods in % inhibition was estimated (eqs 1 and 2).

$$\text{inhibition(\%)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100 \qquad (1)$$
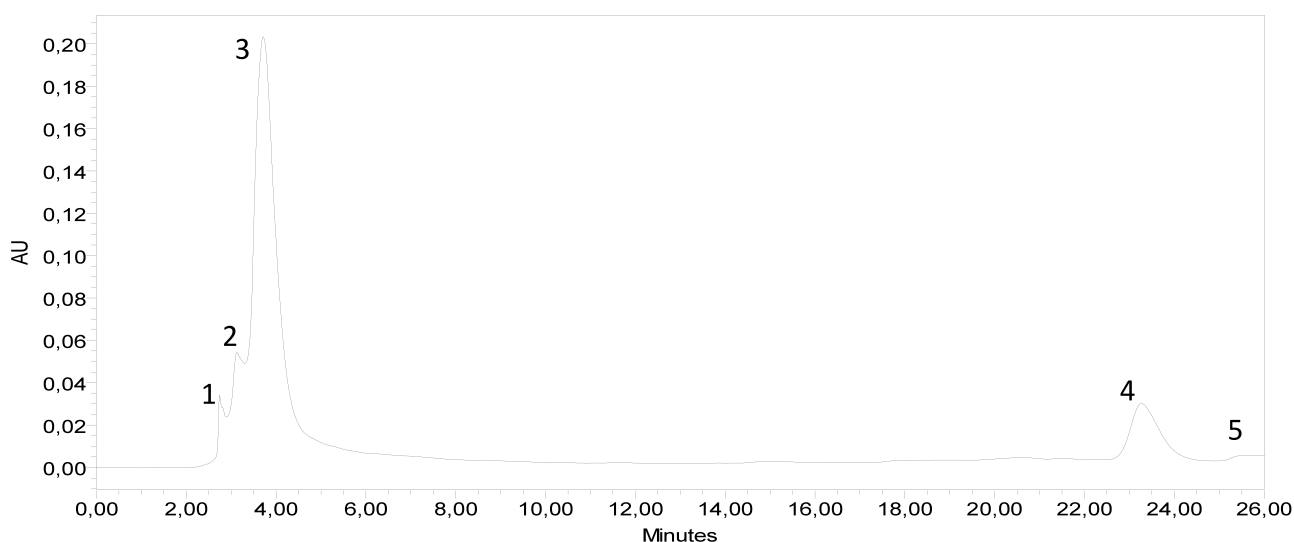
Figure 1. HPLC-DAD chromatogram of *P. crispum* extract (PC-Ext) at 320 nm using the following standards: gallic acid (1), catechin (2), syringic acid (3), quercetin (4), and luteolin (5).

$$\text{inhibition(\%)} = \frac{\text{Abs}(t = 100\text{min})}{\text{Abs}(t = 0)} \times 100 \qquad (2)$$

The results were expressed concentrations required to inhibit 50% (IC_{50}) of DPPH$^\bullet$ and ABTS$^{\bullet+}$ radicals (μg/mL), and BCBT mg/mL.

2.5. Mineral Elements. Mineral elements were determined accordingly, as described in.[33] After burning about 1 g of material for 2 h at 500 °C in a muffle furnace, the ash was treated with hydrochloric acid and nitric acid (65%). The concentrations of B, Ca, Cd, Co, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, Se, and Zn were determined by inductivity-coupled plasma-atomic emission spectrometry (ICP-AES) spectrometry. The results were given as milligrams of mineral (mg/100g) for each 100 g of various parsley parts. Each sample underwent three separate analyses.

2.6. In Vivo Anemia Experimental Design. *2.6.1. Ethical Approval.* Albino rats weighing between 250 and 300 g were housed under standard control conditions at the animal facility of the Faculty of Science, Sidi Mohamed Ben Abdellah University, Fez, Morocco. Rats were acclimatized in a controlled environment maintained at 25 ± 1 °C, with a 12 h photoperiod (12 h of light and 12 h of darkness). They had unrestricted access to both tap water and food *ad libitum*. The procedures conducted in this study were carried out in accordance with ethical guidelines and were approved by our institutional committee on animal protection. This approval is reported under reference number L.20. USMBA-SNAMOPEQ 2023-03.

2.6.2. Induction of Anemia. Intravenous injections of 40 mg phenylhydrazine/kg, body mass were given on four successive days to induce anemia,[7] at 40 mg/kg for the initial 2 days and subsequently dose of 10 mg phenylhydrazine/kg, body mass/kg on days 3 and 4. Rats were considered anemia-induced when RBC levels as well as hemoglobin content of the blood reduced by 30% relative to the controls.[11]

2.6.3. Experimental Animal Protocol. Animals were divided into 5 groups including 10 rats in each group ($n = 50$). The experimental design was carried out as follows: Group I (control) rats were administered distilled water (1 mL/kg/day, po) for a period of 30 days; Group 2 (anemic control) received phenylhydrazine by intravenous injection for 4 days at a concentration of 40 mg/kg, bm; Group 3 (normal control) rats were treated with PC-Ext (500 mg/kg bm dissolved in distilled water) orally by gastric gavage for 30 consecutive days; and Group 4 received phenylhydrazine by intravenous injection for 4 days at a concentration of 40 mg/kg and was then treated with PC-Ext (500 mg/kg bm) orally for 5 weeks. The treatment duration and phenylhydrazine doses were selected according to Diallo et al. study.[7]

The hematological analysis was conducted within 24 h of blood collection. The first set of blood samples from all animals in the entire group was collected prior to the induction of anemia (Day 0). Subsequently, the second set of blood samples was obtained 3 days after the induction of anemia (Day 3). The third and fourth blood samples were collected on days 15 and 30.

2.6.4. Analysis of Hematological Parameters. A refined retro-orbital bleeding (ROB) technique was used, adhering to the lateral method outlined by Sharma et al.[34] to obtain a sufficient volume of high-quality blood samples. For this purpose, sterile glass Pasteur transfer pipettes with flat edges were used. The rats were made to feel very sleepy by gently inhaling diethyl ether and then having their eyelid gently pulled back to reveal the eye. Placing the pipet flat edge at the lateral canthus, it was inserted at a 45° angle about the sagittal and coronal planes toward the back of the skull. Blood was observed to flow from the capillaries draining the orbital sinus[35] after a cautious rotation was carried out with pressure applied against the orbital bone, directly in front of the zygomatic arch. In this way, capillary action draws blood into the tube. Blood samples were collected and centrifuged for 15 min at 10,000×*g* to separate the serum, which was then kept at −20 °C pending biochemical analysis.

In blood: An automatic counter (Sysmex K21, Tokyo, Japan) was used to measure the following parameters: hemoglobin concentration (HGB), red blood cells (RBC), hematocrit (HCT), mean capsulated hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelets (PLT).

In serum: iron, ferritin.

2.7. In Vitro Osmotic Fragility Test. The osmotic fragility test measured the capacities of red blood cells to withstand various osmotic stresses of concentrations of saline by use of the method of Dacie (1954). Briefly, a blood sample from the rats

was taken into EDTA tubes, and the RBC mass was purified. At room temperature, 50 μL of cell suspension was added to tubes, each containing 5 mL of buffered (pH 7.4) sodium chloride solution osmotically equivalent to sodium chloride at the graduate concentrations (0.3; 0.35; 0.4; 0.45; 0.5; 0.55; 0.6; 0.65; 0.7; 0.75; 0.8; 0.85%). The tubes were incubated at room temperature for 30 min and centrifuged (10.000g) for 5 min.[20]

The relative amount of hemoglobin in the supernatant was determined spectrophotometrically at 540 nm with a 0.85% NaCl sample serving as a blank. The percent hemolysis is calculated (eq 3).

$$\text{percentage haemolysis} = \frac{\text{optical density of test solution}}{\text{optical density of standard solution}} \times 100 \quad (3)$$

2.8. Statistical Analyses. To compare the results, GraphadPad Prism version 8.0 software was used. For comparing data involving only two groups, we conducted a student's t test. We employed Tukey's multiple comparison test when dealing with three or more groups relative to a control group with a significance threshold of Type I error of $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Phenolic Composition of the Aqueous PC-Ext. A total of five compounds were identified in the plant: gallic acid (3.48%), catechin (9.96%), syringic acid (74.33%), quercetin (11.82%), and luteolin (0.41%). HPLC chromatograms of the identified polyphenols are presented in Figure 1 and Table 1. Structures of these phytoconstituents are provided (Figure 2).

Table 1. HPLC Chromatographic Analysis of Compounds Identified in Extracts of *P. crispum*

peak	polyphenolic compounds	formula	retention Time	area	% area
1	gallic acid	$C_7H_6O_5$	2.740	354,806	3.48
2	catechin	$C_{15}H_{14}O_6$	3.128	1,016,951	9.96
3	syringic acid	$C_9H_{10}O_5$	3.713	7,589,268	74.33
4	quercetin	$C_{15}H_{10}O_7$	23.273	1,206,831	11.82
5	luteolin	$C_{15}H_{10}O_6$	25.443	42,126	0.41

3.2. Soluble Carbohydrate and Protein Content of the Aqueous PC-Ext. Mean carbohydrate content was approximately 4.18 ± 0.2 g/100 g dm (Table 2), But carbohydrate content can vary from sample to. In addition to carbohydrates, proteins emerge as the predominant components within the apiaceous plant, another member of the botanical realm. Soluble proteins were dominant with a concentration of 12.41 ± 1.2 mg of BSA/100g.

3.3. Phenolic and Flavonoid Content of the Aqueous PC-Ext. The total phenolic content of *P. crispum* was 19.99 ± 0.84 mg GAE/g dm (Table 2). In addition, the observed flavonoid content of PC-Ext was 5.77 ± 0.16 mg QE/g parsley, dm (Table 2).

3.4. Antioxidant Activity. When five recommended *in vitro* assays, including DPPH, ABTS, BCBT, RP, and TAC, were used to measure the antioxidant activity of the investigated *P. crispum* aqueous extracts of parsley, varied significantly. For the DPPH and ABTS tests, the greatest antiradical capacities were attributed to the concentrations required to inhibit 50% of DPPH$^{\bullet}$ and ABTS$^{\bullet+}$ radicals, which were 50.17 ± 1.9 and 312.42 ± 0.16 μg/mL, respectively. However, these values are

greater than those previously reported by our research team in a new antioxidant formulation with three Apiaceae plants, when parsley recorded the lowest trapping potential. Aqueous extracts of parsley leaf reported elsewhere ranged from 18 to 55% DPPH scavenged.[21] Other studies in Saudi Arabia have determined that the scavenging effect (%) of parsley green parts on 1,1-diphenyl-l-2-picrylhydrazyl radical (DPPH) was 88.91%.[22] In a different study, the IC$_{50}$ value of parsley grown in Turkey was 4.21 μg/mL compared to the positive control BHT.[23] In the β-carotene bleaching test, the inherent antioxidants in the PC-Ext were measured at 2.98 ± 0.9 mg/mL. These values were notably higher than those reported by Zhang et al., who found a concentration of 5.12 mg/mL.[24]

Reducing power (RP) of extracts of parsley had an EC$_{50}$ value of 2.019 ± 0.07 mg/g, dm, which was relatively great compared to that of ascorbic acid, which was 0.031 ± 0.07 mg/g. These results are significantly more critical than those reported for Egyptian *P. crispum*, with an EC$_{50}$ of 0.93 mmol/L.[6]

The TAC assay is based on reducing phosphomolybdate ions in the presence of an antioxidant, forming a green phosphate/MoV complex, which is measured spectrophotometrically.[25] PC-Ext had a total antioxidant capacity value of 104.31 ± 5.40 mg AAE/g compared to standards (Table 2). These results are consistent with the range of those reported by others, where the TAC value was 166.83 ± 1.96 acid eq/g extract.[26]

3.5. Mineral Composition of PC-Ext. Of the 15 elements measured, potassium (K) and calcium (Ca) emerged as the most abundant metals in all examined plant parts, with concentrations ranging from 1000 to 2061.4 mg/100g for K and from 817.15 to 1000 mg/100 g for Ca (Table 3). Magnesium (Mg), sodium (Na), and phosphorus (P) were also present in substantial amounts, with average values of 462.8, 593.09, and 154.57 mg/100g dm, respectively. Boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn), categorized as minor minerals, exhibited values ranging from 2.52 (stem) to 31.43 mg/100g (leaves) for B, from 1.11 (leaves) to 16.59 mg/100g dw (stem) for Fe, and from 2.88 (stem) to 8.91 mg/100g dm (leaves) for Zn. Cadmium (Cd), cobalt (Co), nickel (Ni), lead (Pb), and selenium (Se) were undetected in all three plant parts.

3.6. *In Vivo* Evaluation. *3.6.1. Effect of PhZ and PC-Ext on Body Weight.* Body mass (bm) of rats was significantly ($p \leq 0.05$) less in the PhZ group compared to control rats on day D21 (Figure 3). Several groups of antianemic standards and the PhZ+PC-Ext extract significantly improved bm ($p \leq 0.05$). There was no significant change ($p \leq 0.05$) between the control and normal groups. In addition, treating rats with PC-Ext with induction of anemia reversed the loss of mass (g) to a moderate level (g) on day D30. In contrast, the highest significant increase ($p < 0.05$) in final mass was found in group V compared to group I on days 15 and 30.

3.6.2. Effect of PC-Ext Treatment on In Vitro Erythrocyte Osmotic Fragility of Rats Exposed to Phenylhydrazine. Complete hemolysis (100%) was observed in the control solvent containing a NaCl concentration of 0.0% (Figure 4). There was no significant difference ($P > 0.05$) in hemolysis between rats in treatment groups at 0.1, 0.3, 0.35, and 0.4% NaCl. There were, however, significant changes in the percentage of fragile erythrocytes between the 0.5 and 0.7% NaCl groups. When PC-Ext was given concurrently with phenylhydrazine-treated and normal rats in a 0.5% sodium chloride dose, hemolysis percentages of 3.41 ± 0.01 and 2.17 ± 0.4%, respectively, were less when compared to the phenylhydrazine-poisoned group, with 55%. The percentage of
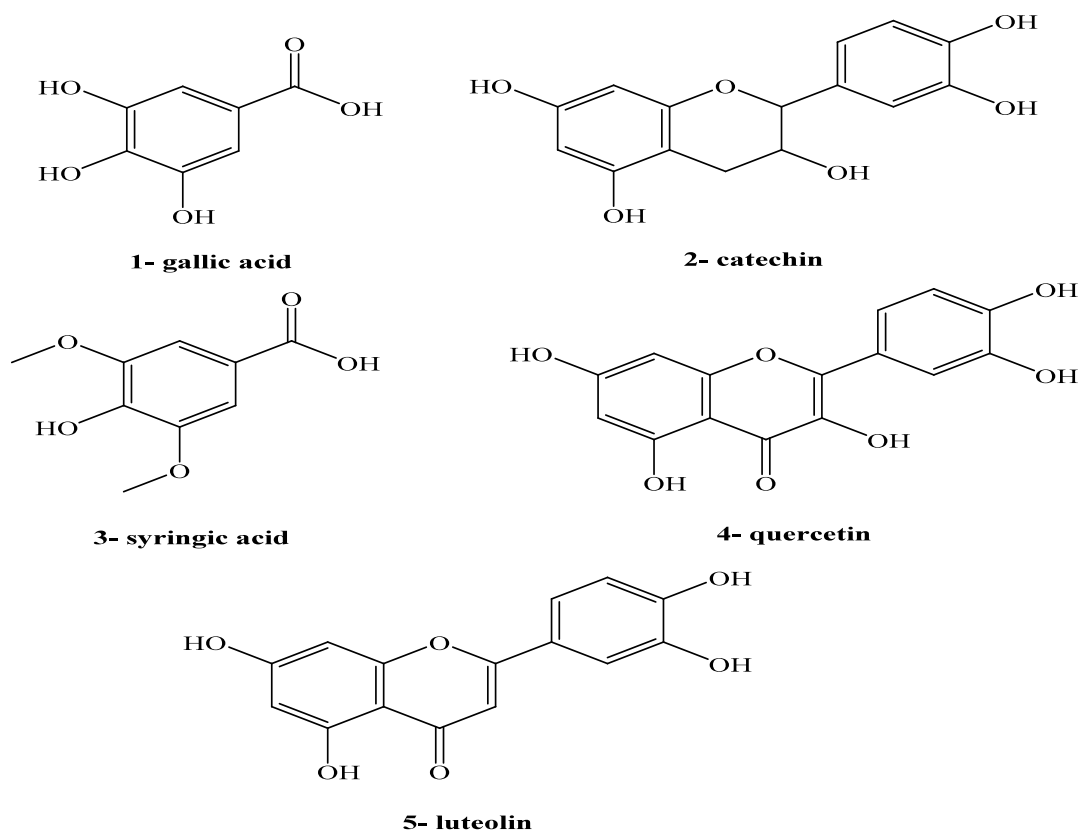
Figure 2. Structures of phytoconstituents of PC-Ext.

erythrocyte fragility age was greatest in the PhZ group compared with other treatment groups at different levels of NaCl concentrations.

3.6.3. Effect of P. crispum Aqueous Extract on the Levels of Hematological Parameters. Rats in the experimental group (PHZ) exhibited significantly lesser values of RBC, HGB, and HCT compared to the normal group ($p < 0.05$) on day 30, with reductions exceeding 50% in red blood cells and 30% in hemoglobin levels (Table 4). This substantial effect indicates the presence of anemia, as previously defined, when a reduction of greater than 30% occurs in these parameters.[27] Results from this study highlight that those rats treated with PC-Ext (500 mg/kg) in conjunction with PHZ experienced a significant restoration of RBC, HGB, and HCT values compared to the positive group. Additionally, phenylhydrazine initially elevated platelet levels during the 15-day experiment and subsequently declined on the final day. Serum heme concentrations were significantly elevated, particularly at 15 days, in rats receiving PHZ. However, this increase was considerably reversed through a combination supplementation therapy involving PC-Ext.

3.6.4. Effect of Interventions on Serum Iron and Ferritin Levels. Concentrations of both serum iron and ferritin exhibited a significant elevation in the PhZ+PC and normal groups compared with the PhZ group (Figure 5). In the group afflicted by phenylhydrazine-induced anemia, a number of red blood cells was observed, leading to diminished Hb and HTC (Table 4). Consequently, it is plausible for concentrations of iron in blood serum to be less, given the reduced iron availability stemming from the breakdown of red blood cell hemoglobin.

4. DISCUSSION

While numerous bioactive compounds in parsley are known to affect various diseases, including diabetes and cancer,[28] limited attention has been given to examining parsley's potential to prevent phenylhydrazine-induced hemolytic anemia. Phenolic compounds, known for their antioxidant, cholesterol inhibition, anticancer, antiaging properties, and antibacterial effects,[4] have demonstrated antianemic effects, potentially correcting blood disorders.[1] Hemolytic disease, associated with increased oxidative stress, can be detrimental to the blood cells. The phenolic compounds in parsley neutralize free radicals, protecting cells from oxidative damage, suggesting that consuming a certain number of phenolic acids might prevent and alleviate the effects of various diseases. Results of another study revealed that apple vinegar enhanced the antioxidant defense system, reducing enzyme leakage into the plasma.[29] These findings are consistent with previous results where antioxidant and organ-protective effects of parsley oil were reported; these effects may be attributed to the attenuation of lipid peroxidation through the potential antioxidant activity of parsley oil and its main components, myristicin and apiol. These components are believed to be responsible for the therapeutic effects of parsley.[30] The enduring significance of parsley as an agricultural crop lies in its remarkable nutritional value, particularly as a rich source of natural antioxidants.[31] The average carbohydrate content is approximately 4.18 ± 0.2 g/100 g dry mass. However, this seemingly constant value belies the nuanced reality that parsley's carbohydrate content can fluctuate. This variability is due to multiple factors, including age, ambient growing conditions, and the specific plant component under scrutiny, whether the verdant leaves, sturdy stems, or tenacious roots.[24] Beyond carbohydrates, proteins

**Table 2. Nutritional Parameters and Antioxidant Activity of PC-Ext[a]**

parameters	carbohydrates (mg GLc/100g)	Soluble protein (mg BSA/100g)	TPC (mg GAE/g)	TFC (mg QE/g)	DPPH IC$_{50}$ (μg/mL)	ABTS IC$_{50}$ (μg/mL)	BCBT IC$_{50}$ (mg/mL)	RP (mg/g)	TAC (mg AAE/g)
PC-Ext	4.18 ± 0.2[e]	12.41 ± 1.2[d]	19.99 ± 0.84[d]	5.77 ± 0.16[e]	50.17 ± 1.9[c]	312.42 ± 0.16[a]	2.98 ± 0.9[f]	2.019 ± 0.07[f]	104.31 ± 5.40[b]

[a]Mean ± SD of 3 experiments. Values in the line followed by the same letters are not significantly different by Tukey's multiple range test (p <0.05). TPC: total phenolic compounds; TFC: total flavonoids content; DPPH: 2,2-diphenyl-1-picrylhydrazyl assay; ABTS: 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assay; RP: reducing antioxidant power assay; TAC: total antioxidants capacity.

Table 3. Mineral Composition of Different Parts of Parsley Plants[a]

samples	B	Ca	Cd	Co	Cu	Fe	K	Mg	Mn	Na	Ni	P	Pb	Se	Zn
leaves	31.43 ± 5.02	817.15 ± 22.3	n.d	n.d	0.21 ± 0.05	1.11 ± 0.6	1000 ± 4.02	364.8 ± 4.56	1.97 ± 0.05	724.77 ± 6.3	n.d.	167.37 ± 5.3	n.d.	n.d.	8.91 ± 1.45
stem	2.52 ± 0.13	1000 ± 3.72	n.d	n.d	0.69 ± 0.06	16.59 ± 1.23	1491.42 ± 41.2	491.02 ± 3.21	3.57 ± 0.3	169.43 ± 2.43	n.d.	138.95 ± 2.15[c]	n.d.	n.d.	2.88 ± 0.71
root	4.50 ± 1.45	856.97 ± 7.8	n.d	n.d	0.47 ± 0.05	3.71 ± 0.4	2061.4 ± 16.4	532.45 ± 7.67	2.33 ± 0.6	885.07 ± 5.17	n.d.	157.39 ± 4.23	n.d.	n.d.	5.43 ± 1.78

[a]The concentration (mg/100 g dw) mean ± SD of three experiments is used to express the values of mineral elements. Tukey's multiple range test indicates that values in the same column separated by the same letter do not differ significantly (p <0.05). n.d.: not detected; dw: dry weight. Boron (B), calcium (Ca), cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), nickel (Ni), phosphorus (P), lead (Pb), selenium (Se), and zinc (Zn).
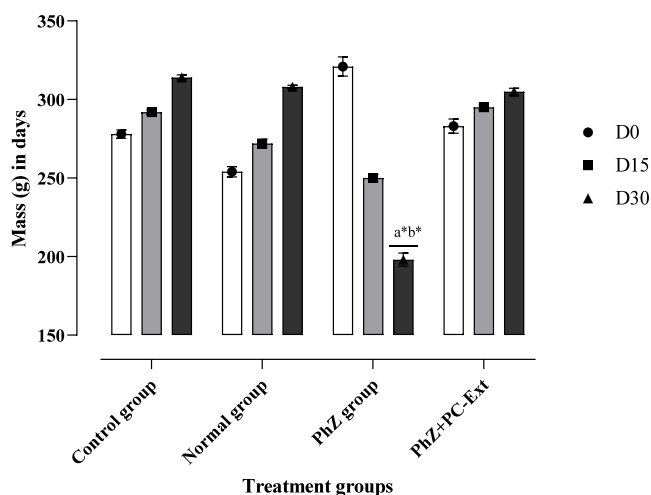
Figure 3. Body mass level in the tested groups. The data are presented in the form of mean ± SEM; [a]comparison between the control group and all groups and [b]comparison between the PhZ group and remaining groups, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.
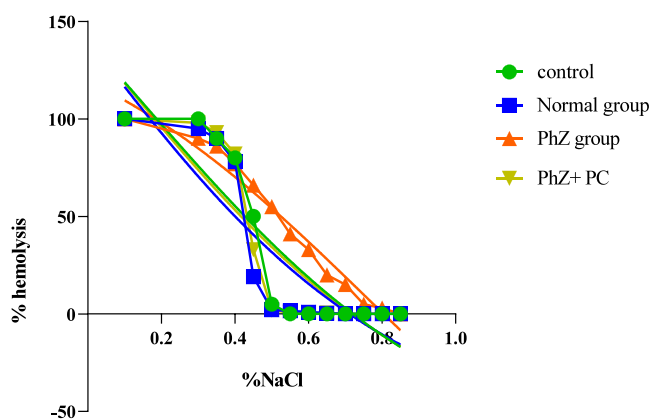
Figure 4. Percentage erythrocyte osmotic fragility of rats treated with phenylhydrazine or *P. crispum*, extract, or a combination of the two.

have emerged as prominent components within Apiaceae plants. Protein contents observed in the study results of which are presented here are greater than those reported previously to be 5.2−6.12 mg/g in parsley leaves.[31] This divergence in protein content might be attributed to the multifaceted interplay of environmental variables, plant genetics, and the intriguing vicissitudes of the growth stages. As parsley responds to its surroundings, it yields variations in nutritional.[32] Phenolic compounds, as the most abundant secondary metabolites in plants, are essential for growth and protection against the harmful effects of pathogens, parasites, and UV−visible rays.[33] The human diet comprises various phenolic compounds with diverse health benefits and biological properties. Parsley has been recognized as a polyphenol-rich food with health-promoting benefits. As indicated in the results, *P. crispum* presents a high phenolic content. Parsley from Spain had the greatest phenolic content with 388.35 ± 21.7 GAE/(mg/L).[35] Similar concentrations of TPC were reported for parsley from Egypt.[36] Flavonoid contents of PC-Ext were less than that reported by El-Sayed et al., who collected it from Giza governorate, Egypt (106.45 ± 2.18 mg RE/extract).[26] Quantitative and qualitative compositions of natural antioxidants in parsley are variable and depend on numerous factors,

including pedoclimatic characteristics, botanical origin, and storage conditions. This variability could contribute to differences in total phenolic content and total flavonoid content observed in various research studies.[37] The antioxidant activity of natural products and their extracts is a topic of significant interest in food, pharmaceutical, and cosmetic industries.[38] Identification of the appropriate active ingredients is a crucial first step in determining the antioxidant capacity of these active compounds. Four recommended *in vitro* assays, including DPPH, ABTS, BCBT, RP, and TAC, were employed to measure the antioxidant activity of the investigated *P. crispum* aqueous extract. Parsley leaf extracts exhibited significant variations in antioxidant capacities, often attributed to both primary and secondary metabolites and their distinct phytochemical components.[39] Furthermore, the extract demonstrated substantial total antioxidant capacity, suggesting its potential as a bioactive agent that could protect and prevent organ injuries caused by toxic agents. Nevertheless, conducting an antianemic experiment involving animal models was necessary to validate *in vitro* effects *in vivo*. The presence of various minerals in the human diet is essential due to their crucial role in the physiological and metabolic processes of animal and human organisms,[40] while their deficiency is the main reason for aging of red blood cells. Potassium (K) and calcium (Ca) were identified as the most abundant metals in all of the plant parts examined. They were recognized as the main representative minerals at different stages of ontogeny, specifically in microgreens and baby greens of *P. crispum*.[32] These two minerals are essential for regulating electrolyte exchange, maintaining the human body's acid−base balance, modulating the proliferation and differentiation of hematopoietic precursors,[41] and regulating erythrocyte biochemical functions. Virginie et al. demonstrated that the effects of the aqueous extract of *P. crispum* on phenylhydrazine-induced anemia may be attributed to the vitamin, mineral, and chemical composition inherent in *P. crispum* leaves. Consequently, this plant exhibits antianemic activity, aligning with its acknowledged efficacy in traditional.[42] These findings are consistent with the results reported previously.[43] Magnesium (Mg), sodium (Na), and phosphorus (P) are also present in significant quantities. Sodium (Na) was 1384.90 mg/100g of dw, differing from the present study; therefore, most mineral contents were found in small amounts. Additionally, phosphorus is a crucial nutrient for energy storage and is needed for nucleic acid synthesis, adenosine triphosphate (ATP), and phospholipids.[44] Furthermore, a considerable concentration of phosphorus is commonly stored in plant seeds for embryonic development, germination, and seedling growth.[45] Moreover, magnesium supplementation prevents oxidative stress and improves glucose homeostasis by enhancing glucose uptake and insulin sensitivity.[46] The iron (Fe) and copper (Cu) contents of herbs play a crucial role in determining the ultimate product quality and in their nutritional and biological functions when they are involved in specific alternative reactions. However, the presence of large amounts of Fe and Cu, due to adventitious contamination, can be detrimental to product quality. In their ionized form, they catalyze lipid oxidation reactions, leading to the development of undesirable flavors. It has been demonstrated that iron availability can modulate myeloid cell differentiation,[47] and its homeostasis regulates osteoclast development.[48] The results of several studies have confirmed the critical role of Zn in the expression of muscular strength and good cardiorespiratory functioning. Zinc is necessary for creating DNA, growing cells,

Table 4. Effects of PC-Ext on the Hematological Indices[a]

	treatment duration (days)			
parameters	day 0	day 4	day 15	day 30
Group 1				
RBC ($10^6/\mu L$)	7.65 ± 0.24	7.56 ± 0.44	7.68 ± 0.29	7.76 ± 0.31
HGB (g/dL)	13.23 ± 0.21	13.52 ± 0.95	12.78 ± 0.26	13.01 ± 0.26
HCT (%)	42.38 ± 1.43	42.27 ± 2.09	41.19 ± 1.01	41.98 ± 1.18
MCV (fL)	56.68 ± 1.44	57.73 ± 2.23	61.50 ± 0.87	59.00 ± 1.00
MCHC (pg)	17.5 ± 1.1	17.50 ± 0.29	17.00 ± 0.58	17.50 ± 0.26
PLT ($10^5/\mu L$)	7.27 ± 0.37	7.65 ± 1.3	7.87 ± 0.17	7.8 ± 1.00
Group 2				
RBC ($10^6/\mu L$)	7.26 ± 0.19	7.74 ± 0.15	7.82 ± 0.55	8.07 ± 0.47
HGB (g/dL)	14.10 ± 0.90	13.85 ± 0.20[b*]	12.60 ± 0.07[b*]	13.10 ± 0.12 [b*]
HCT (%)	41.23 ± 2.03	42.18 ± 1.13[a***b***]	42.78 ± 3.00[a***b***]	42.85 ± 2.56[a***b***]
MCV (fL)	59.65 ± 0.33	57.50 ± 0.87[a***b***]	58.50 ± 1.50[a***b***]	59.70 ± 1.50[a***b***]
MCHC (pg)	17.57 ± 0.47	17.57 ± 1.37[a*b***]	17.40 ± 0.56[a*b***]	17.67 ± 0.62[a*b***]
PLT ($10^5/\mu L$)	7.57 ± 0.63	7.65 ± 0.85	7.38 ± 0.16	7.92 ± 0.14
Group 3				
RBC ($10^6/\mu L$)	7.33 ± 0.43	3.83 ± 0.40	3.92 ± 0.32	3.85 ± 0.32
HGB (g/dL)	14.50 ± 0.22	9.01 ± 0.48	10.22 ± 0.33	9.35 ± 0.27
HCT (%)	45.33 ± 0.55	25.15 ± 1.10[a***]	29.64 ± 1.33[a***]	27.35 ± 1.15[a***]
MCV (fL)	57.60 ± 2.94	44.68 ± 2.39[a***]	46.30 ± 0.57[a***]	47.13 ± 1.23[a***]
MCHC (pg)	17.00 ± 0.28	12.75 ± 0.45	13.95 ± 1.41	13.5 ± 1.25
PLT ($10^5/\mu L$)	7.62 ± 0.11	4.64 ± 1.09	5.03 ± 0.11	4.43 ± 0.23
Group 4				
RBC ($10^6/\mu L$)	7.03 ± 0.37	3.84 ± 0.51	5.14 ± 0.50	7.04 ± 0.34
HGB (g/dL)	14.70 ± 0.29	9.92 ± 0.37	11.25 ± 0.48	13.67 ± 0.40[b*]
HCT (%)	46.70 ± 1.14	22.72 ± 1.00[a***b***]	40.73 ± 1.33[a***b***]	41.75 ± 0.81[a***b***]
MCV (fL)	60.60 ± 1.61	46.68 ± 1.44[a***b***]	59.65 ± 0.33[a***b***]	60.63 ± 2.19[a***b***]
MCHC (pg)	17.50 ± 0.29	12.59 ± 1.05[b*]	15.75 ± 1.12[b**]	17.00 ± 1.5[a*b*]
PLT ($10^5/\mu L$)	7.63 ± 0.54	4.83 ± 0.19	6.74 ± 0.09	7.03 ± 0.11

[a]RBC: red blood cells, HBG: hemoglobin, HCT: hematocrit, MCV: mean corpuscular volume, MCHC: mean corpuscular hemoglobin concentration, PLT: platelet. The data are presented in the form of mean ± S.E.M. [a]Comparison between the control group and all groups; [b]comparison between the PhZ group and remaining groups, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.
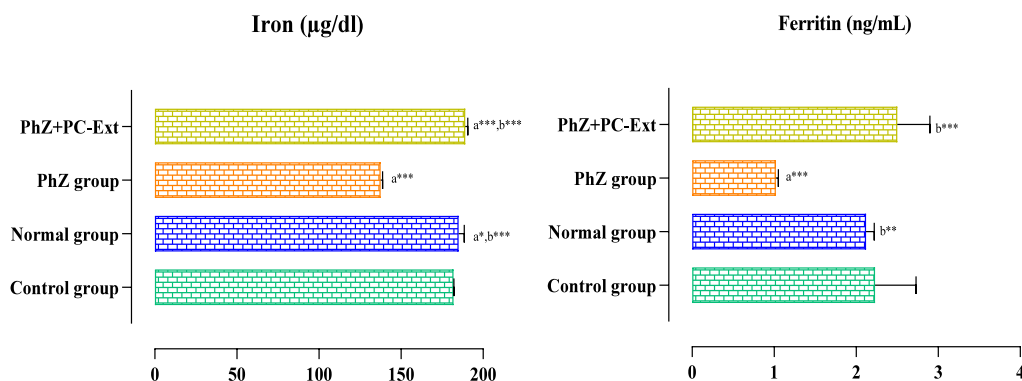

Figure 5. Concentrations of iron and ferritin in the blood serum. The data are presented in the form of mean ± SEM; [a]comparison between the control group and all groups and [b]comparison between the PhZ group and remaining groups, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

building protein, healing damaged tissue, and supporting a healthy immune system.[49] There are three main ways that zinc is related to anemia: (a) zinc deficiency causes anemia, (b) excessive zinc intake causes anemia, and (c) anemia causes abnormal blood levels of zinc[62] in the body. A zinc deficit may, to a lesser degree, compound with other factors to cause anemia.[65] Anemia is not solely caused by a zinc deficiency; other components must work together for anemia to occur.[11] On the other hand, a high zinc intake prevents copper from being absorbed, which causes a copper deficit and anemia.[66] Research conducted on animal models suggests that, in anemia,[67] zinc is transferred from bone and plasma to the bone marrow for the production of new red blood cells. A body with an abnormal zinc status can result from anemia, but anemia itself can also be caused by an excess or deficiency of zinc.[22] Clearly, soil type, climate, and genetic variety all affect how much mineral is in an herb. Furthermore, the impact of soil composition on the macro- and microelement composition of spices and aromatic herbs can be attributed to the ease with which these elements are absorbed from the soil. This process is influenced by the plants' aerial structures and root systems.[43] Phenylhydrazine is a chemical

compound that is toxic to the body and alters various tissues upon entering the body.[28]

The mass loss in the PHZ-treated group suggests that nutrition deteriorated due to oxidative losses in oxygen-free tissue radicals due to the auto-oxidation of PHZ, a strong oxidant.[4] PhZ and its derivatives are known antipyretic drugs that possess the property of inducing hemolytic anemia in both humans and rats. This effect is characterized by a range of detrimental mechanisms, including destroying the erythrocyte's protein framework, peroxidation, alterations in membrane phospholipids, oxidative degradation of hemoglobin, depletion of glutathione and ATP, and reduced membrane deformability.[50] Hb plays a pivotal role as the respiratory pigment within RBCs, and intravascular hemolysis of RBCs leads to the release of hemoglobin and the accumulation of free heme.[51] Moreover, it has been established that phenylhydrazine can bind to erythropoietin receptors, a hormone that regulates hematopoiesis and promotes erythrocyte production.[52] PC-Ext contains substances capable of acting similarly to erythropoietin by binding to its receptors, potentially competing with PhZ, or using an alternative mechanism to stimulate erythrocyte production. Furthermore, the partial or complete restoration of MCV and MCHC suggests that parsley facilitates the normalization of erythrocyte size and hemoglobin content, indicative of enhanced cell multiplication and normal hemoglobin synthesis. The administration of PC-Ext effectively mitigates the fluctuations in the studied parameters induced by PHZ treatment over 30 days, suggesting its potential utility as an antianemic agent in managing anemia. Various studies have successfully evaluated the antianemic potential of various medicinal plants using multiple experimental animal models. For instance, it was demonstrated that combination therapy exhibited the greatest antianemic potential, followed by *Piper betel* leaves and *Triticum aestivum* grass, respectively.[1] Additionally, a study revealed that the coadministration of apple vinegar demonstrated the capacity to ameliorate changes induced by phenylhydrazine.[29] Furthermore, Elaby et al. found that consuming 5% dried beet greens for 42 days significantly increased RBC, HGB, and HCT levels compared to the anemic control group.[5] Parsley's rich antioxidant and phytochemical content, including flavonoids and polyphenols,[53] can potentially counteract the oxidative stress induced by PHZ, thereby strengthening cellular antioxidant defenses. The osmotic fragility test enables the diagnosis of erythrocyte abnormalities in several diseases, including autoimmune hemolytic anemia, thalassemia, and hereditary spherocytosis.[54] A preliminary evaluation of the safety profile of drugs and the discovery of hidden pathologies requires such analysis.[55] The erythrocyte fragility test is a concentration analysis method. It can measure the degree and proportion of hemolysis. Low osmotic resistance can cause intravascular hemolysis, shortening red blood cell life expectancy.[56] The purified erythrocyte mass is placed in a solution of a different medium and subjected to different osmotic stress levels.[57] Osmotic fragility is influenced by various variables, including the erythrocyte composition, the integrity of the cell membrane, and the surface-to-volume ratio.[58] Erythrocyte hemolysis is the hemoglobin release process into plasma due to membrane rupture.[57] Despite having several biological defenses against intracellular oxidative stress, erythrocytes are susceptible to oxidative damage from toxic chemical products.[59] According to the results of the current study, coexposure to PhZ can cause increased lipid peroxidative damage to the rat erythrocyte membrane, which, in turn, causes

the onset of hemolytic anemia. This increased lipid peroxidative damage is what causes erythrocyte fragility. This actual study revealed that the parsley showed vigorous antioxidant activity, which could guard against and stop organ damage brought on by toxic agents.[60] Serum iron levels serve as a reflection of the iron circulating within the bloodstream.[61] Ferritin, a crucial intracellular iron reservoir, is pivotal in this scenario.[62] When anemia is initiated, the response might be to involve the mobilization of Fe from ferritin stores to compensate for the reduced iron intake occasioned by the breakdown of red blood cells.[63] Consequently, amounts of ferritin in blood might surge in response to the augmented iron release from these storage sites.[64] The intervention of parsley effectively replenishes depleted Fe, including serum Fe. Additionally, concerning ferritin regulation, it is imperative to strike a delicate balance between replenishing iron stores and meeting the immediate iron requirements necessary for sustaining red blood cell production.[65] The ultimate goal is to boost hemoglobin levels without leading to an excessive buildup of iron stores. The various components of *P. crispum* (comprising leaves, stems, and roots) contribute to a multifaceted approach, combining Fe supplementation with other strategies to address the root causes of anemia, such as reducing oxidative stress.[11] This factor can significantly impact iron metabolism. Furthermore, the high antioxidant content in parsley plays a pivotal role in neutralizing free radicals and toxic substances, thus maintaining hemostasis.[66] This is particularly important because free radicals harm biological cell membranes, including those of red blood cells.[67] They induce the peroxidation of unsaturated fatty acids, culminating in pathological alterations.[68] Therefore, the *Petroselinum* genus emerges as a key player in preventing and treating a spectrum of disorders, including hemolytic anemia, through its robust free radical-scavenging capabilities.[69] In summary, this study underscores the multifaceted role of parsley in combating and preventing various disorders, with a specific focus on hemolytic anemia, by addressing iron deficiency, managing oxidative stress, and safeguarding hemostasis from the pernicious effects of free radicals. Consequently, parsley may serve as a valuable therapeutic tool for addressing diseases related to oxidative stress. Nevertheless, further research is imperative to develop an appropriate polyherbal formulation to ensure therapeutic efficacy in countering phenylhydrazine-induced hemolytic anemia.

■ CONCLUSIONS

The results of the present study offer valuable insights into the physicochemical properties, antioxidant activity, mineral profile, and protective effect of *P. crispum* from Morocco against phenylhydrazine-induced hemolytic anemia in albino rats. However, it is important to acknowledge the limitations of our study. First, the study was conducted on animal models, and while it provides valuable information, further research is needed to assess the efficacy and safety of parsley supplementation in humans. Additionally, the mechanisms underlying the observed protective effects of *P. crispum* against anemia were not fully elucidated in this study. Further investigations are warranted to understand the molecular pathways involved. Moreover, while our findings suggest a potential role for parsley in addressing both iron deficiency and hemolysis-related anemia, clinical trials are necessary to confirm these effects in human populations. Despite these limitations, our study provides scientific validation for the ethnomedicinal use of parsley and highlights its potential utility in pharmaceutical development.

ETHICAL APPROVAL

The experimental protocols received approval from the ethics committee of Sidi Mohamed Ben Abdallah University Mohammed, Fez, adhering to the principles outlined in the Declaration of Helsinki. Approval for the study, under the reference USMBA-SNAMOPEQ 2023-03, was granted by the Laboratory of Natural Substances, Pharmacology, Environment, Modeling, Health, and Quality of Life at the Faculty of Science Dhar Mahraz in Fez, Morocco.

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Conceptualization, methodology, writing—original draft preparation, G.N.; supervision, F.Z.L., F.K., S.T., and N.S; editing and statistical analyses, J.P.G., A.M.S., M.A.-S.; data curation, software, validation, B.L. and E.D. All authors have read and agreed to the published version of the manuscript.

Notes

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