Journal of Biosciences and Medicines, 2023, 11, 154-168
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Aqueous Leaf Extract of *Moringa oleifera* (Moringaceae) Effectively Treats Induced Hemolytic Anemia in Wistar Rats

Semado Franck Médard[1], Tchogou Atchadé Pascal[1], Medoatinsa Seinde Espérance[1], Agbogba Félicienne[1], Abissi Yollande[1], Kassa Roxane[1], Lokonon Jacques Ezéchiel[1], Houngbeme Alban[2], Agbonon Amegnon[3], Gbénou Joachim Djimon[4], Baba-Moussa Lamine[5], Senou Maximin[1]*

[1]Experimental and Clinical Biology Unit (UBEC), Medical and Pharmaceutical Biotechnology Research Laboratory (LaRBiMeP), National School of Applied Biosciences and Biotechnology of Dassa-Zoumé (ENSBBA), National University of Sciences, Technologies, Engineering and Mathematics of Abomey (UNSTIM), Abomey, Benin
[2]National Pharmacognosy Laboratory (LNP), Institute for Research and Experimentation in Traditional Medicine and Pharmacopoeia (IREMPT), Porto Novo, Benin
[3]Laboratory of Physiology/Pharmacology, Faculty of Sciences, University of Lome, Lome, Togo
[4]Doctoral School (ED-STIM), National University of Sciences, Technologies, Engineering and Mathematics (UNSTIM), Abomey, Benin
[5]Laboratory of Biology and Molecular Typing in Microbiology, University of Abomey-Calavi, Cotonou, Benin
Email: *senouxim@yahoo.fr

How to cite this paper: Médard, S.F., Pascal, T.A., Espérance, M.S., Félicienne, A., Yollande, A., Roxane, K., Ezéchiel, L.J., Alban, H., Amegnona, A., Djimon, G.J., Lamine, B.-M. and Maximin, S. (2023) Aqueous Leaf Extract of *Moringa oleifera* (Moringaceae) Effectively Treats Induced Hemolytic Anemia in Wistar Rats. *Journal of Biosciences and Medicines*, **11**, 154-168.
https://doi.org/10.4236/jbm.2023.118013

Received: July 2, 2023
Accepted: August 19, 2023
Published: August 22, 2023

Abstract

Introduction: *Moringa oleifera* was a medicinal plant generally used by populations in the food and therapeutic fields. It's used to treat anemia has been observed in the Djougou Zone in northern Benin. To our knowledge, there were no scientific data available that have evaluated its efficacy in the treatment of haemolytic anemia. This was what justifies this research work in which the phytochemical analysis, extraction and evaluation of the anti-anemic effect were carried out. **Methods:** Five groups of five Wistar rats each were formed. All the rats were rendered anemic by injection of phenylhydrazine hydrochloride on the first two days D0 and D1 except those in the negative control group. From the second day, the anemic groups were force-fed either with the aqueous extract of *Moringa oleifera* leaves at 200 or 300 mg/kg body weight/day, or with vitafer, the reference drug against anemia. The positive control group (anemia) was not treated. Blood samples were taken from all the rats on different days: D0, D2, D7, D10 and D15 to evaluate the data of the hemogram and the osmotic resistance of the red blood cells. **Results:** Phytochemical analysis revealed the presence of tannins, flavonoids, leucoanthocyanins, saponosides, triterpenes and mucilages. A

good yield was obtained at the extraction. Both the extract and the reference drug vitafer completely corrected anemia within two weeks after stimulating hemoglobin synthesis and early release of immature red blood cells into the bloodstream. Its effect seemed dose-dependent and specific. **Conclusion:** *Moringa oleifera* leaves showed good therapeutic efficacy and can be considered and exploited for transformation into improved traditional medicines (ITM) in the treatment of anemia.

Keywords

Moringa oleifera, Hemolytic Anemia, Red Blood Cells, Wistar Rats

1. Introduction

Anemia was a very common syndrome in the world and especially in poor countries. The World Health Organization (WHO) estimated that 1.62 billion people suffer from anemia, *i.e.* a global prevalence of 24.8% [1]. Pregnant women and children under five were the most affected with a prevalence of 40% and 42% respectively [2]. The highest prevalences were recorded in Africa and South-East Asia with 67.7% and 65.5% respectively [1]. In Benin, 72% of children aged 6 - 59 months suffered from anemia, of which 3% in its severe form and 41% in its moderate form, then 58% of women aged 15 to 49 suffered from anemia: 26% in its mild form, 30% in its moderate form and 2% in its severe form [3]. Anemia was defined as a syndrome in which the number of red blood cells or the level of hemoglobin they contain was lower than normal [2]. It caused symptoms such as fatigue, weakness, dizziness and shortness of breath, among others. The most common causes of anemia were nutritional deficiencies, particularly iron deficiency although folate, vitamin B12 and vitamin A deficiencies were also important causes; haemoglobinopathies; and infectious diseases such as malaria, tuberculosis, HIV infection and parasitoses [2]. The treatment varied according to the type of anemia depending on whether it was central anemia due to a defect in bone marrow production, and peripheral anemia due to destruction of red blood cells. It could be a supply of iron, vitamin B12 or B9 by mouth, treatment with immunosuppressants or corticosteroids, injections of erythropoietin, blood transfusion, or even bone marrow transplantation. bone [4] [5]. Given the high cost of treatment, the inaccessibility and adverse effects of conventional synthetic drugs used in developing countries, the search for alternative and/or complementary strategies was necessary for the use of synthetic antianaemics and other conventional drugs. Herbal medicines were used by the majority of the world's population to cure various diseases. WHO estimated that 80% of rural populations in developing countries depend on traditional medicine [6] [7] [8]. In Benin, out of the 30,700 plant species inventoried in forest ecosystems [9], many plants were used by local populations as food plants and others as medicinal plants, including *Moringa oleifera* [9]. *Moringa oleifera* leaf

powder has been found to contain most of the essential nutrients needed for good health [10] [11] [12] [13]. The leaf powder was rich in multiple minerals and vitamins, including iron, vitamin A (carotenoid) and vitamin C, which are important for iron metabolism. In addition, Moringa has an added benefit in addressing multiple malnutrition issues as it was rich in all essential amino acids, which were building blocks of proteins needed for cell growth [10] [11] [14] [15]. Although *Moringa oleifera* was one of the medicinal plants widely used by people in Benin, scientific data on its use as an anti-anaemic were almost non-existent, while it was the subject of several therapeutic uses, particularly in the treatment of infectious diseases. It was therefore essential for scientists to carry out biological studies in order to promote the use of this plant efficiently as an anti-anaemic by communities and to create a synergy between traditional medicine and conventional medicine. It was for this reason that this study was undertaken with the aim of evaluating the anti-anaemic efficacy of the aqueous extract of *Moringa oleifera* leaves. Such an evaluation would allow not only their rational use but also their eventual transformation into improved traditional medicines (ITM) available to poor populations.

2. Materials and Study Methods

2.1. Animal Material

The animal material used in this study consisted of albino Wistar strain rats aged 4 to 5 months and with an average body weight of approximately 210 g. These rats were acclimatized to the ambient conditions at a constant temperature of 25˚C ± 1˚C in the animal facility of the Experimental and Clinical Biology Unit (UBEC), of the National School of Applied Biosciences and Biotechnologies (ENSBBA) of Dassa, National University of Sciences, Technologies, Engineering and Mathematics (UNSTIM) in Benin. Breeding was carried out in a well-ventilated room with a cycle of 12 hours of light and 12 hours of darkness. Rats were kept in wire mesh cages with feeders and drinkers with free access to drinking water and food. They were supplied with drinking tap water and their daily diet was made up of a mixture of granulated feed presented in the form of kibble and marketed by the "Véto Services" group (Benin). The breeding enclosure was regularly cleaned to guarantee optimal development of the animals protected from any infection.

2.2. Plant Material

It was composed of aqueous extracts of powder from the leaves of *Moringa oleifera*. The samples studied were fresh leaves harvested in the North Benin region, specifically in Djougou in the Department of Donga and brought back to the laboratory for drying. The samples were spread out at the Laboratory of the Experimental and Clinical Biology Unit (UBEC), of the National School of Applied Biosciences and Biotechnologies (ENSBBA) of Dassa, National University of Sciences, Technologies, Engineering and Mathematics (UNSTIM) in Benin for drying at a temperature of 22˚C for about 14 days, after which the samples were

brittle and were practically anhydrous. Then the dry samples were reduced to powder using an electric grinder. The powders thus obtained were sieved with a sieve with a diameter of 710 µm.

2.3. Phytochemical Analyzes

Phytochemical analysis was based on the differential reactions (coloration and precipitation) of the main groups of chemical compounds contained in plants according to the classic method [16] and which is widely used in the literature with success [17] [18]. This analysis includes:

✓ Search of alkaloids: It was done through two tests:

- General acid test

5 g of the powder was mixed with 25 mL of 5% dilute hydrochloric acid. The mixture was macerated for 24 hours. 1 mL of the filtrate was collected, to which 5 drops of Mayer's reagent were added. If alkaloids were present, a yellow or cloudy precipitate was observed in the tube.

- Extraction of alkaloids

5 g of powder was placed in 5 mL of 50% ammonia. To this mixture, 25 mL of chloroform ether was added and left to macerate for 24 hours in a stoppered flask. The filtrate was dried over anhydrous sodium sulfate and then extracted with 5 mL of 5% hydrochloric acid twice in succession. To the exhausted filtrate, 5 drops of Mayer's reagent were added. If an alkaloid was present, a precipitate was observed in the tube.

✓ Search of polyphenolic compounds.

In an Erlenmeyer flask, put 5 g of powder to which 100 mL of boiling water is added. The mixture was left for 15 minutes with continuous stirring, then filtered. This filtrate divided into 6 portions was used for the following research:

- Tannins:

To the first portion of the filtrate, a few drops of 1% ferric chloride were added. The observation of a dark blue, green or black color indicated the presence of tannins.

- Catechin tannins

To 30 mL of the second portion, 15 mL of Stiasny's reagent was added and the mixture was heated in a water bath at 90˚C. for 15 minutes. The appearance of a pink precipitate indicated the presence of catechin tannins.

- Galic tannins

After collecting the filtrate, it was saturated with sodium acetate to which a few drops of 1% ferric chloride were added. A blue or black tint indicates the presence of gallic tannins.

- Flavonoids

To 5 mL of the third portion, add 5 mL of hydrochloric alcohol and a pinch of magnesium powder: this was the cyanidin reaction, known as the Shinoda reaction. The appearance of an orange, red or purple color indicated the presence of flavonoids.

- Anthocyanins

A few drops of 5% hydrochloric acid were added to 1 mL of the fourth portion. This mixture was then made alkaline by adding a few drops of 50% ammonia. A red color that increases and turned blue-purple or greenish indicated the presence of anthocyanin.

- Leuco-anthocyanin

To 5 mL of the last portion, add 5 mL of hydrochloric alcohol. The mixture was then heated for 15 minutes in a water bath at 90˚C. The observation of a cherry red or purplish color indicated the presence of leuco-anthocyanin.

✓ Search of quinone derivatives

In an Erlenmeyer flask, 2 mL of 5% HCl and 2 g of powder were mixed. To this mixture, 20 mL of chloroform was added and we leave stirring continuously for 24 hours. After maceration, 5 mL of ammonia was added to the previous mixture: this was the Born-Trager reaction. A pink or purplish-red color indicated a positive reaction.

✓ Search for saponosides

The decoction of 1 g of powder was prepared for 30 minutes in 100 mL of distilled water with moderate boiling. The filtrate cooled then adjusted to 100 mL was distributed in 10 test tubes (height 16 cm × 16 mm in diameter) in a geometric series at the rate of 1/10th concentration of the decoction. After adjusting to 10 ml with water and 30 shakings in 15 seconds, the tube was left to stand for 15 minutes. The height of the foam was measured. If it was ≥1 cm in one of the tubes, the dilution in this tube was the desired foam index.

✓ Search for triterpenoids and steroids

For this research, 10 mL of ethyl alcohol at 70˚C was added to 1g of powder. To this mixture, 10 mL of distilled water was added then 2 mL of 10% lead acetate at equal volume V/V. After standing for 15 minutes, 2 ml of 10% aqueous sodium phosphate solution was added to the filtrate. After 15 minutes of rest, the filtrate was collected in a separatory funnel and extracted three times with 5 mL of chloroform ($CHCl_3$). The chloroform solutions were dried over sodium sulphate then divided into two portions and evaporated to dryness (bain-marie).

At the first portion was dissolved by a few drops of acetic acid. To the mixture obtained, 3 mL of a mixture of acetic anhydride-sulfuric acid is added. A purple, blue or green color indicated the presence of triterpenoids.

To the second portion, add 2 drops of an alcoholic solution of dinitrobenzoic acid and 2 drops of 1N sodium hydroxide. The appearance of a purple or wine-red color indicated the presence of steroids.

✓ Search for cyanogenic derivatives

To 15 mL of distilled water, was added 2 g of the powder then stopped immediately and left to macerate for 1 hour. The neck of the Erlenmeyer flask was covered with paper soaked in picric acid and heated for a few minutes. The appearance of a brown color indicated the release of HCN.

✓ Search for mucilages

1 mL of 10% decoction was introduced into a test tube and 5 mL of absolute alcohol was added. The appearance of a fluffy precipitate indicated the presence of mucilage after about ten minutes.

✓ Search for coumarins

To 20 mL of ether, 1 g of powder was added then immediately plugged in a small Erlenmeyer flask and left to macerate for 24 hours. The filtrate was adjusted to 20 mL with ether. 5 mL of filtrate was evaporated in a dish in the open air. To the residue obtained, 2 mL of hot water was added and the solution was divided into two test tubes. In one of the tubes, 0.5 mL of 25% ammonia was added. The second tube represented the control. The fluorescence of the two test tubes was observed under UV at 365 nm. Intense fluorescence in the test tube indicated the presence of coumarins.

✓ Search for reducing compound

The 10% decoction was obtained by moderate boiling for 3 minutes of a mixture of 50 mL of distilled water and 5 g of powder. After cooling, the filtrate was adjusted to 50 mL with distilled water. 5 mL of filtrate is introduced into a test tube. After heating in a water bath at 90˚C. for a few minutes, 1 mL of Fehling's reagent (Fehling's liquor A + Fehling's liquor B in equal volume) was added. The filtrate was heated a few minutes later. The observation of a bright red precipitate indicated the presence of reducing compound.

✓ Search of anthracene derivatives

- Free anthracenes

To 1 g of powder, add 10 mL of chloroform and heat carefully for 3 minutes in a water bath. After hot filtration, the mixture was made up to 10 mL with chloroform. 1 mL of the chloroform extract was added with 1 mL of ammonia diluted to 1/2 then stirred. The appearance of a more or less intense red color indicated the presence of free anthracenes.

- Combined anthracenics

• O-heterosides

To part of the residue exhausted with chloroform, 10 mL of distilled water and 1 mL of concentrated hydrochloric acid were added. The test tube was kept in a boiling water bath for 15 min was then cooled under a stream of water. The hydrolyzate was obtained after filtration and adjustment to 10 mL. 5 mL of the hydrolyzate was taken and shaken with 5 mL of chloroform. The drawn-off organic phase was introduced into a test tube and added with 1 mL of ammonia diluted to 1/2 then stirred (the aqueous phase is kept). The presence of anthracene was revealed by the more or less intense red coloring. If the reaction was negative or weakly positive, the O-heterosides with reduced genins were sought. To do this, take 5 mL of hydrolyzate and add 3 to 4 drops of 10% $FeCl_3$ (ferric chloride). The mixture, heated in a water bath for 5 min, was then cooled under a stream of water and then stirred with 5 ml of chloroform. To the chloroform phase drawn off and introduced into a test tube, 1 mL of ammonia was added to 1/2 and then stirred. A more or less intense red color indicated the presence of O-heterosides with reduced genins.

- C-heterosides

To the aqueous phase preserved above, 1 ml of 10% $FeCl_3$ is added. The mixture was brought to the boil in a boiling water bath for 30 minutes and then cooled. After stirring with 5 mL of chloroform, the chloroform phase was drawn off and collected in a test tube. Add 1 mL of ammonia diluted to 1/2 and stir. A more or less intense red color indicated the presence of C-heteroside genins.

✓ Cardiotonic glycosides

Prepare an extract from 1 g of drug powder and 10 mL of 60% alcoholic ethanol and 5 mL of a 10% neutral lead acetate solution. Bring to the bain-marie for 10 minutes and filter. Extract with chloroform and divide the organic phase between 3 test tubes, evaporate to dryness and resume with isopropanol. Then enter in:

- tube 1 Baljet's reagent;
- tube 2 Kedde's reagent;
- tube n˚3 the Raymond-Marthoud reagent;

Finally, introduce 5 drops of 5% KOH in alcohol into each tube. The presence of cardenolides results in a coloration:

- Orange in tube 1;
- Red-violet in tube n˚2;
- fleeting violet in tube n˚3.

2.4. Preparation of Extract

The extraction of the total chemical principles was done using the method of maceration in accordance with the traditional use of plants. Based on the extraction techniques cited in the literature [17]. 50 g of powder was dissolved in 500 mL of distilled water. The mixture was left stirring continuously for 48 hours. After cooling, the mixture obtained was filtered (3 times in a row) on absorbent cotton and the filtrate was transferred to a 1000 mL flask then subjected to evaporation until dryness at 40˚C. Using a rotavapor (Heidolph Laborota 4000 efficient) coupled to a water chiller (Julabo FL 300). The dry residue obtained represented the maceration. Finally, the various dry residues obtained are weighed and the yield was calculated according to the expression:

$$\text{Yield}(\%) = \frac{\text{Mass of dry extract}}{\text{Initial mass of powder}} \times 100$$

2.5. Induction of Anemia

Anemia was induced in animals according to a standardized protocol from Seidou, 2013 [5]. According to this protocol, anemia is set in two days after the end of the injections of phenylhydrazine [19] [20] [21]. Phenylhydrazine hydrochloride was diluted in physiological saline, before being administered to the rat by the intraperitoneal route IP, at a dose of 40 mg kg d for two days (D0 and D1). An animal was considered anemic if its hemoglobin level was below normal [20] [21]. The control animals (not anaemic) received only physiological saline.

2.6. Evaluation of the Anemia-Correcting Effect of Moringa Oleifera Leaf Extracts

To evaluate the correction of anemia by extracts of *Moringa oleifera*, the rats were divided into five (5) experimental groups at the rate of five (5) animals per group: Group 1: Negative control, consisting of rats which received 0.9% saline solution only from D2 to D2; and distilled water from D2 to D15; Group 2: Positive control, consisting of rats which received phenylhydrazine on D0 and D1 and distilled water from D2 to D15; Group 3: Reference control, consisting of rats which received phenylhydrazine on D0 and D1 and 1 ml/kg/d of body weight of vitafer (Reference drug), from D2 to D15; Group 4: Test group, consisting of rats which received phenylhydrazine on D0 and Dd1 and 200 mg/kg/d of body weight of *Moringa oleifera* leaves extract from D2 to D15; Group 5: Test group, consisting of rats which will receive phenylhydrazine on D0 and D1 and 300 mg/kg/d of body weight of *Moringa oleifera* leaves extract from D2 to D15. The extract and the reference medicinal product vitafer are administered by force-feeding using a gastric tube.

2.7. Hematological Analyzes

Blood samples were taken during the manipulation on different days: D0, D2, D7, D10 and D15 for all the groups. Blood was collected by orbital puncture after anesthetizing the rats with chloroform. These samples will make it possible to assess the evolution of hematological parameters in each group [5]. The hemoglobin level, the number of red blood cells, the mean corpuscular volume (MCV), the mean corpuscular hemoglobin concentration (MCHC), the thrombocyte count and the osmotic resistance of hematic were determined. These parameters are measured using the PLC SYSTEM KX 21. Osmotic resistance is the ability of blood cells to resist hemolysis in a hypotonic solution. Blood was diluted 1/200 in two salt solutions of different concentrations. One was isotonic (0.9% NaCl) and the other hypotonic (0.45% NaCl). Red cells were counted with a Malassez cell. The ratio of the number of red blood cells counted in the hypotonic solution over that of the isotonic solution was the percentage of red blood cells resistant to hemolysis. This test was used to assess the rate of young red blood cells.

2.8. Statistical Analysis

Graphs were plotted using Graphpad software. In each group, the different means were compared to that of D0 using ANOVA one way, Dunnett's Multiple Comparison Test. The significance level was set at 5%.

3. Results

3.1. Chemical Groups of *Moringa oleifera* Leaves Studied

The results of the phytochemical screening carried out on the powders of the species analyzed were summarized in Table 1 below:

Table 1. Identified chemical groups.

GROUPES CHIMIQUES	*Moringa oleifera* leaves
Catechic tannins	+
Gallic tannins	+
Flavonoids	+
Leuco-Anthocyanins	+
Anthocyanins	+
Alkaloids	−
Reducing compounds	−
Mucilages	+
Saponosides	+
Cyanogenic derivatives	−
Triterpenes	+
Steroids	−
Coumarins	−
Quinone derivatives	−
Free antracenes	−
C-Geosides	−
O-Heterosides	−
Cardiotonic derivatives	−

(+): positive; (−): negative.

Table 1 showed that the species analyzed contained all the various secondary metabolites. *Moringa oleifera* contains 7 chemical groups such as catechic tannins gallic tannins, flavonoids, leucoanthocyanins, mucilages, triterpenes and saponosoides. The organ studied did not contain toxic chemical groups, namely cyanogenic and cardiotonic derivatives; which gave it a priori a certain security as to its use. In addition, we noted that all the organs studied contain catechic tannins, flavonoids, triterpenes and mucilages.

3.2. Yield of Extracts

The yield calculated for the prepared extract was recorded in Table 2 below:

The yield obtained with the aqueous extract of *Moringa oleifera* leaves was significant. The plant was very rich in polar compounds, the extraction was made with water which was a recognized very polar solvent.

3.3. Evaluation of the Efficacy of *Moringa oleifera* Leaf Extracts on Wistars Rats

Hemoglobin (Hb) level

Figure 1 showed the evolution of the hemoglobin level in the groups of rats.

Table 2. Yield of the extract.

Sample	*Moringa oleifera* leaves
Yield ± (%)	13.50 ± 0.13

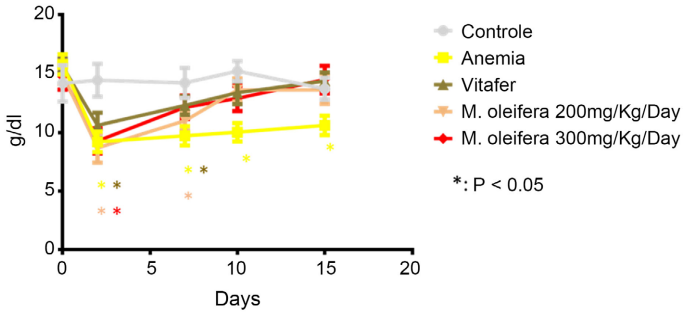

Figure 1. Evaluation of hemoglobin level.

The hemoglobin level varied from 14.2 ± 1.4 to 16.2 ± 1.1 g/dL in the various groups of rats on D0. Phenylhydrazine significantly lowered the Hb level on D2, thus creating anemia. This decrease was completely corrected on D10 by Vitafer® and by *Moringa oleifera* extract at 200 mg/Kg of body weight and on D7 by *Moringa oleifera* extract at 300 mg/Kg of body weight. Only the group of untreated anemic rats did not experience such a correction. The hemoglobin level did not vary significantly in non-anemic controls.

Red blood cell count

Figure 2 showed the evolution of the number of red blood cells in the groups of rats.

The number of red blood cells varies from 8.3 ± 0.7 to 9.2 ± 1.0 T/L in the various groups of rats on D0. Phenylhydrazine significantly lowered the number of red blood cells on D2, reflecting hemolysis of red blood cells. This decrease was corrected on D10 by Vitafer® and by the extract of M. oleifera at 300 mg/Kg of weight/D and on D15 by the extract with the extract of M. oleifera at 200 mg/Kg of weight /D. Only the group of untreated anemic rats did not have such a correction. The number of red blood cells did not vary significantly in non-anemic controls.

Mean corpuscular volume (MCV)

Figure 3 showed the evolution of the mean globular volume in the groups of rats.

The Mean Globular Corpuscular varied from 55 ± 4 to 59 ± 5 fl in the various groups at D0. The MCV increased significantly from D10 in the anemic and treated groups, reflecting a release of macrocyte.

Mean Corpuscular Hemoglobin Concentration (MCHC)

Figure 4 showed the evolution of mean corpuscular hemoglobin concentration in groups of rats.

The mean corpuscular hemoglobin concentration varied from 28.9 ± 1.2 to 32 ± 2.7 g/dl in the various groups of rats at D0. It did not change significantly during the experiment.

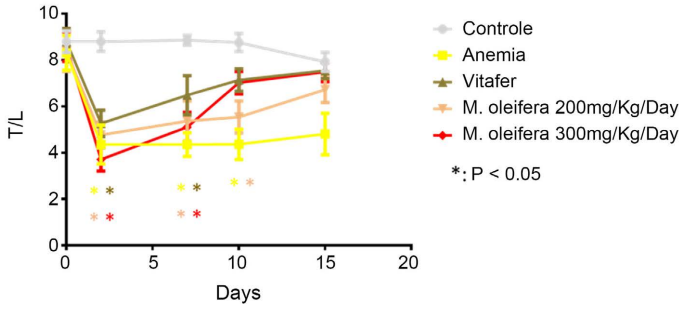

Figure 2. Evaluation of red blood cell count.

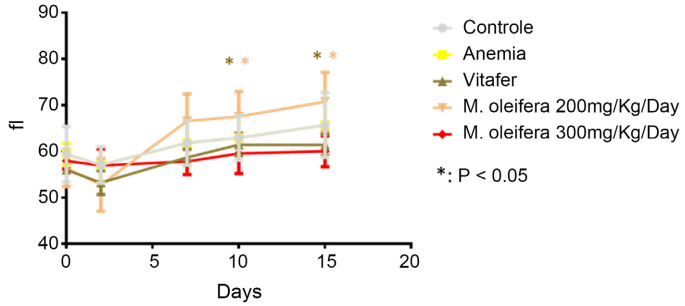

Figure 3. Assessment of mean corpuscular volume.

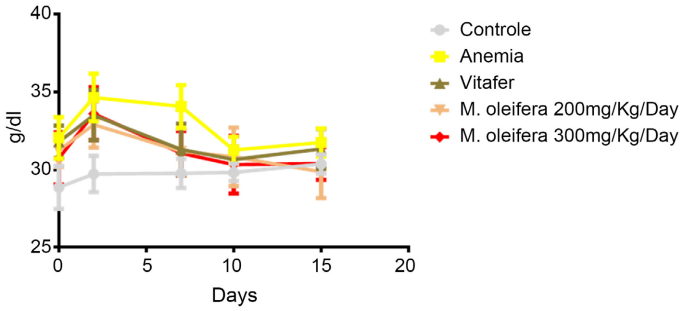

Figure 4. Evaluation of mean corpuscular hemoglobin concentration.

Blood platelet count (BPC)

Figure 5 presented the evolution of the number of blood platelets in the groups of rats.

The number of platelets varied from 822 ± 71 to 1297 ± 218 G/L on D0 in the different groups of rats. It did not change significantly in the groups during the experiment, indicating an absence of treatment effect on the thrombocyte lineage.

Osmotic resistance of red blood cells (OR)

Figure 6 showed the evolution of Osmotic resistance of red blood cells in the groups of rats.

The osmotic resistance of red blood cells varied from 54% ± 6% to 68% ± 14% on D0. It significantly increased in all anemic groups treated from D10 and in the untreated anemic group on D15, reflecting a massive release of young red blood cells. In the group treated with vitafer (reference drug), the increase was significant on D7. The osmotic resistance of red blood cells did not vary significantly in non-anemic controls.

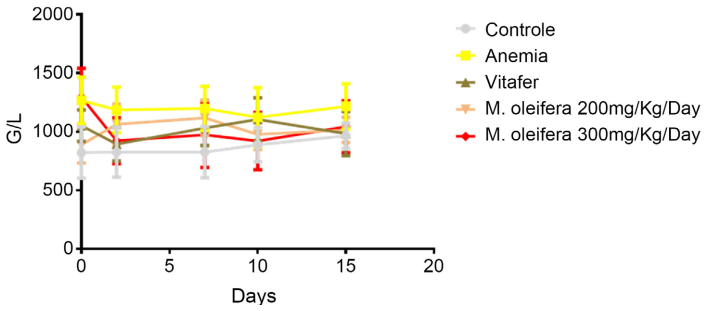

Figure 5. Evaluation of the number of blood platelets.

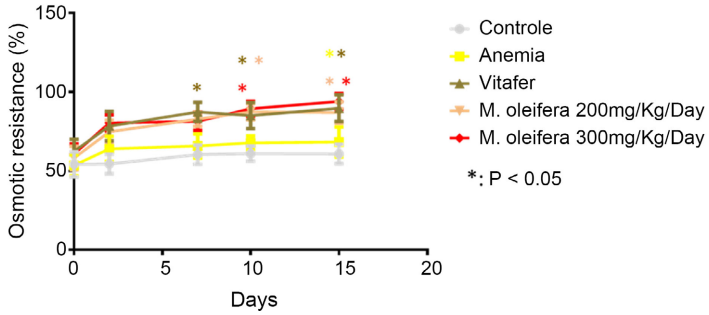

Figure 6. Evaluation of osmotic resistance of red blood cells.

4. Discussion

This work was an experimental study of the anti-anemic properties of the extract of the leaves of *Moringa oleifera*, a plant used in traditional medicine in Benin to treat anemia. The phytochemical analysis of the leaves of this plant revealed the presence of chemical groups such as flavonoids, mucilages, leucoanthocyanins, saponosoides, triterpenes, and tannins. These results were similar to those of [19] [20] [21] [22] [23] who detected tannins, flavonoids and leucoanthocyanins in the root bark of Psorospermum febrifugum, in the roots of Cocos nucifera and in the leaves of Justicia secunda Vahl (Acanthaceae), which are anti-anaemic plants used in Benin. Analysis of anti-anaemic properties revealed that *Moringa oleifera* leaf extract corrected phenylhydrazine-induced anemia by correcting hemoglobin level on D7 at a dose of 300 mg extract/kg body weight/day and day 10 at a dose of 200 mg/kg of weight/day. Such a dose-dependent correction of the hemoglobin level was also observed with the bark of the roots of Psorospermum febrifugum, in the roots of Cocos nucifera [20] [21]. The evolution of the number of red blood cells followed that of the hemoglobin level. The extract corrected the drop in the number of red blood cells following the hemolysis caused on D2 by phenylhydrazine on D10 at a dose of 300 mg of extract/kg of weight/D and on D15 at a dose of 200 mg/kg of weight/D. Such a correction was also obtained with extracts of Tectona grandis leaves in Togo [24]; leaves of Sorghum bicolor in Benin [25]. Osmotic resistance was proportional to the youth of the red blood cells. It significantly increased from D10 in all the anemic and treated groups, reflecting a massive release of young red blood cells into the bloodstream to compensate for the anemia. Its evolution was moderate in un-

treated anemic rats. Such an observation was also made on the root bark of Psorospermum febrifugum [21] and the roots of Cocos nucifera [9]. The evolution of the Mean Corpuscular Volume (MCV) followed that of the osmotic resistance of red blood cells and reflected a release of macrocyte, red blood cells that had not completed their differentiation in the blood, following an increased stimulation of the bone marrow from D10 to compensate for anemia [27]. To verify the specific action of the extract on erythropoiesis, the evolution of the number of blood platelets was also monitored during the experiment. Indeed, the number of platelets was not significantly modified by the treatment, which indicated a lack of stimulation of the thrombocyte lineage. This result suggested that the extract did not stimulate all hematopoietic lineages and therefore showed some specificity of action on the erythroid. The same observation was made with the aqueous extract of the calyx of Hibiscus sabdariffa or the roots of Cocos nucifera [9] [26] [27].

5. Conclusion

The aqueous extract of *Moringa oleifera* leaves showed an anemia-correcting effect. This property would be linked to many groups of chemical compounds contained in these leaves and which were also found in other hematopoietic plants. This result could contribute to the management of anemia by medicinal plants. It would pave the way not only for its scientific valorization but also for its possible transformation into Improved Traditional Medicine (ITM) for better accessibility of poor populations to anti-anaemic drugs. However, the study must be continued by an exploration of the toxicological effects.

Conflicts of Interest

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

References

[1] WHO (2008) Prevalence of Anemia 1993-2005: WHO Global Database on Anemia. https://apps.who.int/iris/handle/10665/43894

[2] WHO (2020) Anémie. https://www.who.int/fr/health-topics/anaemia

[3] WHO (2001) Promotion du rôle de la médecine traditionnelle dans le système de santé: Stratégie de la région africaine. https://www.afro.who.int/sites/default/files/sessions/working_documents/AFR%20RC50%209_0.pdf

[4] WHO (2008) Cibles mondiales de nutrition. Pour améliorer la nutrition chez la mère, le nourrisson et le jeune enfant. https://www.unicef.org/media/108926/file/%20Nutrition%20Strategy%202020-2030%20(Document)%20-%20French.pdf

[5] Movaffaghi, Z., Hasanpoor, M., Farsi, M., Hooshmand, P. and Abrishami, F. (2006) Effects of Therapeutic Touch on Blood Hemoglobin and Hematocrit Level. *Journal of Holistic Nursing*, **24**, 41-48. https://doi.org/10.1177/0898010105282527

[6] Seidou, A. (2013) Evaluation de quelques proprietes de "Forte" utilisée pour traiter

l'anemie. FSS/UAC, Bénin, 100 p.

[7] WHO (2001) Iron Deficiency Anemia Assessment, Prevention and Control. Organisation Mondial de la Santé, 60 p. https://www.who.int/publications/m/item/iron-children-6to23--archived-iron-deficiency-anaemia-assessment-prevention-and-control

[8] Kar, A. and Borthakur, S.K. (2008) Medicinal Plants Used against Dysentery, Diarrhoea and Cholera by the Tribes of Erstwhile Kameng District of Arunachal Pradesh. *Natural Product Radiance*, **7**, 176-181.

[9] Chaddha, S. and Mittal, A. (2016) Role of Flavonoids in the Treatment of Hemolytic Anaemia—A Review. *European Journal of Pharmaceutical and Medical Research*, **3**, 212-216.

[10] Sènou, M., Tchogou, A.P., Assogba, F., Agossadou, A., Dougnon, T.V., Agbangnan, D.C.P., Lalèyè, A. and Loko, F. (2017) Study of Biological Tolerance of Aqueous Extract of *Sorghum bicolor*. *Journal of Applied Biosciences*, **109**, 10640-10648. https://doi.org/10.4314/jab.v109i1.8

[11] Tchogou, A.P., Sènou, M., Dougnon, T.V., Agossadou, A., Assogba, F., Kinsiclounon, E.G., Ewedjè, E., Agbangnan, D.C.P., Gbénou, J., Lalèyè, A. and Loko, F. (2016) The Aqueous Extract of *Cocos nucifera* L. (Arecaceae) Effectively Treat Induced Anemia. Experimental Study on Wistar Rats. *International Journal of Biology*, **8**, 1-9. https://doi.org/10.5539/ijb.v8n3p1

[12] Tchogou, A.P., Sènou, M., Agbangnan, D.C.P., Agossadou, A., Assogba, F., Dougnon, T.V., Klotoé, J.R., Gbénou, J., Sèzan, A., Lalèyè, A. and Loko, F. (2017) Test of the Safety of *Cocos nucifera* L. (Arecaceae) Root Aqueous Extract. *Journal of Chemical, Biological and Physical Sciences*, **7**, 282-291.

[13] Tchogou, A.P., Sènou, M., Agbogba, F., Lokonon, J.E., Medoatinsa, S.E., Agbangnan Dossa, C.P., Loko, F., Agbonon, A. and Sèzan, A. (2021) Efficacy of Butanolic Fraction of Cocos Nucifera's Root Aqueous Extract on Induced Anemia Treatment. *International Journal of Biology*, **13**, 8-15. https://doi.org/10.5539/ijb.v13n1p8

[14] Diallo, A., Gbeassor, M., Vovor, A., Eklu-Gadegbeku, K., Aklikokou, K., Agbonon, A., *et al.* (2008) Effect of *Tectona grandis* on Phenylhydrazine-Induced Anemia in Rats. *Fitoterapia*, **79**, 332-336. https://doi.org/10.1016/j.fitote.2008.02.005

[15] Gbenou, J.D., Tossou, R., Dansou, P., Fossou, M. and Moudachirou, M. (2006) Etude des propriétés antianémiques de *Justicia secunda Vahl* (acanthaceae) chez les rats de souche wistar. *Pharmacopée et Médecine Traditionnelles Africaines*, **14**, 45-54.

[16] Yapi, H.F. (2015) Anti-Anaemic Activity of Aqueous and Ethanolic Extracts of Entandrophragma Angolense Bark on Phenylhydrazine-Induced Anaemic Rats. *Asian Journal of Biochemical and Pharmaceutical Research*, **3**, 12-21.

[17] Houghton, P.J. and Raman, A. (1998) A Laboratory Handbook for the Fractionation of Natural Extracts. Chapman & Hall, London, 199 p.

[18] Houngbeme, A.G., Gandonou, C., Yehouenou, B., Kpoviessi, S.D.S., Sohounhloue, D., Moudachirou, M. and Gbaguidi, F.A. (2014) Phytochemical Analysis, Toxicity and Antibacterial Activity of Benin Medicinal Plants Extracts Used in the Treatment of Sexually Transmitted Infections Associated with HIV-AIDS. *International Journal of Pharmaceutical Sciences and Research*, **5**, 1739-1745.

[19] Ombouma, J.G., Houngbeme, A.G., Mboma, R., Deguenon, P., Houinato, M.R.B. and Gbaguidi, F.A. (2021) Phytochemistry, Cell Tolerance and Chromatography Profile of Plants Used against Diarrhea in Mitsogho in Gabon. *World Journal of Pharmacy and Pharmaceutical Sciences*, **10**, 96-108.

[20] Félicienne, A., Isabelle, S.T., Pascal, T.A., Espérance, M., Rose, K.E., Eugène, A., *et al.* (2019) The Aqueous Extract of the Root Bark of *Psorospermum febrifugum* Spach Effectively Corrects Anaemia. Experimental Study on *Wistar* Rats. *Journal of Applied Biosciences*, **139**, 14137-14146. https://doi.org/10.4314/jab.v139i1.1

[21] Nwinuka, N.M., Monanu, M.O. and Nwiloh, B.I. (2008) Effect of Aqueous Extract of *Mangifera indica* L. (Mango) Stem Bark on Haematological Parameters of Normal Albino Rats. *Pakistan Journal of Nutrition*, **7**, 663-666. https://doi.org/10.3923/pjn.2008.663.666

[22] Ogbe, R.J., Adoga, G.I. and Abu, A.H. (2010) Antianemic Potentials of Some Plant Extracts on Phenylhydrazine-Induced Anemia in Rabbits. *Journal of Medicinal Plants Research*, **4**, 680-684.

[23] Assobayire, F.S., Adou, P., Davidson, L., Cook, J.D. and Hurrel, R. (2001) Prevalence of Iron Deficiency with and without Concurrent Anemia in Population Groups with High Prevalences of Malaria and Other Infections: A Study in Côte d'Ivoire. *The American Journal of Clinical Nutrition*, **74**, 776-782. https://doi.org/10.1093/ajcn/74.6.776

[24] Ogwumike, O.O. (2002) Hemopoietic Effect of Aqueous Extract of the Leaf Sheath of *Sorghum bicolorin* Albinos Rats. *African Journal of Biomedical Research*, **5**, 69-71.

[25] Bonina, F., Puglia, C., Avogadro, M., Baranelli, E. and Cravotto, G. (2005) The Topical Protective Effect of Soybean-Germ Oil against UVB-Induced Cutaneous Erythema: An *in vivo* Evaluation. *Archiv der Pharmazie*, **338**, 598-601. https://doi.org/10.1002/ardp.200500159

[26] Sènou, M., Tchogou, P.A., Agbogba, F., Lokonon, E.J., Medoatinsa, E., Kanfon, R.E., Dougnon, V.T., Agbangnan, D.C.P., Boko, C., Loko, F., Lalèyè, A., Agbonon, A., Baba-Moussa, L.S. and Sèzan, A. (2020) The Ethyl Acetate Fraction of *Psorospermum febrifugum* Spachroot Barks Aqueous Extract Is a Good Stimulator of Hematopoiesis. *International Journal of Advanced Research*, **8**, 1018-1026. https://doi.org/10.21474/IJAR01/11929

[27] Sènou, M., Agossadou, A., Agbangnan, D.C.P., Tchogou, A.P., Assogba, F., Klotoé, J.R., Dougnon, T.V., Gbénou, J., Lalèyè, A., Sèzan, A. and Loko, F. (2016) The Aqueous Extract of *Hibiscus sabdariffa* L. Calyces Effectively Corrects Induced Anemia. *International Journal of Pharmacy and Pharmaceutical Research*, **7**, 254-265.