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Original Research Article

Methanolic extract from the fruit kernel of *Mangifera indica* (Anacardiaceae) improves blood count, serum iron levels and transaminases in anemic rats

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ABSTRACT

Background: *Mangifera indica* (Anacardiaceae), known for its many therapeutic potentialities is used in traditional Ivorian medicine to treat anemia. The aim of this study was to evaluate the effects of methanolic extract from the fruit kernel of *Mangifera indica* (EMMI) on blood pressure, iron and transaminase levels in rat's anaemic by phenyl hydrazine (50 mg/kg, per os).
Methods: The determination of iron, tannins, polyphenols and total flavonoids in EMMI samples was performed, using specific reagents. Different groups of rats were treated with EMMI (100, 500 and 1000 mg/kg) or Folifer (50 mg/kg) by gavage for 14 days. The determination of hematological and biochemical parameters following the different treatments was carried out, using an URIT 3000 PLUS machine.
Results: Phytochemical analyses show that EMMI (10 mg/ml), is rich in polyphenols (38.97±0.04 mg GAE/g), flavonoids (26.83±0.02 mg QE/g), tannins (33.87±0.06 mg TAE/g). and iron (52.91±0.01 mg/100 g). The observed anti-anaemic effects were dose-dependent. After 14 days of treatment, EMMI (1000 mg/kg) induced normalization of RBC, Hb, Hct and serum iron levels ($p<0.001$), with a decrease in ASAT and ALAT levels respectively of about 28 and 34% in anaemic rats ($p<0.001$). These effects were comparable to those of Folifer ($p>0.5$).
Conclusion: Altogether, this study showed that EMMI normalizes blood count, serum iron and transaminases in rats made anaemic by administration of phenyl hydrazine. This beneficial effect would be attributed to its richness in secondary metabolites and iron.

Keywords: *Mangifera indica*, Traditional medicine, Anemia, Rats

INTRODUCTION

Anemia is a pathology characterized by a decrease in the number of red blood cells or the concentration of circulating hemoglobin below normal values. It is diagnosed when hemoglobin levels are less than 13 g/dl and 12 g/dl, respectively, in men and women.[1] In 2019, around 1.76 billion people worldwide were affected by anemia.[2] The prevalence rate of anemia varies according to age groups or physiological status. According to the World Health Organization (WHO), it was estimated at 40%, 37% and 30%, respectively, among children aged from 6 to 59 months, pregnant women and women aged 15 to 49. This prevalence remains more critical in developing countries with values of 60% among pregnant women, 50% among children under 4 years and 45% among school-aged children.[3] In Côte d'Ivoire, a survey conducted by the National Institute of Statistics and its partners in 2016 showed that 66% of Ivorian women were affected by anemia4.[4] This condition is a risk factor for mortality of women in childbirth and their babies or miscarriages. It is thus a major public health problem.[5,6]

The management of anemia requires hygienic-dietary measures. It also uses anti-anaemic molecules such as foldine or vitamin B9, vitamin B12, iron or erythropoietin.[7] In the Ivorian public health system, the high cost of these pharmaceutical specialties is a limiting factor for the patient, given its low purchasing power. In addition, these are often associated with the occurrence of serious adverse events.[8] Therefore, Ivorian populations frequently use medicinal plants derived from the pharmacopoeia. Thus, *Mangifera indica* or mango, plant of the family Anacardiaceae, known for its many therapeutic potentialities is used in traditional Ivorian medicine to treat anemia. Several parts of this plant species can be used for the constitution of anti-anaemic drug recipes.

In Ivorian folk medicine, the infusion, decoction or maceration of the fruit kernel (mangoes) of *Mangifera indica* are used against anemia. Despite its anti-anaemic potential, information from the literature on the pharmacological effectiveness of the mango kernel remains insufficient. Therefore, this study was conducted, in order to evaluate the effects of methanolic EMMI, on the blood count, iron and transaminase levels in rat's anaemic by oral administration of phenyl hydrazine.

METHODS

Plant material

The plant material was represented by nuclei of the fruit of *Mangifera indica*, harvested in Sinémentiali (northern of Côte d'Ivoire) between the month of April and May 2023.

Animal material

The animals used in this work were Wistars albino rats (*Rattus norvegicus*) aged 3 months, with an average weight ranging between 150 g and 200 g purchased from Institute Pasteur of Côte d'Ivoire (IPCI). As a first step, rats were maintained to the vivarium of the Ecole Normale Supérieure (ENS, Abidjan), placed in airy metal cages containing wood chips regularly renewed. They were fed with IVOGRAIN pellets and had free access to water. In a second step, rats were transferred at the laboratory of biochemistry of UFR of Biological Sciences of Peleforo Gon Coulibaly University (Côte d'Ivoire) and acclimatized for 14 days in the same conditions previously described before the beginning of experiments. Protocols used in this study were approved by the local ethical committee of UFR biological sciences of Peleforo Gon Coulibaly University (Côte d'Ivoire) and in accordance with the guide for the care and use of laboratory animals.[9]

Preparation of methanolic extract from the kernel of Mangifera indica

The harvested *Mangifera indica* samples were thoroughly washed. After extracting the pulp core, it was cut into small pieces and dried in the shade at room temperature for two weeks. The dried pieces were ground mechanically, until a fine powder was obtained. Subsequently, one hundred grams (100 g) of this powder was dissolved in one litre of methanol and then homogenized using a blender. After several cycles of homogenization, the homogenates obtained were filtered successively, twice on hydrophilic cotton and once on Whatman filter paper. The filtrates were then oven dried at 50°C for two days. The brown evaporation obtained constituted the EMMI.

Phytochemical analyses of EMMI

Iron dosage

The iron content of the extract was carried out according to the AOAC method.[10] Briefly, 1g of EMMI powder was taken from a crucible, then calcined in a muffle oven for two hours. The ash obtained was dissolved in a test tube containing 4 ml of concentrated nitric acid and the solution homogenized for 1 minute. The homogenate was evaporated dry on a hot plate at 100 C, then evaporated calcined at 550 C in an oven for 1 hour. After cooling to the desiccant, the ash from the evaporator was taken back into 10 ml of hydrochloric acid and then filtered into a standard 50 ml dilution tube. The filtrate was homogenized for 2 minutes and the homogenate retained for the determination of Iron. A calibration line was established from a concentration range of 0, 2.5, 5, 7.5, 10 and 20 µg/ml, obtained from a stock iron solution concentrated at 100 µg/ml. The reading of the samples was done with Pekin Elmer analyst flame atomic absorption spectrophotometer (SAA), with a wavelength of 400 and 248 nm. The iron content was calculated from the expression below and expressed in mg/100 g.

Content (mg/100 g) = C_{50}×100Ts/×1000

C=Concentration of the element to be dosed in mg/l. Ts=Test sample in g.

Determination of total polyphenols

Determination of total phenol content in EMMI samples was performed using the Folin-Ciocalteu method.[11] To do this, 200 µl of EMMI (1 mg/mL) was added to 1 mL of Folin-Ciocalteus reagent in three test tubes. After 5 min incubation at room temperature, 800 µl of aqueous sodium carbonate solution (7.5%) was added to the tubes. The mixtures were homogenized and incubated in a water bath for 10 minutes.

The absorbance was read at 765 nm against a white, using a biochemical automaton Roche Hitachi 902 (Germany). A parallel calibration curve was performed under the same operating conditions, using gallic acid as standard, at different concentrations (10, 20, 30, 40, 50, 60, 70, 80 and 100 µg/ml). The total phenol concentration was expressed in milligrams of gallic acid equivalent per gram of extract (mg GAE/g).

Determination of total flavonoids

Determination of the total flavonoid content in EMMI samples was carried out by the Aluminium trichloride ($AlCl_3$) method.[12] A volume of 1 ml of EMMI (1 mg/ml) was put into three test tubes, then 1 ml of AlCl3, 1% (m/v) was added to each tube. The mixtures were homogenized and incubated at room temperature for 10 min, then the absorbance was read at the Roche Hitachi 902 spectrophotometer (Germany) at 760 nm. The flavonoid content was determined from the regression equation of the quercetin calibration line, established with the respective concentration range of 10, 20, 30, 40, 50, 60, 70 and 80 µg/ml. This flavonoid content was expressed in equivalent milligrams of quercetin per gram of EMMI (mg QE/g).

Tannin dosage

Tannin content was determined using the method previously described12. A volume of 500 µl of EMMI samples (diluted to 1/50th in methanol) is added to 2.5 ml of an aqueous solution of KIO3 (2.5%). The mixture was kept for 2 min at 30°C in a water bath, so that the reaction between the reagents could take place. The absorbance was measured at 550 nm, against a white one using a spectrophotometer. A calibration curve was performed in parallel, under the same operating conditions, using tannic acid as standard at different concentrations (0, 10, 20, 40, 60, 80 and 100 µg/ml). The tannin concentration in the samples was expressed in milligrams of tannic acid equivalent per gram of EMMI (mg of TAE/g).

Pharmacological studies

Experimental induction of anemia in rats by phenylhydrazine

Experiments were carried out at the laboratory of biochemistry of UFR of biological sciences (Peleforo Gon Coulibaly University) in June 2023. Briefly, induction of anemia was performed according to the method previously described with minor modifications.[13] A total of 30 rats were used in this pharmacological protocol. After determining their hematological profile, the rats were divided into 2 groups. The first group, considered as a control, included 5 rats. The second experimental group, used for pharmacological tests, was composed of 25 rats. The animals in the experimental group received phenylhydrazine (20 mg/kg) daily by gavage for one week.

Those in the control group received during the same period of observation as the distilled water, administered by gavage, at the rate of 1 ml/100g/BW. At the end of the experimental induction of anemia, the blood of the rats was taken by puncture in the sinus retro-orbital using sterile pasteur pipettes, to determine blood levels of hemoglobin, red blood cells, hematocrit and transaminases (AST, ALT).[14] Rats with a decrease in hemoglobin levels of more than 30% after 7 days of observation, were considered anaemic and selected for further pharmacological tests.

Antianaemic effect of EMMI in rats

In this protocol, rats were divided into 6 groups of 5 rats depending on the treatment received. The first group of rats, used as a negative control, received distilled water by gavage at 1 ml/100 g/BW. The second group of rats, considered as a negative control, consisted of untreated anaemic rats, given orally 1ml phenylhydrazine, 20 mg/kg. The third, fourth, and fifth groups were anaemic rats treated with EMMI (pers os) at 100, 500, and 1000 mg/kg BW, respectively, for 14 days. The sixth group consisted of anaemic rats treated with Folifer, a reference molecule used in the management of anemia, at a rate of 50 mg/kg for 14 days.

Hematological and biochemical analyses

At the end of the treatment, the blood of the animals was taken by puncture in the retro-orbital sinus as previously described, for the determination of hematological and biochemical parameters, such as the content of red blood cells (RBC), hemoglobin (Hb), hematocrit (Hct), serum iron and transaminases, including Aspartates Aminotransferases (ASAT) and Alanine Aminotransferases (ALAT), using an automaton (URIT 3000 PLUS).

Statistical analysis

Statistical analysis and graphical representation of the data were performed using GraphPad Prism 8.4.3 software (San Diego, USA). The values were expressed as a Standard Error average over the Average. Statistical differences between the results were measured using variance analysis (ANOVA), followed by the Tukey-Kramer multiple comparison test. Results were considered significant, with $p<0.05$.

RESULTS

Content of secondary metabolites and iron in EMMI samples

The secondary metabolite assay with EMMI samples at 10 mg/mL showed that this extract was rich in polyphenols, flavonoids, and tannins, with EMMI concentrations of 38.97±0.04 mg EQA/g, 26.83±0.02 mg QE/g, and 33.87±0.06 mg TAE/g, respectively (Table 1). The iron content was estimated to about 52.91±0.01 mg/100g of EMMI (Table 1).

EMMI improves blood count and serum iron content in rats anaemia with phenylhydrazine

Administration of phenylhydrazine at 20 mg/kg BW for seven days resulted in significant disruption of the blood count in anaemic rats ($p<0.001$; Figure 1). This led to a

significant decrease in RBC levels (6.82±0.05 versus 4.03±0.03 106/mm^3;p<0.001; Figure 1), Hb (13.56 0.06 versus 8.60±0.4; p<0.001; Figure 1) and Hct (37.40±0.05 versus 20.45 0.4%; p<0.001; Figure 1), in untreated anaemic rats, compared to control rats. Serum iron levels also decreased significantly in untreated anaemic rats compared to control rats (6.20±0.01 versus 4.83±0.02 mmol/l; p<0.001; Figure 2).

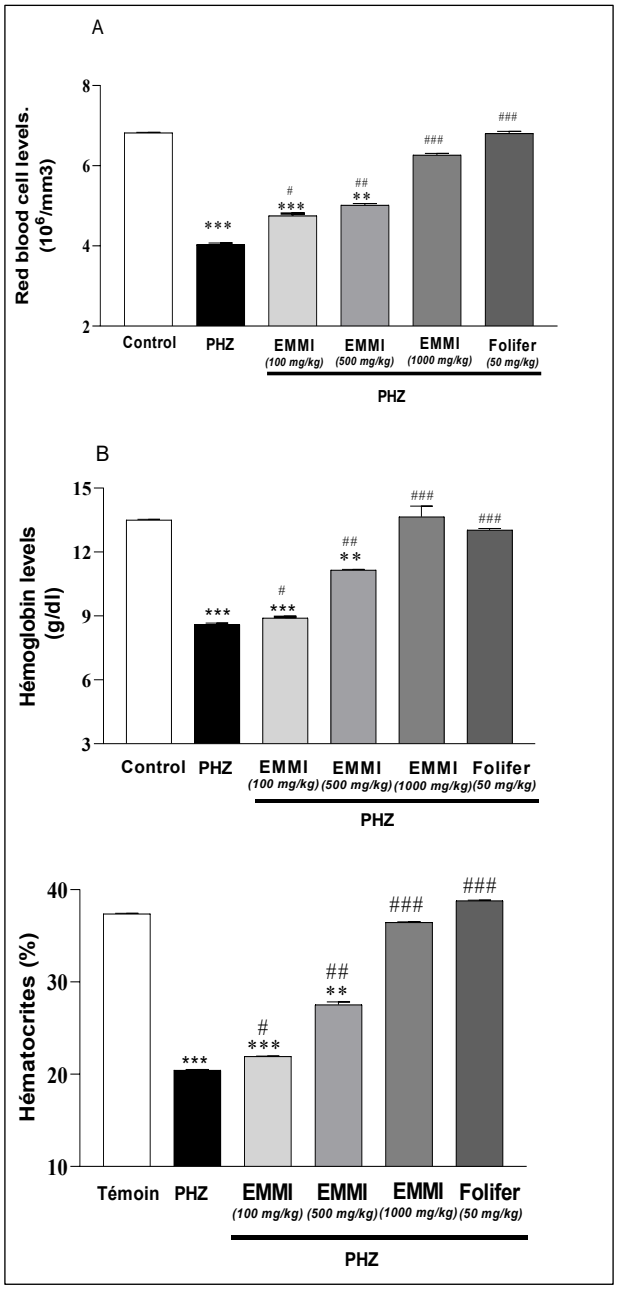

Figure 1: Effect of EMMI on transaminase expression in rats anaemized by phenylhydrazine. The data were expressed as average ESM (n = 5 rats/group).

Note:**p<0.001: Significant difference compared to control group rats. #p<0.05; ##p<0.01; ###p<0.001: Significant differences compared to rats in the untreated anemic group after phenylhydrazine (PHZ) administration, at a dose of 20 mg/kg P.C.(A): Content of Aspartates Aminotransferases (ASAT); (B):Content of Alanine Aminotransferases (ALAT).

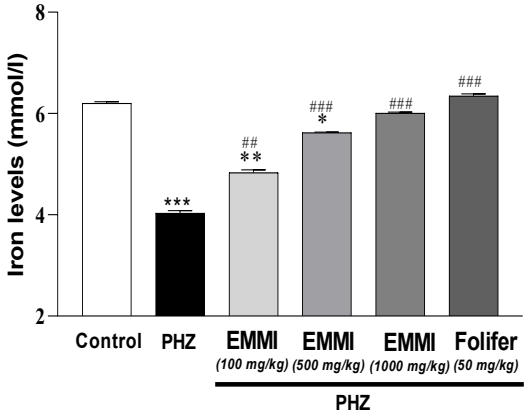

Figure 2: Effect of EMMI on serum iron content in rats anaemized by phenylhydrazine. The data were expressed as average ESM (n = 5 rats/group).

Note: **p<0.05;***p<0.01; ***p<0.001: Significant differences compared to rats in the control group. #p<0.05; ##p<0.01; ###p<0.001.

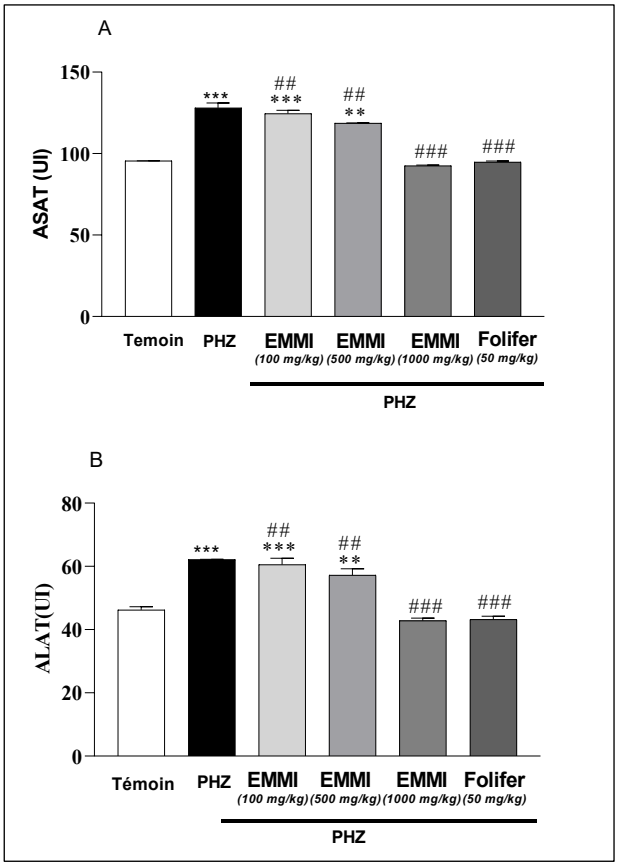

Figure 3: Effect of EMMI on transaminase expression in rats anaemized by phenylhydrazine. The data were expressed as average ESM (n = 5 rats/group).

Note: **p<0.001: Significant difference compared to control group rats. #p<0.05; ##p<0.01; ###p<0.001: Significant differences compared to rats in the untreated anemic group after phenylhydrazine (PHZ) administration, at a dose of 20 mg/kg P.C. (A): Content of Aspartates Aminotransferases (ASAT); (B) : Content of Alanine Aminotransferases (ALAT).

After 14 days of treatment, the EMMI samples (100, 500 and 1000 mg/kg BW), promoted a significant improvement in blood count parameters ($p<0.01$; $p<0.001$; Figure 1) and iron content ($p<0.05$;$p<0.01$;$p<0.001$; Figure 2) in anaemic rats. This improvement was dose-dependent. Thus, the administration of EMMI at the maximum dose (1000 mg/kg BW), induced a respective normalization of RBC (6.27±0.04×106/mm3; <0.001; Figure 1), Hb (13.04±0.05 g/dl; $p<0.001$; Figure 1), Hct (36.47±0.05 %; $p<0.001$; Figure 1) and serum iron (6.01±0.02 mmol/l; $p<0.001$; Figure 2) in anaemic rats. The observed beneficial effects of EMMI (1000 mg/kg BW) were comparable to those of Folifer (50 mg/kg), the reference molecule used for the management of anemia, which resulted in an improvement in the RBC, Hb, Hct. and serum iron levels, respectively, to about 69, 52, 90 ($p<0.001$; Figure 1) and 58% ($p<0.001$; Figure 2) in anaemic rats compared to healthy rats.

EMMI reduces overexpression of transaminases in rats with phenylhydrazine anemia

After one week of administration of phenylhydrazine (20 mg/kg BW), levels of ASAT (128. 01±0.6 versus 95.51±0.1 IU; $p<0.001$; Figure 3) and ALT (62.14±0.7 versus 46.20±0.3 IU;$p<0.001$; Figure 3), increased significantly in untreated anaemic rats compared to control rats. This overexpression of transaminase was reduced following EMMI at doses between 100 and 1000 mg/kg BW. The effects of EMMI on transaminases increased with the dose administered. Administration of EMMI at 1000 mg/kg BW resulted in a significant reduction in AST and ALT levels, respectively, of approximately 28 and 34% ($p<0.001$; Figure 3). This normalization of transaminase levels was similar to that induced by folifer (50 mg/kg BW), which resulted in a significant improvement in ASAT and ALAT levels of about 26% and 29% respectively in anaemic rats ($p<0.001$, Figure 3).

Table 1: Secondary metabolites and iron content in EMMI samples.

Phytochemical composition	Polyphenols (mgEG/Ag)	Flavonoids (mg QE/g)	Tannins (mg TAE/g)	Iron (mg/100g).
Levels	38,97± 0.04	26,83±0.02	33.87±0.06	52.91±0.01

DISCUSSION

The development of new molecules to address population health problems has become a concern for scientific research15. Thus, several investigations from the pharmacopoeia, allow to list various medicinal plants, such as Mangifera Indica (mango), whose core is used in traditional medicine, to fight anemia. This study showed that EMMI is rich in polyphenols, flavonoids in tannins, with respective contents of 38.97±0.04 mg EGA/g extract, 26.83±0.02 mg QE/g extract and 33.87±0.06 mg TAE/g extract. These secondary metabolites could give the mango kernel several therapeutic virtues.

The administration of phenylhydrazine in rats resulted in disruption of blood pressure parameters, serum iron content and transaminases. Blood samples taken eight (8) days after induction of anemia showed a decrease in RBC, Hb and Hct levels in anaemic rats compared to control rats by approximately 50, 36 and 45%, respectively. These results corroborate the effectiveness of anemia and the validity of the experimental model in this study. Moreover, they agree with previous work, which showed that a reduction of more than 30% in RBC and Hb levels was sufficient to confirm hemolytic anemia.[14]

Phenylhydrazine is responsible for the high production of free radicals, with oxidation of hemoglobin to senescent hemoglobin, whose degradation releases heme and iron.[16] Iron released following the destruction of red blood cells is recycled by the bone marrow for hemoglobin neoformation.[17] This mechanism could explain the decrease in iron levels in rats following the induction of anemia. In addition, high levels of transaminases in anaemic rats may be linked to liver damage. Indeed, in hemolytic anemia the released and non-recycled heminic iron is absorbed by the liver, with toxic consequences on liver cells, resulting in liver cirrhosis. The treatment of pathological rats with EMMI normalizes the blood count, serum iron level and value of transaminases. This effect could be attributed to its richness in polyphenols and also to its high iron content known for its key role in erythropoiesis. This process promotes the production of red blood cells in the bone marrow, using two-thirds of the iron present in the body and transported by a protein called transferrin.[18] Thus, the iron supplement brought by the administration of EMMI for 14 days in rats could be at the origin of the normalization of blood cell levels, observed in this study.

Tannins and flavonoids are compounds capable of capturing free radicals due to their antioxidant properties.[19] Their actions are believed to be responsible for neutralizing the free radicals formed by phenylhydrazine, thus protecting red blood cells from lipid peroxidation. Similar results were obtained with extracts of the leaves of Jatropha gossypiifolia which showed potentiality in the restoration of hematological parameters in anaemic rats.[20]

In addition, the presence of flavonoid in EMMI extracts could activate hematopoiesis and therefore blood production in the bone marrow. This result is consistent with previous work highlighting the potential of flavonoids in the management of anemia.[21,22] The administration of EMMI in rats also induced a decrease in serum levels of transaminases, until their normalization. This effect could be explained by the presence of flavonoids and

polyphenols present in *Mangifera indica* extracts. These metabolites are able to neutralize hepatotoxicity induced by non-recycled heme iron and absorbed by the liver. The hepatoprotective effects of *Mangifera indica* extracts are comparable to those of *Peganum harmala* extracts.[23] In addition, the results of this study are similar to those obtained with the aqueous extract of the bark of the stem of *Magnifera indica*, formulated at doses of 25, 50 and 75 mg/kg, which have shown effectiveness against iron deficiency anemia, with improved functioning of the hematopoietic system in rats.[24]

CONCLUSION

In this study, the plant material was harvested in the north of Côte d'Ivoire, with geographical specify. It is well established that ecology is a factor strongly influencing the phytochemical composition of plants. These could be a limitation in case of extrapolation of our data at national level. The main findings, of this study indicate that methanolic extract of the *Mangifera indica* kernel would be an interesting therapeutic alternative for the management of anemia in Ivorian traditional medicine, due to its rich in iron and secondary metabolites such as polyphenols, flavonoids, and tannins.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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