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Mineral composition, in-vivo hematinic and antioxidant potential of *Jatropha gossypiifolia* n-hexane root extract in hemolytic anemic rats

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Abstract

This study screened for the mineral composition and in-vivo hematinic and antioxidant properties of *J. gossypiifolia* root n-hexane extract in Wistar rats. Standard protocols were used for the mineral constituents of *J. gossypiifolia* root. Forty-five male Wistar rats were randomly selected for this study. They were administered 40 mg/kg phenylhydrazine hydrochloride to induce-hemolytic anemia for five days and thereafter treated with graded oral doses of 30, 50, and 100 mg/kg *J. gossypiifolia* extract for 14 days. Hematological, antioxidant, peripheral blood smear and histopathological evaluations of the blood cells and bone marrow were carried out. The results of the mineral composition of *J. gossypiifolia* physiological quantity include; calcium, potassium, sodium, chloride, manganese, iron, zinc, magnesium, and phosphorus. Calcium and magnesium were abundant at (33.14 and 8.34 mg/1kg) of the plant root. The results for the treatment groups at graded doses include; red blood cells at days 7 (4.68, 4.72, 4.82 x 10^6/ul) and 14 (6.51, 6.59, 6.82 x 10^6/ul), hematocrit at days 7 (40.30, 46.13, 48.63 %) and 14 (40.30, 41.47, 45.30%) and hemoglobin at days 7 (10.18, 10.92, 11.82 g/dl) and 14 (11.40, 11.87, 12.90 g/dl) when compared with untreated control ($p<0.05$). The peripheral blood smear showed normal blood morphology across the treatment groups compared to untreated control rats. The histopathological architectural framework of the bone marrow elicited an excitatory effect of myeloid/erythroid cell ratio in the treatment groups when compared with phenylhydrazine control. *J. gossypiifolia* extract displayed hematinic potential, which concurred with its ethnomedicinal report.

Keywords: mineral constituents, hematinic, antioxidants, *Jatropha gossypiifolia*

1. Introduction

In Nigeria, plants have been widely used as an alternative medicine since the event of orthodox medicine is prone to drug resistance, side-effect, non-economical, and non-readily available. Hence, herbal remedies with plant-based medicine have been scientifically proven with their potency, availability, less or no adverse effect, and very economical (1). This classification has helped World Health Organization set a standard in evaluating herbal products' possible safety, efficacy, and quality (2).

Jatropha gossypiifolia is a shrub plant that belongs to the family Euphorbiacea (2). It is generally known as Bellyache-bush, pignut, and wild cassava in English; botuje pupa (Yoruba), Cini da zugu, or Binidi zugu (Hausa), and ake mbogho (Igbo) (3). *J. gossypiifolia* has been used to treat several infectious diseases globally. Various parts of the plant have been used to manage several diseases, such as anemia, venereal disease, and so many diseases in ethnomedicines (4).

Anemia is a blood disorder that frequently affects people in all age brackets. People prone to higher risk include; adolescent women of child-bearing age, infants, and older adults (5). Anemia is of various types, such as hemolytic, sickle, and many more (6). Pregnant women are susceptible to anemia, resulting in several complications during fetal development (7). Anemia is one of the leading causes of death, either due to a lack of vital mineral constituents capable of triggering blood-related diseases like anemia. Thus, orthodox medicine has failed due to its verse adverse effect and drug resistance. This study aimed to screen for the mineral constituents of the plant and determine the hematinic and in-vivo antioxidant potential of *J. gossypiifolia* in rats. The investigation of the hematinic property of *Jatropha gossypiifolia* root extract was carried out.

2. Materials and Methods

2.1. Collection of Plant material

The young root of *Jatropha gossypiifolia* Linn. was collected in August from Oluku Estate, Ovia North East LGA, Benin City, Edo State. Dr. O. Timothy from the Department of Plant Biology and Biotechnology, Life Sciences, University of Benin, Nigeria, identified the plant. The plant was authenticated by Dr. H. A. Akinnibosun in the Herbarium Unit

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of Plant Biology and Biotechnology, Life Sciences, University of Benin, Nigeria, with voucher specimen number UBH-M511.

2.2. Preparation of Plant Material

The root of *Jatropha gossypiifolia* was rinsed, chopped into pieces, and air-dried at room temperature. The plant materials were further dried using an oven at a regulated temperature of 40°C for 10 minutes before being pulverized using a British mechanical grinder. Four thousand grams (4000 g) of the pulverized root was extracted with 5,000 ml of n-hexane via a Soxhlet extractor. The extract was concentrated semi-solid using (HH-S Water Bath; Search Tech Instruments) at a controlled temperature (45°C). Percentage yields were calculated via the formula (% Yield=extract weight/powder sample weight x 100/1).

2.3. Determination of mineral elements' composition

"The mineral element analysis was carried out using a modified procedure described by Silva et al. (8). The analysis of minerals, including calcium, potassium, sodium, chloride, manganese, iron, zinc, and phosphorus, was conducted using an Agilent FS240AA atomic absorption spectrophotometer. Magnesium was measured twice, once as a separate measurement and once as magnesium phosphorus. Approximately 2 g of the dried sample was placed into a digestion flask, and 20 ml of the acid mixture (650 ml conc. HNO_3; 80 ml perchloric acid; 20 ml conc. H_2SO_4) was added. The flask was heated until a clear digest was obtained. The digest was diluted with distilled water to the 100 ml mark. A series of standard metal solutions in the optimum concentration range were prepared. The reference solutions were prepared daily by diluting the single stock element solutions with water containing 1.5 mL concentrated nitric acid/liter. A calibration blank was prepared using all the reagents except the metal stock solutions. A calibration curve for each metal was prepared by plotting the absorbance of standards versus their concentrations."

2.4. Experimental animals

Forty-five (45) healthy whisker (albino) rats (males) weighed 180-250 g. The animals were acquired from Animal and Environmental Biology at the University of Benin animal house. They were housed in well-ventilated wooden cages in a standard laboratory state (12 hours light/dark cycle: 23 ± 2°C) and fed using a standard diet. Food and water were administered at free choice (ad libitum) to the animals used for experiments. The animals were handled correctly using the ethics of Laboratory animals' approval from the ethical committee of the Faculty of Life Sciences with the ethical number LS21009.

2.5. Experimental Design

Phenylhydrazine hydrochloride was given to the entire group to induce anemia using a modified method by Sanni et al. (9). Five groups (n=9) received the following scheduled treatment. The reference group was pre-exposed to ferrous (iii) - hydroxide poly-maltose 5 mg/kg orally p.o., and the untreated group was administered with phenylhydrazine hydrochloride 40 mg/kg p.o. Other groups were pre-exposed to graded doses (30, 50, and 100 mg/kg derived from the pilot study) of *Jatropha gossypiifolia* n-hexane root extract. Animals were fasted overnight before administering phenylhydrazine hydrochloride for five days according to their body weight. Three (3) rats were sacrificed from each group on days 0, 7, and 14, and the blood sample, bone marrow, and other organs were analyzed for histopathological evaluation.

2.6. Hematological analysis

Blood samples in the EDTA bottles were injected into the chamber of the human-automated hematology system analyzer and diluted with an isotonic saline solution. Indices analyzed included hemoglobin, red blood cell count, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration (it takes the volume or size of the red blood cell), mean corpuscular hemoglobin (mass or weight of red blood cell), platelet count, white blood cell count and differential white blood cell count (10).

2.7. Determination of antioxidant properties of the extract

Superoxide Dismutase (SOD) method was earlier reported by Bagul et al. (11) and Magili and Bwatanglang (12). Catalase assayed method was reported by Bagul et al. (11). Malondialdehyde activity was examined using a described method by Bagul et al. (11) and Magili and Bwatanglang (12).

2.8. Histopathological of Bone marrow

The isolated Bone marrows of Wistar rats were fixed in neutral buffered formalin. Affixed organs were utterly dehydrated, prepared, and interpreted via a modified method by Drury and Wallington (13).

2.9. Data Analysis

Results were analyzed with Graph pad prism version 6. Data were presented as Mean±S.E.M, and statistical significance was calculated using one-way ANOVA, followed by Dunnett's multiple comparison tests where $p<0.05$ were considered statistically significant.

3. Results

3.1. Mineral composition

Table 1 shows the mineral composition (calcium, potassium, sodium, chloride, manganese, iron, zinc, lead, magnesium, and phosphorus) of *J. gossypiifolia* n-hexane root extract at various concentrations.

3.2. Hematological Indices

The results in Fig. 1 showed a significant increase in red blood cell count and hemoglobin value on days 7 and 14 of 50 and 100 mg/kg root extract when compared with untreated control. This elicited that the plant at 50 and 100 mg/kg triggered the release or synthesis or rapidly matured the RBC and HGB, which could be responsible for the availability of the quantity of mineral content (iron) present.

Results in Fig. 2 exhibited a significant increase in

hematocrit and MCV values across the treatment groups on days 1, 7, and 14 of graded doses (30, 50, and 100 mg/kg) when compared with untreated control. It was observed that the plant extract displayed a quick onset of action of hematocrit and MCV, which will serve as a viable anti-anemic agent.

Table 1. Minerals composition of *Jatropha gossypiifolia* root extract

Minerals Composition	Concentration (mg/1kg)	Limit of Detection (LOD) ng/L	Limit of Quantitation (LOQ) ng/L
Calcium	33.14	11.16	33.82
Potassium	0.98	0.33	1.0
Sodium	0.1	0.03	0.10
Chloride	0.76	0.26	0.78
Manganese	0.021	0.01	0.02
Iron	0.65	0.22	0.66
Zinc	0.12	0.04	0.12
Magnesium	8.34	2.81	8.51
Phosphorus	0.73	0.25	0.75

The results in Fig. 3 indicated a significant increase in MCHC and MCH values across days 1, 7, and 14 of the treatment groups (30, 50, and 100 mg/kg) of *J. gossypiifolia* when compared with the untreated control.

Results in Table 2 showed a significant increase in white blood cell count in the treatment groups (30, 50, and 100 mg/kg) *J. gossypiifolia* on days 1, 7, and 14, compared with the untreated control, showed a significant decrease in WBC count.

3.3. In-vivo antioxidant

Glutathione and catalase are enzymatic antioxidants that elicited a significant increase in the scavenging capacity of graded doses of the extract when compared with the control in rats. Superoxide dismutase (SOD) is an enzymatic antioxidant that displayed a non-significant difference in the scavenging capacity of the extract when compared with the control in rats. Malondialdehyde (MDA) is a non-enzymatic antioxidant that exhibited a significant reduction, thereby enhancing the scavenging capacity of the extract when compared with the control in rats, as displayed in Table 3.

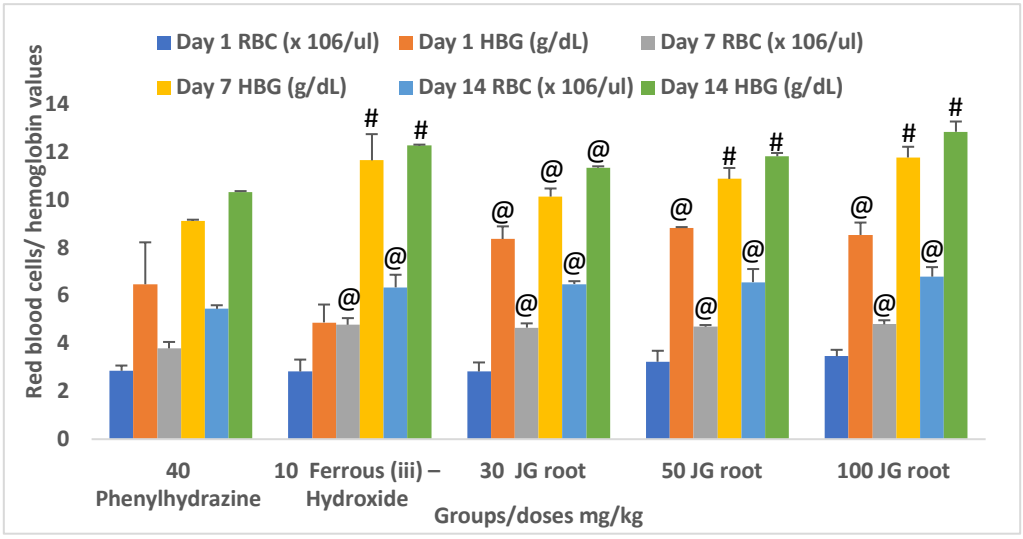

Fig. 1. Effects of *Jatropha gossypiifolia* root extract on phenylhydrazine-induced anemia in rats' red blood cells and hemoglobin. *p-value <0.05* showed the level of significant, superscript [@] and [#] indicated a significant increase (@ indicated significant increase while # indicated highly significant increase), JG; *Jatropha gossypiifolia*

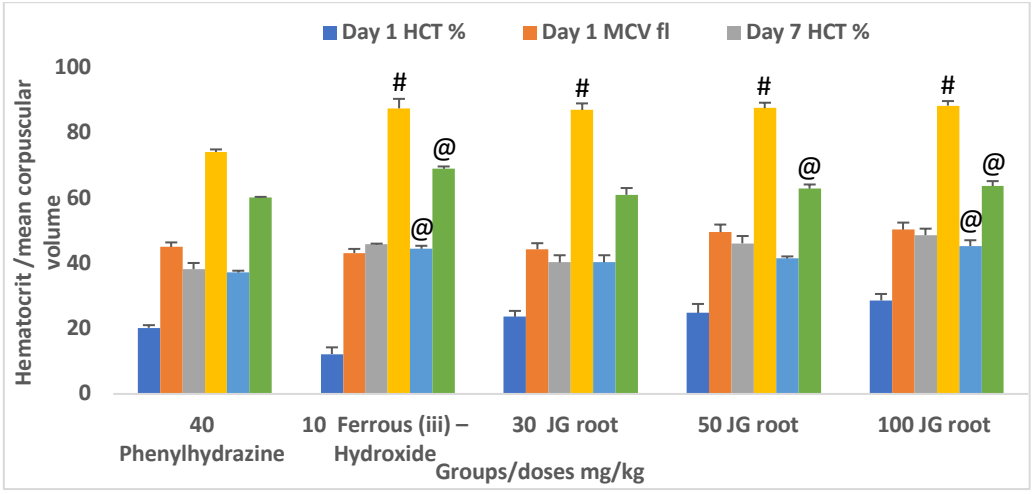

Fig. 2. Effects of *Jatropha gossypiifolia* root extract on phenylhydrazine-induced anemia in rats' hematocrit and mean corpuscular volume. *p-value <0.05* showed the level of significant, superscript [@] and [#] indicated a significant increase (@ indicated significant increase while # indicated highly significant increase) JG; *Jatropha gossypiifolia*

Table 2. Effects of *Jatropha gossypiifolia* root extract against phenylhydrazine-induced anemia in rats' white blood cells

Groups	Doses (mg/kg)	Mean±SEM DAY 1 WBC x 10^3/µl)	Mean±SEM DAY 7 WBC (x 10^3/µl)	Mean±SEM DAY 14 WBC (x 10^3/µl)
Phenylhydrazine	40	19.43±2.20[a]	6.70±5.24[a]	3.00±0.53[a]
Ferrous (iii) – Hydroxide	10	47.87±8.49[c]	7.80±0.40[a]	9.77±0.09[c]
JG root	30	44.13±3.64[b]	7.47±0.03[a]	7.40±0.12[b]
JG root	50	44.47±2.32[b]	7.80±1.27[a]	9.87±0.90[c]
JG root	100	48.27±0.72[c]	8.27±0.72[b]	10.30±3.52[c]

P-value <0.05 showed the level of significant, and superscript indicated non-significant JG; *Jatropha gossypiifolia*

Table 3. Effects of *Jatropha gossypiifolia* root extracts against phenylhydrazine-induced anemia on *in-vivo* antioxidant assay in rats

Groups	Doses (mg/kg)	Mean±SEM Glutathione (µg/ml)	Mean±SEM SOD (µg/ml)	Mean±SEM Catalase (µg/ml)	Mean±SEM MDA (10^{-4})
Phenylhydrazine	40	77.57±1.80[a]	0.043±0.00[a]	0.247±0.14[a]	5.80±0.10[a]
Ferrous (iii) – Hydroxide	10	85.37±1.93[b]	0.045±0.01[a]	0.480±0.01[b]	4.20±0.90[b]
JG root	30	83.40±0.51[b]	0.040±0.01[a]	0.466±0.02[b]	4.47±0.260[b]
JG root	50	81.01±1.01[a]	0.035±0.00[a]	0.480±0.01[b]	4.30±0.520[b]
JG root	100	82.25±0.95[a]	0.043±0.00[a]	0.483±0.01[b]	4.93±0.090[a]

P-value < 0.05 showed the level of significant, and superscript indicated non-significant JG; *Jatropha gossypiifolia*

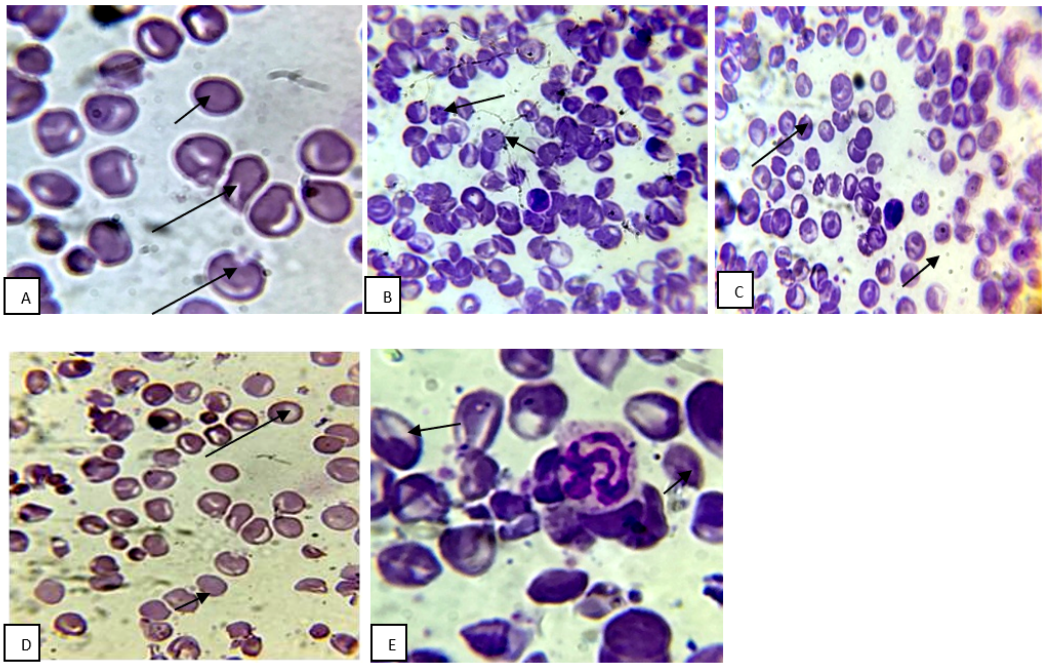

Fig. 4. Effects of the n-hexane root extract of *Jatropha gossypiifolia* on peripheral blood smear; **A,** Untreated group: Erythrocytes showed macrocytes, hypochromic, polychromatic cells, and Sickle shape. Normal WBC and platelet; **B**, Ferrous (iii) – Hydroxide: Erythrocytes showed normocytic and normochromic cells. Adequate and normal WBC and platelet; **C**, 30 mg/kg extract: Erythrocytes appeared normocytic, normochromic, and lysed cells with no polychromatic cells. Adequate and normal WBC and platelet; **D**, 50 mg/kg extract: Erythrocytes showed normocytic, lysed, and normochromic cells. Normal WBC and platelet; **E**, 100 mg/kg extract: Erythrocytes appeared in normocytic and normochromic cells with no polychromatic cells—adequate and normal WBC and platelet.

3.4. Peripheral blood smear

Fig. 4 displays normal blood normocytic, normochromic, and homochromatic cell morphology and structures across graded doses of the treatment groups (root extract of *J. gossypiifolia*) when compared with untreated control that displayed a macrocytic, hypochromic, polychromatic cells and Sickle blood shape.

3.5. Bone marrow analysis of rats fed with *Jatropha gossypiifolia*

The bone marrow in the treatment groups (root extract of *J. gossypiifolia*) of the extract displayed an increase in the level of Mylo-erythroid cells when compared with anemic control that had reduced Mylo-erythroid cells, as shown in Fig. 5.

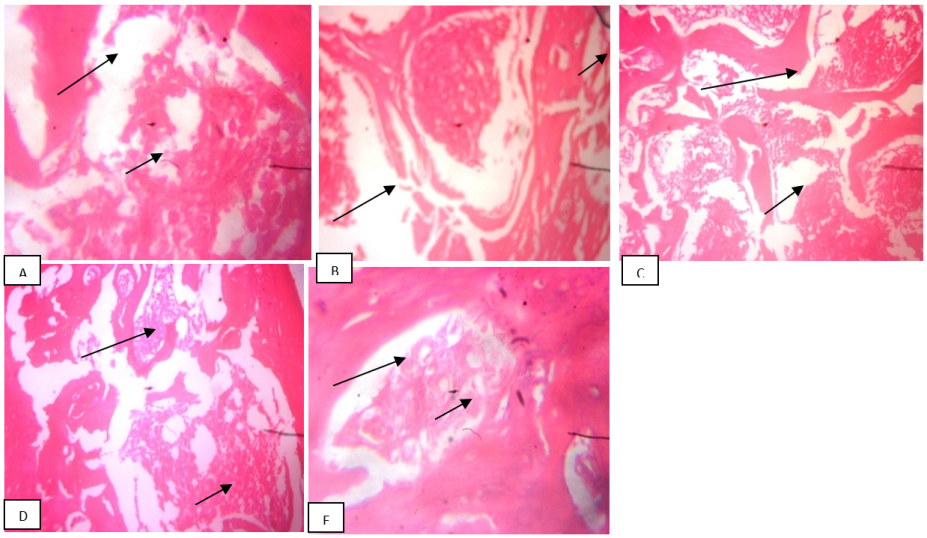

Fig. 5. Effects of n-hexane root extract of *Jatropha gossypiifolia* on the bone cell regeneration; **A**, Untreated group: The number of bone cells (Mylo-erythroid cells) decreased with cartilage surface areas appearing exfoliated with cracks. The bony trabeculae appear loose, irregular, and slender. All types of cells were scattered and sparsely distributed; **B**, Ferrous (iii) – Hydroxide: The bone marrow cavity was rich in bone marrow cells (Mylo-erythroid cells), with few fat cells—the visible histologic appearance of the bone trabeculae, bone marrow, and cartilage; **C**, 30 mg/kg extract: The bone marrow cavity has fewer bone marrow cells (Mylo-erythroid cells) and no fat cells. Normal morphology was noted, revealing a normal histologic appearance of bone trabeculae, bone marrow, and cartilage; **D**, 50 mg/kg extract: The bone marrow cavity has scanty bone marrow cells (Mylo-erythroid cells) with bone trabeculae, bone marrow, and cartilage; **E**, 100 mg/kg extract: The bone marrow cavity has fewer bone marrow cells (Mylo-erythroid cells) and no fat cells. Normal morphology revealed a normal histologic appearance of bone trabeculae, bone marrow, and cartilage.

4. Discussion

The present study showed that the calcium content level present in the root of *J. gossypiifolia* (Table 1) plays a functional role in strengthening bone marrow, which can stimulate erythrocyte regeneration, especially in anemic conditions. Additionally, as Faokunla et al. (14) reported, muscles, heart, bones, and teeth require calcium to function properly, with an increase in the quantity required for vital biological functions. Hence, the presence of this mineral content in *J. gossypiifolia* root, whose calcium level had a higher concentration than a similar report by Mustapha et al. (15) working on a related elemental constituent found in *Vitex doniana* sweet (black plum) stem bark, having calcium content (Table 1). The World Health Organization (WHO) places a safe calcium content limit at 3.6-80 mg/kg (16). The root of *J. gossypiifolia* showed the presence of magnesium content, which slightly exceeds the safety limits recommended by WHO. Therefore, caution is needed with the quantity consumed. The results of this present study are not significantly different from the recommended values, unlike the work of Faokunla et al. (14), who worked on Amaranthus hybridus leaves (23.18 mg/100g) and *Cassia siamea* (400 mg/100 g), whose results are greater than the recommended values. It is proposed that the root of *J. gossypiifolia* could be a viable source of magnesium (Mg) useful in managing extracellular fluid, bone, and plasma for osmotic balance. It is suggested that the safety limits for magnesium should not exceed 0.1-0.2 mg/kg, as recommended by WHO. Hence, the obtained doses present in the root of *J. gossypiifolia* slightly exceeded the recommended doses, and caution is needed with the quantity consumed (16).

The results obtained from this present study showed that the root of *J. gossypiifolia* possesses a slight increase in potassium content above the recommended safe limit (17). Therefore, the results obtained from the present study are better than those reported in the work of Uzama et al. (18) on *Securinega virosa* leaves at 3.67 mg/g. WHO (16) and Faokunla et al. (14) suggested that the harmless potassium limit is 0.01-0.1 mg/kg. The root of *J. gossypiifolia* is a restrained source of sodium with a slight reduction from the recommended safe limit. Moreover, the quantity of sodium in the root of *J. gossypiifolia* is almost within the safe limit, better than the reported values from the work of Swati et al. (17); Uzama et al. (18) and Okwu and Josiah (19), who compared the values of 20.31 mg/kg of *Securinega virosa* leaves with 0.02 mg/g in *Bryophylum pinnatum* and *Aspilia Africana*. WHO (16) suggested that the protective sodium limit is 0.4-0.5 mg/kg. Zinc reduces the amount of hemoglobin associated with red cell membranes, thereby inhibiting the effect of calcium in causing hemoglobin retention by membranes (20, 21). The zinc content in the root of *J. gossypiifolia* falls within the required safety limit. This present study concurred with the report of Faokunla et al. (14), which closely relates to the result of *Mucuna sloanei* content (0.25 mg/kg) and the leaves of *I. astragelina* (0.11 mg/kg), but is lower compared to the 6.85 mg/kg found in the leaves of *Cassia siamea*. Zinc is comparatively harmless despite being a microelement like manganese, which is vital to human health and cannot be overstated (22). "The root of *J. gossypiifolia* is rich in crucial micronutrients, as shown in Table 1, and falls slightly below

the recommended safe limit. This is consistent with previous studies on other plants, including *Securinega virosa* (1.50 mg/g), *Mucuna sloanei* (0.65 mg/100g), green vegetable leaves (0.98 mg/g), and the leaves of *I. astragalina* (0.43 mg/g). The leaves of *Momordica balsamina L*. were found to contain 11.6 mg/g, the root of *Boerhavia diffusa* had 9.09 mg/kg, *Catharanthus roseus* leaves had 37.2 mg/kg, and the leaves of *Phyllanthus amarus* had 64.5 mg/kg, according to a report by Djama et al. (24)." According to the World Health Organization (WHO) (16), the safety limit for manganese is 0.1-20 mg/kg (25). WHO also recommends harmless limit values for phosphorus at 0.1-0.2 mg/kg, while the National Research Council (NRC) (26) provides a Recommended Dietary Allowance (RDA) for phosphorus at 700 mg/day for adults. Phosphorus deficiency can lead to osteomalacia, tooth decay, and rickets, as previously indicated in the report by Michael (27). *J. gossypiifolia* root is an excellent source of phosphorus, as indicated in Table 1. The plant root's phosphorus values fall within the safe limit, stimulating the bone marrow to synthesize more red blood cells and promoting a healthy kidney to release erythropoietin. This study agrees with the NRC's report on the RDA values for phosphorus at 700 mg/day for adults (27).

This present study displays the results of the anti-anemic property of *J. gossypiifolia* root n-hexane extract, which significantly enhances the synthesis and regeneration of blood cells across the treatment groups when compared with the control. This could be due to certain phytochemical constituents or certain mineral components present in the extract that is implicated in the regeneration of blood cells from bone marrow. It could also stimulate the instigation of erythropoietin present in the kidney cells responsible for triggering the synthesis of blood cells from the mature bone marrow of the spleen. The results obtained from the hematological index in the untreated control at days 7 and 14 (RBC, HGB, and HCT) when compared with graded doses of the treatment groups (root extract) at days 7 and 14 (RBC, HGB, HCT), which elicited a significant increase as displayed in Figures 1 and 2. The presence of iron and other required mineral constituents in *J. gossypiifolia* root, though in smaller amounts, contributed to the synthesis and regeneration and facilitated the maturation of red blood and hemoglobin cells. The present study showed the efficacy of *J. gossypiifolia* root extract against hemolytic anemia, thereby agreeing with the work of Yamoto and Maude (28).

The anti-anemic properties of the standard drug (10 mg/kg ferrous (III) – hydroxide) and *J. gossypiifolia* root extract at a graded dose (30, 50, and 100 mg/kg) on days 1, 7, and 14 demonstrated a curative effect in blood regeneration and quick maturation of red blood cells, making it unique in its capacity to promote blood cells. The study had similar results to the report of Yakubu et al. (29). The recovery process of the blood cells could result from the mechanism of action from the mineral component or stimulation of the kidney to release erythropoiesis needed in the bone marrow to regenerate blood cells. Hemoglobin (which carries oxygen in the blood), RBC, HCT, MCV, MCH, MCHC, and WBC exhibited a significant increase in the treated groups, as shown in Fig. 1-3 and Table 2. These findings agree with the work of Cole (30) on the ethanol extract of *Bougainvillea spectabilis*.

Malondialdehyde (MDA) is a product of lipid peroxidation (LPO), which is shown to be reduced by *J. gossypiifolia* root extract. The extract reduces the over-synthesis of free radicals in erythrocytes, as evidenced by the significant reduction in MDA levels in the treatment groups compared to the control (Table 3). This suggests that *J. gossypiifolia* controls the level of oxidative stress, possibly due to the presence of natural antioxidants present in the plant extract. This finding is in agreement with the report of Bagul et al. (11) on the evaluation of the free radical scavenging properties of two classical polyherbal formulations. Glutathione (GSH) and Catalase (CAT) levels exhibited a significant increase in the treatment groups when compared with the control, as they have direct radical-scavenging properties and are crucial constituents of glutathione peroxidase (GPx), capable of eliminating diverse hydroperoxides. This is similar to the work of Dickinson and Forman (31) on cellular glutathione and thiol metabolism. Superoxide dismutase (SOD) levels showed no significant difference in the treated groups when compared with the untreated control (32).

The n-hexane extract of *J. gossypiifolia* root triggered the production and regeneration of blood cells, either due to the presence of mineral constituents or other unestablished mechanisms of action. This resulted in a normal blood cell morphology, with cells being normocytic and normochromic within adequate ranges compared to the untreated control and standard drugs in the peripheral blood smear (see Fig. 4). This study showed better results than the report by Yakubu and Afolayan (33), who worked on the effect of *Fadogia arggrestis* stem aqueous extract and *Bulbine natalensis* stem aqueous extract on anemic rats. The bone marrow is known to be a source of blood production, regeneration, and facilitation of immature cells. The treated groups' bone marrow (*J. gossypiifolia* root n-hexane extract) showed an increase in the level of myelo-erythroid (M.E.) cells when compared with the untreated control. Meanwhile, a decrease in M.E. cells level may lead to a severe anemic state. These findings agreed with the earlier study of Claro et al. (34), which reported significant changes and an increase in red blood cell percentage and morphology in myelo-erythroid cells ratio, as shown in Fig. 5. Hence, *J. gossypiifolia* root extract elicited hematinic properties specifically on days 7 and 14 of the treatment groups. MacDonald Idu et al. (35) also found similar results in their report on the phytochemical screening, antioxidant study, and hematinic properties of Mojeaga herbal remedy using an animal model, which showed an enhancement or stimulation of blood formations.

The root of *J. gossypiifolia* extract demonstrated the presence of essential mineral constituents responsible for various biochemical and hematological functions in anemic rats, particularly on days 7 and 14 of treatment. Hence, further investigation is needed to isolate, elucidate, and purify the compounds for potential clinical applications.

Ethical Statement

The animals were handled correctly using the ethics of Laboratory animals' approval from the ethical committee of the Faculty of Life Sciences with the ethical number LS21009.

Conflict of interest

No competing conflict of interest.

Funding

Not applicable to this section.

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Authors' contributions

Concept: B.O.G., M.I., Design: B.O.G., M.I., Data Collection or Processing: B.O.G., M.I., Analysis or Interpretation: B.O.G., M.I., Literature Search: B.O.G., M.I., Writing: S B.O.G., M.I.

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