# Haemopoietic Actions of *Justicia secunda* Leaf Extracts in Mice

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## Abstract

**Objective:** To evaluate the haemopoietic effects of *Justicia secunda* leaf ethanol, n-hexane, ethyl acetate, and n-butanol extracts in mice and compare these effects with the effects of standard antianaemic agents.

**Methods:** Sixteen groups of mice, six in each group, were used for the study. Anemia was induced in Groups 1 to 12 using 20 mg/kg i.p. phenylhydrazine (PHZ) daily for 2 days, followed by either ethanol, n-hexane, ethyl acetate or n-butanol extracts for 6 days. Groups 13 and 14 were induced for anemia and then received 200 mg/kg ferrous sulphate and vitamin $B_{12}$ for 6 days. Group 15 (positive control) received 20 mg/kg PHZ i.p. only, while group 16 (negative control) was untreated. Blood was collected from the retro-orbital plexuses of the mice into EDTA-containing bottles on the 7th day and analyzed for hemoglobin (Hb) level, packed cell volume, mean cell hemoglobin concentration, and mean cell volume. Red blood cell, white blood cell, and platelet counts were also measured.

**Results:** The ethanol leaf extract of *J. secunda* significantly increased the hematological parameters of mice compared to the positive and negative controls ($p$<0.05). However, the n-hexane, ethyl acetate, and n-butanol extracts showed greater hemopoietic effects ($p$<0.001) than the ethanol extract and standard antianemic drugs. The extract of *J. secunda* leaf tended to stimulate erythropoiesis comparable to the standard antianemic drugs, especially the n-hexane.

**Conclusion:** *Justicia secunda* leaf extracts exert hemopoietic actions in mice, while the n-hexane extract shows greater haemopoietic activities than ferrous sulphate and vitamin $B_{12}$.

**Keywords:** Anaemia, haemoglobin, haemopoietic effect, *Justicia secunda*, phenylhydrazine

## Introduction

Anemia is a condition in which the number of red blood cells (and consequently the oxygen-carrying capacity, hemoglobin) is insufficient to meet the body's physiologic needs. It results when the hemoglobin (Hb) concentration in the blood is lower than normal and affects about one-third of the world's population[1] and over 800 million women and children.[2] Anaemia in children is a major global public health concern and one of the major causes of childhood mortality, especially in developing countries.[3] It has significant consequences for human health as well as social and economic development in low-, middLe- and high-income countries.[4] The burden of anemia in some developing countries is 40% times higher than in most developed countries, with an average prevalence of 60 % among children aged 6–59 months having been reported in 27 Sub-Sahara African countries.[5]

Diagnosis of anemia is made when the Hb concentration falls below established cut-off values of 13 g/dL in men (15 years and above);

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12 g/dL in non-pregnant women (15 years and above); and 11 g/dL in pregnant women and children (6–59 months old).[6] When the hemoglobin concentration decreases, the ability of the blood to transport oxygen to tissues is compromised, leading to symptoms like fatigue, reduced physical work capacity, and shortness of breath, among others.[4] In two national representative cross-sectional surveys in Nigeria: the Nigeria Demographic and Health Survey (2018 NDHS) and the National Human Development Index (2018 NHDR), the prevalence of anemia among children in Nigeria was found to be 68.1%, with Zamfara State having the highest prevalence (84%), and Kaduna the least (50%).[7]

There are three main mechanisms underlying the development of anemia in mammals: ineffective erythropoiesis (when the body makes too few red blood cells), hemolysis (when red blood cells are destroyed), and blood loss. The most common contributors to anemia are nutritional deficiencies, diseases, and genetic hemoglobin disorders,[1] while the three top causes of anemia globally are iron deficiency, hemoglobinopathies, and malaria.[4] Iron deficiency is the most common cause of anemia (nutritional or otherwise) and is estimated to contribute to about 50% of all cases of anemia among non-pregnant and pregnant women, and 42% of cases in children under 5 years of age worldwide.[2] Haemolytic anemia is associated with oxidative stress within the erythrocytes,[8] with oxidative stress being involved in the aging and apoptosis of erythrocytes, thus inducing hemolysis.[9,10] Oxidative stress plays a role in anemia, which can manifest in the elevation of both oxidants and antioxidants (as a compensatory mechanism). It has been shown that levels of both would be ideal in arriving at a reasonable conclusion of oxidative status in anemia.[10] This concept is supported by the fact that hemolytic damage is accompanied by the generation of reactive oxygen species (ROS), glutathione depletion, Hb oxidation, and Heinz body formation in RBCs. Hemolytic agents have been reported to cause membrane lipid peroxidation and denaturation of cytoskeletal protein.[8] Factors associated with red blood cell destruction include infections, drugs, and hemoglobinopathies, which lead to reduced ability of blood to carry oxygen.[11] Evidence has shown that up to 130 drugs can induce hemolytic anemia through several mechanisms.[12] Phenylhydrazine treatment has been shown to induce changes in various blood cell counts causing haemotoxicity and consequently leading to hemolytic anemia;[13] hence its use in the induction of anemia in experimental models.

For many years, medicinal plants were the only sources of treatment for diseases in humans and many of today's drugs have been isolated from medicinal plants. Herbal remedies have relied heavily on the use of natural products as active ingredients.[14] The WHO reported that up to 80% of the population in Africa depends on traditional medicine to help meet their healthcare needs.[15] A number of natural products, including herbs, are widely used in folk medicine to prevent and/or alleviate anemia.[16] *Justicia Secunda* is an evergreen perennial plant with stems that sometimes become more or less woody, growing up to 90–200 cm tall. The plant comprises almost 250 genera, with 2500 species, and is harvested from the wild bush for local use as medicine. The plant species are widespread in tropical regions and are poorly represented in temperate regions. The leaf decoction of *J. Secunda* is used for the treatment of various ailments including anemia, fever, malaria, cough, and cold.[17] This study, therefore, was set to evaluate the basis for the use of *J. Secunda* leaf for the treatment of hemopoietic disorders.

## Methods

Healthy albino mice (both male and female) weighing 18–30 g were sourced from the Animal House Facility of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Agulu Campus. The animals were housed in cages and fed with commercial rat pellets and tap water at the Pharmacology Laboratory of the Faculty of Pharmaceutical Sciences of our University, where the study was carried out, from October to December 2019. Before administration of the extract, the animals were acclimated to laboratory conditions for two weeks. The animals were allowed access to food and water *ad libitum.* The study protocol was approved by Nnamdi Azikiwe University Teaching Hospital, Ethics Committee, Nnewi, Nigeria NAUTH/CS/66/vol.11/187/2018/122).

*Justicia Secunda* leaves were collected from a local farm at Abakiliki, South-East Nigeria. The plant was identified by a plant taxonomist, Mr. A. Ozioko of 110 Aku Road, Nsukka, Nigeria. A voucher specimen of the plant was deposited at the Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, University of Nigeria,

Nsukka, Nigeria, with the voucher number *Justicia Secunda*-interceded/29648. Standard anti-anemic agents, ferrous sulfate (Reagan Remedies Ltd, Owerri, Nigeria) and vitamin $B_{12}$ (Mason Vitamins Inc. Miami, USA), and the anemia-inducing agent phenylhydrazine (Sigma, Steinheim, Switzerland) were all bought from Index Pharmacy, Nnewi, Nigeria. All the drugs and chemicals used in the study were of analytical grade.

The plant material was extracted in four different solvents by agitation extraction method, which involved successive extraction with solvents of increasing polarity, from non-polar n-hexane to more polar ethanol. This was to ensure the extraction of a wide polarity range of compounds as well as choosing the solvent that will give a higher percentage yield of the extract. This process was done by weighing 1,000 g of the pulverized plant leaf into an Erlenmeyer flask containing 80% ethanol in water for 72 h, using a solid/liquid ratio of 1:10. With intermittent shaking every 2 min in an orbital shaker. This was thereafter filtered with Whatman No.1 filter paper. The filtrate was concentrated using a rotary evaporator at 40°C. The paste weighed 85.4 g and was stored in a refrigerator until use. The phytochemical study was carried out with ethanol extract, using standard methods as recommended by Evans.[18] All measurements were taken in triplicates.

The median lethal dose ($LD_{50}$) was determined in the ethanol extract, using the up and down procedure (UDP) in accordance with Guideline No. 425 of the Organization for Economic Cooperation and Development (OECD).[19] The dose of the extract that reversed anemia in the mice was determined by calculating the median effective dose ($ED_{50}$), using the method of Miller and Tainter as described by Milan *et al*.[20] The experiment was conducted with 30 anemic mice, with anemic mice being mice with Hb<11.5 g/dL. Briefly, the mice were randomly assigned to 5 groups of 6 mice per group. Each group received a single oral dose of either of the extracts at 4, 8, 16, 32, or 64 mg/kg for 7 days. After

**Table 1 Effect of *J. Secunda* Leaf Extracts on Hemoglobin Level (g/dL) in Mice**

| Group | Baseline | Induction | Treatment | F | p-value |
|---|---|---|---|---|---|
| Ethanol extract (mg/kg) | | | | | |
| LD | 14.05±0.97 | 10.20±0.98* | 12.30±0.70* | 20.80 | 0.001 |
| MD | 13.60±0.78 | 11.30±2.19 | 12.33±2.24 | 2.81 | 0.142 |
| HD | 13.95±1.39 | 10.38±0.48* | 13.75±1.12# | 18.73 | 0.018 |
| n-hexane extract (mg/kg) | | | | | |
| LD | 13.58±1.15 | 9.73±1.65* | 12.53±1.46* | 9.51 | 0.019 |
| MD | 14.85±0.58 | 10.38±1.18* | 12.80±0.89* | 46.13 | 0.001 |
| HD | 13.13±1.49 | 11.03±0.83 | 15.13±0.15+# | 11.08 | 0.012 |
| Ethyl acetate extract (mg/kg) | | | | | |
| LD | 13.80±1.27 | 10.60±1.99* | 12.20±0.39* | 7.01 | 0.025 |
| MD | 15.00±0.76 | 10.38±0.78* | 11.78±1.88* | 20.27 | 0.004 |
| HD | 13.97±0.81 | 9.72±0.88* | 13.48±0.85# | 43.08 | 0.002 |
| n-butanol extract (mg/kg) | | | | | |
| LD | 13.98±0.76 | 10.36±1.61* | 11.53±0.67* | 14.51 | 0.003 |
| MD | 14.12±0.52 | 9.52±1.20* | 13.43±1.69* | 20.84 | 0.001 |
| HD | 13.94±0.40 | 8.70 ± 0.53* | 13.70±2.10# | 34.95 | 0.011 |
| $FeSO_4$ (0.2 mg/kg) | 15.70±0.66 | 8.90±1.39* | 13.80±0.88# | 21.50 | 0.044 |
| Vit $B_{12}$ (100µg) | 14.60±0.66 | 9.10±1.32* | 13.40±1.10# | 31.17 | 0.035 |
| Negative control | 14.90±1.66 | 15.34±1.17 | 14.30±1.34 | 1.23 | 0.340 |

LD=low dose; MD=median dose; HD=high dose; *Significantly lower than baseline value ($p<0.05$); +Significantly higher than baseline value ($p<0.05$); #Significantly higher than the induction value ($p<0.05$)

**Table 2 Effect of *J. Secunda* Leaf Extracts on Packed Cell Volume (L/L) in mice**

| Group | Baseline | Induction | Treatment | F | p-value |
|---|---|---|---|---|---|
| Ethanol extract (mg/kg) | | | | | |
| LD | 48.98±2.54 | 34.25±2.95* | 38.90±3.32* | 35.05 | 0.034 |
| MD | 48.92±2.77 | 34.96±4.04* | 39.35±7.05* | 11.73 | 0.009 |
| HD | 46.20±3.20 | 30.20±14.52* | 46.10±6.60# | 20.82 | 0.014 |
| n-hexane extract (mg/kg) | | | | | |
| LD | 44.97±3.54 | 32.30±2.31* | 40.10±7.07*# | 14.35 | 0.036 |
| MD | 49.10±1.86 | 33.93±3.07* | 41.70±4.48*# | 33.83 | 0.010 |
| HD | 43.33±5.98 | 33.70±2.24* | 49.57±1.46+# | 11.54 | 0.016 |
| Ethyl acetate extract (mg/kg) | | | | | |
| LD | 46.35±4.05 | 32.70±8.11* | 40.75±1.46*# | 8.69 | 0.016 |
| MD | 49.90±2.92 | 33.28±1.93* | 39.65±5.51*# | 23.36 | 0.004 |
| HD | 48.70±2.03 | 31.20±3.94* | 45.90±1.28# | 52.10 | 0.019 |
| n-butanol extract (mg/kg) | | | | | |
| LD | 46.62±2.31 | 30.28±5.73* | 37.93±1.05*# | 27.51 | 0.004 |
| MD | 45.70±1.62 | 29.60±2.25* | 44.50±4.10# | 48.60 | <0.001 |
| HD | 46.40±1.57 | 26.40±4.32* | 44.70±5.78# | 37.04 | 0.006 |
| $FeSO_4$ (0.2mg/kg) | 51.90±2.22 | 31.80±1.32* | 46.70±2.71# | 19.47 | 0.049 |
| Vit B12 (100µg) | 47.80±2.20 | 28.90±6.38* | 44.90±5.28# | 52.30 | 0.010 |
| Negative control | 49.34±4.67 | 52.44±4.14 | 50.50±4.49 | 0.53 | 0.551 |

LD=low dose; MD=median dose; HD=high dose; *Significantly lower than the baseline value ($p<0.05$); +Significantly higher than the baseline value ($p<0.05$); #Significantly higher than the induction value ($p<0.05$)

the 7-day treatment period, the hemoglobin concentration of each mouse was determined for the different groups. Mice with Hb≥11.5 g/dL were considered as having their anemia reversed.

Ninety-six healthy albino mice of different sexes, weighing 28–30g, were divided into 16 groups of 6 mice per group. Animals in groups 1–12 were treated with 20 mg/kg (i.p.) phenylhydrazine (PHZ) for 2 days, and from the 3rd day onwards the animals were administered 2.7 mg/kg (p.o.) (low dose, LD), 8.3 mg/kg (medium dose, MD) and 24.9 mg/kg (high dose, HD) of ethanol, n-hexane (NH), ethyl acetate (EA) and n-butanol (NB) extracts of *J. Secunda* leaf for 6 days. The animals in Group 13 were treated with 20 mg/kg (i.p.) PHZ for 2 days and thereafter 200 mg ferrous sulfate, while the Group 14 animals received 20 mg/kg (i.p.) PHZ for 2 days followed by 100 µg vitamin $B_{12}$. The positive control (Group 15) received 20 mg/kg (i.p.) PHZ only, while the negative control (untreated) (Group 16) received only feed and water. All the animals in the positive control (Group 15) died on the 3rd day of treatment.

On the 7th day of treatment, 1ml of blood was collected from the retro-orbital plexus of the animals into ethylenediaminetetraacetic acid (EDTA)-containing bottles. The samples were analyzed for hematological parameters using a hematology automated analyzer machine (Mindrays, Model BC-2800Vet, China). The hematological parameters analyzed were hemoglobin, packed cell volume, total red blood cell count, white blood cell count, mean corpuscular volume, mean corpuscular hemoglobin concentration, and platelet count. All the parameters were estimated thrice.

Data were presented as mean ± standard deviation and analyzed using the one-way analysis of variance (ANOVA), followed by Bonferroni's multiple comparison (post-hoc) test. Statistically significant levels were determined at $p<0.05$.

**Table 3 Effect of *J. Secunda* Leaf Extracts on Mean Corpuscular Hemoglobin Concentration in Mice**

| Group | Baseline | Induction | Treatment | F | p-value |
|---|---|---|---|---|---|
| Ethanol extract (mg/kg) | | | | | |
| LD | 28.92 ± 0.53 | 29.98 ± 1.59 | 30.35 ± 0.29 | 2.58 | 0.171 |
| MD | 29.34 ±0.75 | 33.94 ± 1.47$^{+}$ | 31.88 ± 1.05$^{**}$ | 20.48 | 0.004 |
| HD | 29.95 ± 1.04 | 34.18 ± 0.77$^{+}$ | 30.53 ± 2.04$^{**}$ | 13.04 | 0.009 |
| n-hexane extract (mg/kg) | | | | | |
| LD | 30.28 ± 1.50 | 35.28 ±3.37 | 31.70 ± 1.94 | 5.72 | 0.055 |
| MD | 30.48 ±0.70 | 33.28 ± 3.15 | 31.90 ± 1.56 | 2.77 | 0.187 |
| HD | 29.83 ±0.67 | 32.70 ± 2.01$^{+}$ | 30.50 ± 0.70$^{**}$ | 6.61 | 0.048 |
| Ethyl acetate extract (mg/kg) | | | | | |
| LD | 29.48 ± 0.67 | 32.84 ± 2.12$^{+}$ | 30.18 ± 0.51$^{**}$ | 9.36 | 0.012 |
| MD | 30.40 ± 0.70 | 31.55 ± 1.10$^{+}$ | 29.63 ± 0.87$^{**}$ | 4.93 | 0.035 |
| HD | 28.86 ± 0.63 | 31.70 ± 1.26$^{+}$ | 29.63 ± 1.26$^{**}$ | 9.60 | 0.006 |
| n-butanol extract (mg/kg) | | | | | |
| LD | 29.95 ± 1.01 | 34.84 ± 4.91 | 30.00 ± 1.14 | 5.14 | 0.101 |
| MD | 30.85 ± 0.51 | 32.20 ± 1.84 | 30.10 ± 1.15 | 3.27 | 0.105 |
| HD | 30.00 ± 1.49 | 33.00 ± 3.65 | 30.50 ± 0.70 | 1.95 | 0.223 |
| $FeSO_4$ (0.2mg/kg) | 30.40 ± 0.00 | 28.10 ± 1.32 | 29.90 ± 0.66 | 4.98 | 0.167 |
| Vit $B_{12}$ (100µg) | 30.00 ± 0.44 | 30.20 ± 1.32 | 30.50 ± 0.88 | 4.77 | 0.677 |
| Negative Control | 30.20±0.34 | 29.20±0.26 | 29.42 ± 0.83 | 4.22 | 0.086 |

LD = low dose; MD = median dose; HD = high dose; $^{+}$Significantly higher than baseline value ($p < 0.05$); $^{**}$Significantly lower than the induction value ($p < 0.05$)

## Results

The qualitative phytochemical analysis showed that *J. Secunda* ethanol leaf extract contains saponins ++, tannins ++, flavonoids +, alkaloids +, terpenoids +, carbohydrate +, and reducing sugars +. The quantitative analysis showed that saponins had the highest concentration of 9.2 %, followed by tannins (9.0 %) and flavonoids (7.0 %), while alkaloids had the least concentration (2.4%).

The $ED_{50}$ of *J. Secunda* ethanol leaf extract was determined as 8.3 ± 3.2 mg/kg (C.I.: 5.1-11.5 mg/kg). This value was then used to determine the doses for the study. Administration of 2000 mg/kg (p.o.) of the extract produced no death or any signs of toxicity, so the $LD_{50}$ was taken as > 2,000 mg/kg, following the UDP for $LD_{50}$ determination. The therapeutic index (TI) was subsequently calculated to be 240.96.

The Hb levels of the PHZ-induced anemic mice and those that were treated with LD of ethanol extract, and LD and MD of the NH, EA, and NB extracts were significantly lower than the baseline values (Table 1). Treatment of the anemic mice with LD and MD of all the extracts resulted in a non-significant increase ($p>0.05$) in Hb levels of the animals compared to the induction levels. However, the Hb levels of the animals treated with HD of the extracts were significantly increased ($p<0.05$) in comparison with the induced animals, just like the standard agents (ferrous sulfate and vitamin $B_{12}$) (Table 1). Treatment with HD of NH extract, however, caused a significant increase in Hb level (15.13 ± 0.15) compared to the baseline value (13.13 ± 1.49) ($p=0.012$).

The PCV of the anemic mice and those

**Table 4 Effect of *J. Secunda* leaf Extracts on Red Blood Cell Count ($10^6$cells/$mm^3$) in Mice**

| Group | Baseline | Induction | Treatment | F | p-value |
|---|---|---|---|---|---|
| Ethanol extract (mg/kg) | | | | | |
| LD | 8.28 ± 0.19 | 6.63 ± 0.65* | 5.80 ± 0.65* | 50.04 | 0.004 |
| MD | 3.46 ± 0.50 | 6.16 ± 1.00* | 5.23 ± 0.86* | 14.20 | 0.001 |
| HD | 7.83 ± 0.62 | 5.70 ± 1.02* | 7.60 ± 0.73# | 15.84 | 0.038 |
| n-hexane extract (mg/kg) | | | | | |
| LD | 7.38 ± 0.98 | 4.10 ± 0.93* | 5.73 ± 0.73* | 15.10 | 0.002 |
| MD | 7.82± 0.58 | 5.53 ± 0.62* | 5.85 ± 0.38* | 82.40 | 0.043 |
| HD | 7.35 ± 1.13 | 5.55 ± 0.34* | 8.30 ± 0.10# | 10.60 | 0.020 |
| Ethyl acetate extract (mg/kg) | | | | | |
| LD | 8.08 ± 0.63 | 5.58 ± 1.41* | 6.68 ± 0.88* | 8.48 | 0.010 |
| MD | 8.54 ± 0.67 | 5.48 ± 0.69* | 6.95 ± 0.76* | 21.23 | 0.003 |
| HD | 7.64 ± 0.31 | 4.78 ± 0.73* | 7.70 ± 0.26# | 68.70 | 0.019 |
| n-butanol extract (mg/kg) | | | | | |
| LD | 7.87 ± 0.40 | 4.94 ± 0.96* | 6.73 ± 0.86* | 21.60 | 0.008 |
| MD | 8.12 ± 0.24 | 4.65 ± 0.55* | 7.15 ± 1.07# | 34.90 | 0.001 |
| HD | 7.70 ± 0.23 | 4.15 ± 0.26* | 8.20 ± 0.90# | 85.83 | 0.006 |
| $FeSO_4$ (0.2mg/kg) | 6.93 ± 1.54 | 4.77 ± 0.88 | 6.27 ± 0.66 | 19.17 | 0.159 |
| Vitamin $B_{12}$ (100µg) | 6.60 ± 0.88 | 4.90 ± 0.66 | 6.50 ± 0.88 | 4.16 | 0.194 |
| Negative control | 8.48±0.59 | 8.52±0.76 | 7.84 ± 1.91 | 0.45 | 0.552 |

LD=low dose; MD=median dose; HD=high dose; *Significantly higher than baseline value ($p<0.05$); **Significantly lower than the induction value ($p<0.05$)

treated with LD and MD of the extracts were significantly lower ($p<0.05$) than the baseline group (Table 2). There was a statistically significant increase ($p<0.05$) in the PCV of the animals treated with the HD ethanol extract, and LD, MD, and HD of the other extracts in comparison with the anemic mice, in the same manner as the standard agents. Again, treatment with HD of NH extract caused a significant increase ($p=0.016$) in PCV (49.57±1.46) compared to the baseline value (43.33±5.98).

There were significantly higher ($p<0.05$) MCHC in the MD and HD induction values of the ethanol extract than the baseline values (Table 3). Treatment with MD and HD ethanol and HD n-hexane and LD, MD, and HD ethyl acetate extracts resulted in a significant decrease in MCHC of mice in comparison with the values of the induced mice. Also, the MD and HD induction group of the ethanol, and the HD n-hexane and LD, MD and HD ethyl acetate extracts of the induction group showed a statistical increase ($p<0.05$) in MCHC compared to the baseline values (Table 3).

The RBC counts of the anemic mice and those treated with LD and MD of the extracts were significantly lower ($p<0.05$) than those of the baseline group, but the RBC counts of the animals treated with HD of the extracts were increased significantly ($p<0.05$) when compared with the RBC count of the induced animals (Table 4). Treatment of the anemic mice with the standard agents caused no significant differences in the RBC count (Table 4). The effect of the extracts on the

**Table 5 Effect of *J. Secunda* Leaf Extracts on White Blood Cell Count ($10^9$/L) in Mice**

| Group | Baseline | Induction | Treatment | F | p-value |
|---|---|---|---|---|---|
| Ethanol extract (mg/kg) | | | | | |
| LD | 4.54 ± 0.27 | 3.05 ± 0.39* | 3.87 ± 0.59# | 23.41 | 0.013 |
| MD | 3.46 ± 0.50 | 4.90 ± 2.10 | 4.45 ± 0.40 | 1.68 | 0.271 |
| HD | 4.30 ± 0.53 | 3.10 ± 0.81 | 4.00 ± 0.67 | 4.70 | 0.165 |
| n-Hexane extract (mg/kg) | | | | | |
| LD | 4.28 ± 0.30 | 3.65 ± 1.30 | 3.57 ± 0.57 | 1.17 | 0.326 |
| MD | 3.58 ± 0.54 | 2.25 ± 0.72* | 4.18 ± 0.59# | 11.33 | 0.028 |
| HD | 3.52 ± 0.68 | 3.28 ± 0.98 | 3.00 ± 0.42 | 0.45 | 0.340 |
| Ethyl acetate extract (mg/kg) | | | | | |
| LD | 3.30 ± 1.32 | 4.10 ± 1.54 | 3.00 ± 0.44 | 2.55 | 0.182 |
| MD | 4.10 ± 0.88 | 2.80 ± 0.66 | 2.70 ± 0.44 | 23.19 | 0.079 |
| HD | 4.00 ± 0.88 | 2.80 ± 0.66* | 3.90 ± 1.32# | 15.86 | 0.009 |
| n-butanol extract (mg/kg) | | | | | |
| LD | 3.75 ± 0.28 | 3.84 ± 0.98 | 2.86 ± 0.50 | 2.82 | 0.206 |
| MD | 3.78 ± 0.73 | 3.15 ± 0.57 | 3.60 ± 0.49 | 1.22 | 0.329 |
| HD | 2.70 ± 0.44 | 2.90 ± 0.66 | 4.50 ± 0.00 | 94.66 | 0.0002 |
| $FeSO_4$ (0.2mg/kg) | 3.40 ± 1.10 | 4.90 ± 0.44 | 4.40 ± 0.44 | 1.93 | 0.341 |
| Vit $B_{12}$ (100µg) | 4.10 ± 0.66 | 3.20 ± 0.66 | 4.60 ± 0.66 | 5.146 | 0.163 |
| Negative control | 4.34±0.48 | 2.98±0.64* | 4.33±0.35# | 11.06 | 0.003 |

LD=low dose; MD=median dose; HD=high dose; *Significantly higher than baseline value ($p<0.05$); **Significantly lower than the induction value ($p < 0.05$)

MCV showed that only the HD ethanol extract resulted in a significant increase ($p$=0.011) in the MCV of the treated mice (59.03±1.04 f/L) when compared with the anemic mice (56.15±1.29 f/L).

The LD ethanol, MD n-hexane, and HD ethyl acetate extracts all had a significant increase in the WBC counts of the animals in comparison with those of the animals induced with anemia (Table 5). Platelet counts of the animals in the baseline, induction, and treatment groups did not differ statistically from one another, except in the LD and MD ethanol extract, which caused a significant decrease ($p$<0.05) in comparison with the baseline value. The LD ethanol extract treatment also caused a significant increase ($p$=0.014) in the platelet count (1334.0±348.90) in comparison with the anemic mice (1039.00±220.20) (Table 6).

## Discussion

This study assessed the hemopoietic properties of *J. Secunda* leaf extracts in mice using ethanol, n-hexane, ethyl acetate, and n-butanol solvents. The findings showed that *J. Secunda* leaf contained phytochemicals like saponins, tannins, flavonoids, and alkaloids. The different biological activities and promising drug properties are the consequence of the unique chemical diversity that has arisen in natural products.[21] The presence of these phytochemicals in the extract is in agreement with the findings of Yamoah and co-workers, which also found that *J. Secunda* contained tannins, saponins, alkaloids, flavonoids, glycosides, and sterols.[22] Our findings did not reveal the presence of glycosides and steroids which were reported by Yamoah.

**Table 6 Effect of *J. Secunda* Leaf Extracts on Platelet Counts ($10^9$/L) in Mice**

| Group | Baseline | Induction | Treatment | F | p-value |
|---|---|---|---|---|---|
| Ethanol extract (mg/kg) | | | | | |
| LD | 1850.00±140.60 | 1039.00±220.20* | 1334.0±348.90*# | 14.55 | 0.014 |
| MD | 1622.00±207.20 | 1041.00±159.20* | 1100.0±118.10* | 21.19 | 0.000 |
| HD | 1174.00±81.85 | 983.70±69.10 | 1334.0±214.60* | 5.74 | 0.054 |
| n-hexane extract (mg/kg) | | | | | |
| LD | 1174.00±140.90 | 853.00±490.80 | 957.0±106.90 | 1.57 | 0.265 |
| MD | 1025.00±121.50 | 1063.00±101.80 | 1179.0±153.90 | 1.83 | 0.229 |
| HD | 1135.00±451.80 | 1158.00±237.40 | 1031.0±190.10 | 0.12 | 0.767 |
| Ethyl acetate extract (mg/kg) | | | | | |
| LD | 1408.00±410.40 | 1011.00±41.36 | 1169.0±152.70 | 3.24 | 0.146 |
| MD | 977.30±75.29 | 1113.00±139.80 | 989.30±61.45 | 2.75 | 0.148 |
| HD | 1046.00±85.02 | 1276.00±177.80 | 1115.0±44.23 | 4.41 | 0.106 |
| n-butanol extract (mg/kg) | | | | | |
| LD | 973.70±157.50 | 1020.00±364.60 | 987.0±65.02 | 0.10 | 0.782 |
| MD | 1025.00±112.60 | 1191.00±181.70 | 1129.0±150.50 | 1.67 | 0.237 |
| HD | 986.00±65.22 | 1034.00±71.65 | 976.0±166.60 | 0.39 | 0.561 |
| $FeSO_4$ | 1329.30±291.94 | 1194.25±299.86 | 1212.6±405.02 | 0.13 | 0.882 |
| Vit $B_{12}$ | 1396.00±359.48 | 1024.8±49.94 | 1274.6±205.92 | 1.67 | 0.374 |
| Neg control | 1136.00±125.90 | 1225.00±203.00 | 1156.0±154.70 | 0.42 | 0.561 |

LD=low dose; MD=median dose; HD=high dose; *Significantly higher than baseline value ($p<0.05$); **Significantly lower than the induction value ($p<0.05$)

The differences in the phytochemical contents could be attributed to the environmental conditions, such as soil fertility, pH, water supply, climate, and seasonal variations, at the different geographical locations in which the plant material was grown.

The $ED_{50}$ of the extract (i.e. dose that was able to restore Hb level to ≥11.5 g/dL in 50 % of the anemic mice) was 8.3±3.2 mg/kg. This shows that the plant extract has a very potent hemopoietic property. The acute oral toxicity test revealed that the extract, at a dose of 2000 mg/kg, was not able to cause death or any sign of toxicity after 14 days of exposure, giving a therapeutic index of over 240. This is an indication that the extract has a very wide safety margin and is practically non-toxic. With the economic downturn in society, some patients are unable to afford the conventional drugs used in the management of anemia, coupled with their attendant adverse effects. In contrast, herbal medicines used in therapy are comparatively cheap, readily available, and thought to be less toxic than conventional drugs. However, the use of herbal medicine extracts without safety evaluation could be noxious.

The hemopoietic potentials of the extract were demonstrated by the restoration of some of the hematological parameters to normal levels, after treatment with the various doses of *J. Secunda* leaf extracts in as short as 6 days. Essentially, treatment with high dose n-hexane extract raised the Hb and PCV levels of the animals significantly in comparison with both the induction and baseline values. It is noteworthy that the standard hemopoietic drugs (ferrous sulfate and vitamin $B_{12}$) were not able to raise the Hb and PCV values of the animals as much as the high-dose n-hexane extract. This effect may be due to the presence of phytochemicals in

the plant extracts. Bigoniya *et al*.[23] reported good anti-anemic and hematopoietic activities in *Wrightia tinctoria* bark methanolic extract, which has a rich presence of flavonoid and polyphenolic compounds. Some medicinal herbs used in traditional medicine for the treatment of anemia in Cote d'Ivoire revealed that *J. Secunda* had a Fe content of 26.6 mg/100 g of the extract, while the stem bark of *Khaya senegalensis* (Mahogany), a popular haematinic, had 33.3 mg/100 g.[17] Iron forms the nucleus of the iron-porphyrin haeme ring, and together with globin chains forms Hb. This may be responsible for the hematinic properties of *J. Secunda* in mammals.[22] The exact mechanisms by which the extracts exhibited the reported effect need further investigation. Many plant extracts have been found to raise the Hb levels of mice and thus, used for the treatment of anemia.[17] ExpectedLy, there were no significant differences between the MCHC values of the treatment and baseline groups and also those of the standard drugs, since there were corresponding changes in the Hb and PCV values across the different study groups. There were no significant changes in the white blood cell and platelet counts of the treatment and baseline groups in all the extracts employed. It is plausible that *J. Secunda* leaf extracts do not exert thrombocytic and leucocytic effects.

This study was designed to provide scientific evidence for the plausible hemopoietic action of *J. Secunda* leaf by the local populace. We have been able to prove that the plant extracts possess hemopoietic effects as claimed. Similar findings were also found with the leaf and stalk extract of *Beta vulgaris* by Gheith and El-Mahmoudy.[24] Stevens and co-workers have reported that inadequate progress has been made on anemia in women aged 15–49 years, to meet the World Health Assembly global nutrition target to halve the prevalence of anemia by 2030 and that the prevalence of anemia in children had also remained high.[25] If this target of reducing the prevalence of anemia must be met, alternative treatment approaches, like the use of medicinal herbs, would need to be employed; on this premise, *J. Secunda* finds its relevance.

From the findings, the extracts of *J. Secunda* leaf exert hemopoietic action in PHZ-induced anemic mice, by causing an increase in Hb concentration, and thus justifies the use of the plant leaf in the management of anemia. The n-hexane extract showed better hemopoietic activity than the standard haematinics, ferrous sulfate, and vitamin $B_{12}$. These results show that *J. Secunda* could be a good and promising source for pharmaceutical preparations with hemopoietic actions and associated conditions. The limitations to the study were: firstly, the study was carried out with extracts only. Further studies are required to isolate and characterize the active molecules responsible for the actions. Also, the design was an animal study, which does not always give similar results to man. Relevant safety data assays, followed by clinical studies are recommended before the use of the extracts in man.

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