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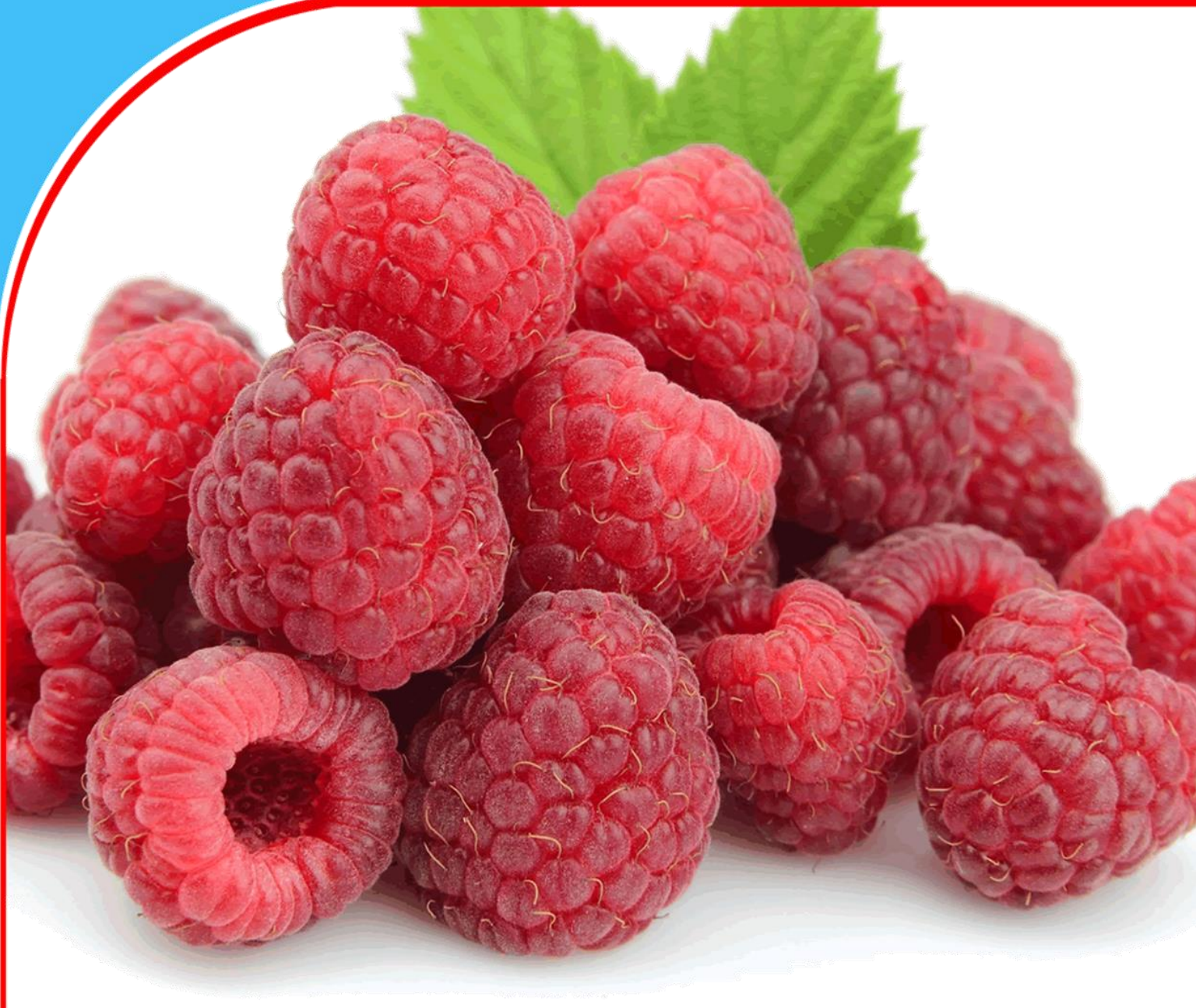

Effect of *Pupalia Lappacea, Iresine Herbstii, Ficus Capensis* and *Morinda Lucida* Leaf Extracts on Phenylhydrazin-Induced Anaemic Adult Male Wistar Albino Rats

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Effect of *Pupalia lappacea, Iresine herbstii, Ficus capensis and Morinda lucida* Leaf Extracts on Phenylhydrazin-Induced Anaemic Adult Male Wistar Albino Rats

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Article History

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Abstract

Purpose: The study evaluated the effect of *Pupalia lappacea, Ipomoea herbstii, Ficus capensis* and *Morinda lucida* leaf extracts on phenylhydrazin-induced anaemic Wistar albino rats.

Materials and Methods: Wild leaves of Pupalia lappacea, Ipomoea *herbstii, Ficus capensis and Morinda lucida* were collected from Egede and Enugu Ngwo communities in Udi Local Government Area, Enugu State, Nigeria. The leaves were cleaned and shade-dried at room temperature for seven days, and milled separately into homogeneous powders of 60 µm using a laboratory hammer mill. Methanol extracts of the powdered samples were prepared by cold maceration. Acute toxicity test (LD_{50}) was conducted to determine safe dosage levels. Adult male Wistar albino rats (n=120) were acclimatized and randomly assigned into five groups of six rats per extract. Anaemia was induced with phenylhydrazine and confirmed before treatment. Based on established safe levels, three doses (10, 100, 1000 mg/kg body weight) were administered orally to Groups B, C, and D, respectively for 14 days. Groups A served as the untreated control, while Group E received a standard anaemia drug (Astymin blood tonic). Blood samples were collected via retro-orbital puncture under ether anesthesia and analyzed for packed cell volume (PCV), red blood cell count (RBC), and hemoglobin (Hb). Data were analyzed using SPSS (version 23), with statistical significance set at $p < 0.05$.

Findings: A significant increase ($p < 0.05$) in PCV, RBC and Hb levels was observed from baseline to end-line in all extract-treated groups. These improvements were comparable to those observed in the group treated with the standard drug.

Unique Contribution to Theory, Practice, and Policy: This study provides evidence supporting the hematopoietic effects *of Pupalia lappacea, Ipomoea herbstii, Ficus capensis,* and *Morinda lucida,* contributing to the understanding of their bioactive properties. Practically, these plants show potential as alternative therapies for anaemia, especially in resource-limited settings. There is need to support further research and regulation to integrate validated herbal treatments into anaemia management strategies.

Keywords: *Anaemia, Heamoglobin, Packed Cell Volume, Leaf Extract, Medicinal Plants*

JEL Codes: *I10, I12, I15, I18*

INTRODUCTION

Anaemia is a major global public health concern, particularly in developing countries where nutritional deficiencies and limited healthcare access contribute to its prevalence. The World Health Organization (WHO, 2021) defines anaemia as a condition characterized by a reduced number of red blood cells or haemoglobin concentration, leading to inadequate oxygen delivery to tissues. Anaemia affects vulnerable populations such as infants, children, adolescents, pregnant women, and the elderly, with global estimates indicating that 40% of children under five and one-third of all women of reproductive age are anaemic. The causes of anemia are multifaceted and include nutritional deficiencies (iron, folate, and vitamin B_{12}), genetic disorders (sickle cell anemia and thalassemia), chronic infections (malaria and tuberculosis), and drug-induced hemolysis.

Medicinal plants have been extensively explored for their potential role in disease prevention and management, including anaemia. Traditional medicine has long utilized plant-derived compounds for their therapeutic benefits, with an estimated 80% of the global population relying on botanical preparations for primary healthcare (Backeberg, 2013). These plants contain bioactive compounds such as flavonoids, tannins, alkaloids, and saponins, (Nweze et al., 2016; Ani & Abel, 2018; Agidew, 2022), which exhibit haematinic properties that may enhance red blood cell production and combat anaemia. Wild green leafy vegetables are known to be nutrient-dense, providing essential macro and micronutrients, antioxidants and phytochemicals. Studies have shown that many underutilized vegetables contain higher concentrations of protein, vitamins, and minerals compared to commonly cultivated varieties such as spinach, lettuce, and cabbage (Aberoumand & Deokule, 2009). In regions with limited access to conventional medicine, these plants provide alternative sources of essential nutrients and medicinal compounds supporting anaemia management and overall health.

Among the wild leafy plants traditionally consumed for their health benefits in Nigeria, *Pupalia lappacea, Iresine herbstii, Ficus capensis,* and *Morinda lucida* are notable for their potential haematinic properties (Nweze et al., 2016). These plants have been widely used in ethnomedicine for managing conditions such as diabetes, cardiovascular diseases, and anaemia (Oji et al., 2020; Ajayi et al., 2017). They are commonly found in forest regions, including Udi district of Enugu State, Nigeria, where local communities have relied on them for generations. Despite their reported health benefits, they remain underexplored due to lack of awareness and scientific validation of their medicinal and nutritional properties (Backeberg, 2013). Pupalia lappacea, commonly known as forest burr, is a plant traditionally used for its antimicrobial and anti-inflammatory properties. It has been reported to contain flavonoids, alkaloids, and tannins (Selvan et al., 2014), which may contribute to its potential role in haemoglobin synthesis and red blood cell production. *Iresine herbstii,* a member of the Amaranthaceae family, is rich in anthocyanins and flavonoids, known for their antioxidative and haematinic effects. *Ficus capensis,* widely known as forest fig has been used in traditional medicine for treating anaemia, owing to its high iron content and bioactive compounds that may support erythropoiesis. *Morinda lucida,* commonly referred to as brimstone tree, has demonstrated antimalarial and antioxidant properties, with evidence suggesting its potential role in improving haematological parameters.

Problem Statement and Study Rationale

Anaemia continues to represent a critical health burden in many low- and middle-income countries, where limited access to effective diagnosis and treatment exacerbates its impact. Despite the known ethnomedicinal use of various wild leafy vegetables in anaemia

management, the scientific basis for their therapeutic claims remains weak or undocumented. *Pupalia lappacea, Iresine herbstii, Ficus capensis,* and *Morinda lucida* are among such plants traditionally used for treating anaemia, yet there is a limited experimental studies demonstrating their efficacy on blood indices in anaemic conditions. Most existing studies have focused on their nutritional or phytochemical properties (Okoroh et al., 2019; Sodimu et al., 2021), with insufficient in vivo validation of their haematinic effects.

This gap in research has important implications, as it limits the development of plant-based therapeutic options that could serve as affordable and accessible alternatives in anaemia management, especially in resource-poor settings. Thus, the rationale for this study is to investigate the effects of methanol extracts from *Pupalia lappacea, Iresine herbstii, Ficus capensis,* and *Morinda lucida* on phenylhydrazine-induced anaemia in adult male Wistar albino rats. Phenylhydrazine is a well-known haemolytic agent that induces anaemia in experimental models, mimicking the pathophysiological conditions observed in human anaemia (Shwetha et al., 2019).

Specifically, the study seeks to answer the following research questions:

- Do the methanol leaf extracts of *Pupalia lappacea, Iresine herbstii, Ficus capensis,* and *Morinda lucida* significantly improve red blood cell count (RBC), haemoglobin (Hb), and packed cell volume (PCV) in phenylhydrazine-induced anaemic rats?
- How do these effects compare to a standard commercial anaemia treatment (Astymin blood tonic)?
- Are there dose-dependent variations in the haematinic responses among the treated groups?

By addressing these questions, the study aims to contribute new knowledge on the functional role of these plants in haematological restoration. It builds upon the ethnobotanical understanding of these species by introducing scientific validation through biochemical and physiological assessments. The results will have practical implications for public health nutrition and herbal medicine by supporting the formulation of plant-based interventions for anaemia, thereby informing future research, policy, and clinical integration of indigenous medicinal plants.

MATERIALS AND METHODS

Study Design

An experimental study design was adopted for this study.

Procurement and Identification of Sample

The leafy vegetables were collected from bushes and fallow lands in the towns of Egede and Enugu Ngwo, located in Udi Local Government Area (LG.A), Enugu State, Nigeria. They were identified at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

Processing of the Samples

Fresh tender leaves of the four vegetables were separately harvested into different bags, plucked and sorted to remove extraneous materials. They were washed thoroughly with clean tap water and allowed to drain for 15 minutes in separate plastic colanders. The leaves were individually shredded using kitchen knives and shade dried at room temperature for seven (7)

days. Each dried sample was separately milled into a homogeneous powder of 60 µm using a laboratory hammer mill (Warburg laboratory blender). The powdered samples of the four leafy vegetables were stored in four (4) different air tight containers till ready for extraction.

Preparation of Methanol Extracts of the Four Leafy Plant Samples

Methanol extracts of the plant samples were separately prepared by cold maceration, as described by Ibeziem et al. (2012) with slight modifications. To prepare the extracts, 2.0 kg of each of the four powdered leaf samples was carefully weighed and divided into four portions of 500 g each. The 500 g portion was transferred into a 2-litre plastic container containing 1 litre methanol. The mixture was stirred with a stainless steel pallet knife to form a semi-solid consistency. The suspension was carefully poured into a 2.5 litre Winchester bottle, which was sealed with a screw cap. The bottle was then firmly clamped and transferred onto an end-over-end mechanical shaker. The shaker was connected to a 240 volt power source, and the bottle was set up for continuous shaking for 24 hours. Shaking was performed intermittently to promote rapid equilibrium, thereby enhancing proper percolation of the menstruum (methanol) into the particle surfaces for exhaustive extraction. The bottles were de-clamped every 8 hours, and the suspension was poured into a muslin cloth and strained to extract the methanol-soluble components into another container. The residue was poured into the Winchester bottle, and more methanol was added. This process continued until the filtrate became colourless. The entire filtrate was then concentrated to a thick syrup-like consistency using a rotary evaporator. Each extract obtained was labelled accordingly, stored in an amber-coloured container with tightly fitting lid, and kept in a refrigerator at 4^0C for further use.

Animal Study

One hundred and twenty (120) adult male albino Wistar rats, weighing between 180 - 200 g were used for the study. The rats were obtained from the animal house of the College of Medicine, University of Nigeria, Enugu Campus. They were housed in sanitized, clean steel-gauze cages and acclimatized for one week under standard temperature condition (25^0C±30^0C) with a 12:12 hour light/dark cycle. The study lasted for 24 days, consisting of 7 days for acclimatization, 1 day for anaemia induction, 2 days for disease establishment, and 14 days of experimental feeding trials with the methanol leafy extracts and standard drugs. After acclimatization, the rats were evenly distributed into five groups of six rats each, consisting of two control groups and three test groups. They were fed with standard rat pellet diet (Guinea Grower's feed, Nigeria Plc.) and water ad libitum. All experimental protocols and animal handling procedures were observed in compliance with the international guidelines for animal experiments as described by McGrath et al. (2010).

Acute toxicity test was conducted to determine the lethal dose (LD_{50}) and establish safe dosage levels for the animal study. The test followed the Up-and-Down Procedure (OECD 425) with slight modifications (OECD, 2008). This method involved sequential dosing, where single mice were administered increasing concentrations of the extracts until signs of toxicity or mortality were observed. The LD_{50} values varied across the four leafy vegetable extracts. Based on the results, doses of 10 mg/kg, 100 mg/kg, and 1000 mg/kg body weight were selected as safe doses for the animal study. These doses were chosen to represent low, moderate, and high exposure levels while remaining within non-lethal limits. The highest dose (1000 mg/kg) was below the estimated LD_{50} values, ensuring that no severe adverse effects were observed in the test animals (OECD, 2008).

Design of the Animal Study

A total of 120 male albino Wistar rats weighing between 180 - 200 g were used for each of the four extracts. The rats were randomly divided and housed into 5 groups (A, B, C, D, and E) of six rats per group and appropriately labelled. Groups A served as the negative control (induced but not treated with extracts or drugs). Groups E served as the positive control (induced and treated with standard anaemia drugs). Groups B, C and D were the test groups, each received an appropriate anaemia induction agent and three different doses of the plant extracts. Based on the LD_{50} test results, graded doses of 10 mg/kg body weight (BW), 100 mg/kg BW and 1000 mg/kg BW of each of the extracts were randomly assigned to groups B, C and D, respectively.

Anaemia was induced according to the method described by Harris & Kuglar (2001). The grouped rats were subcutaneously administered 2.5% neutralized phenylhydrazine hydrochloride (Thermo Fisher Scientific, Waltham, MA, USA) at a dose of 30 mg/kg BW, at six-hour intervals for three consecutive days. After induction, anemia was confirmed by a PCV of ≤ 30 %, a RBC of $\leq 5.0 \times 10^6$ cells/µL and/or a haemoglobin level of ≤ 10 g/dL (Redondo et al., 1995; Criswell et al., 2000).

Administration of the Extracts and Standard Drugs to Experimental Rats

The extract doses were orally administered to Groups B (10 mg/kg BW), C (100 mg/kg BW) and D (1000 mg/kg BW). Group E received a standard drug (Astymin blood tonic- 0.1mg/kg BW) while Group A received no treatment. The administration was performed using intubation tubes once daily for the 14 days feeding trial. The rats were also provided with their normal rat pallet diet and water *ad-libitum.* The effects of the four extracts on heamatological parameters were monitored and compared with those of the standard drug.

Baseline Blood Sample Determination for Anti-Anaemia Study

Baseline blood levels of each of the five rat groups were determined shortly after acclimatization and prior to anaemia induction, and the mean values recorded. Blood samples were collected through retro–orbital puncture under ether anesthesia. Capillary tubes were carefully inserted into the canthus of the eyes to puncture the retro-bulbar plexus to enable outflow of about 2 ml of blood from each rat into heparin plain test tubes with EDTA and another 2 ml into non- heparin containers. The non-heparinized samples were allowed to clot at room temperature (25-37^{0}C) for 30 minutes. Serum samples were collected after centrifugation at 1000 rpm for 10 minutes and immediately subjected to auto-analysis using haematological auto-analyzing machine (Mindray Hematological Analyzer BC 2300 Beacon). All procedures followed the cyano-methaemoglobin method as described by Jain (2020).

Collection and Analysis of the Endline Blood Samples for Anti-Anaemic Study

After an overnight fast (12 hours) on the final day (day 24) of the experimental study, the rats were bled through the retro–orbital puncture under ether anesthesia. The fasting period was necessary to reduce potential variations in haematological parameters that could arise from recent food intake, ensuring more accurate and consistent baseline measurements. Capillary tubes were carefully inserted into the canthus of the eyes to puncture the retro-bulbar plexus to enable outflow of blood into heparin bottles with EDTA. About 4 ml of blood was collected from each group for haematological parameter analysis. The blood samples were thoroughly mixed with EDTA to avoid coagulation and left to stand at room temperature (25-37^{o}C) for 30 minutes.

The clotted blood samples were centrifuged at 1000 revolutions per minute for 10 minutes using a tabletop centrifuge to separate the serum from the clotted blood. The samples were then analyzed immediately after centrifugation using the cyano-methemoglobin method, as described by Jain (2020), with a Mindray hematological auto-analyzer (Home Test Kit Better2Know, UK). The experiment was repeated three times using the same automated hematology analyzer, and the mean values were recorded.

Statistical Analysis

Data from the study were entered into Microsoft excel, cleaned and categorized using standard indicators (cut-off values), before exporting to IBM Statistical Product and Service Solutions (SPSS), version 22 for statistical analysis. To assess the effects of treatment with each of the four leaf extracts on anaemia, Student's t-test was used to compare the mean difference between after induction values and endline (post-treatment values). Statistical significance was set at $P < 0.05$. Results were presented as percentages, means and standard deviations.

FINDINGS

Effects of the Methanol Leafy Extracts of *Pupalia Lappacea, Iresine Herbstii, Ficus Capensis,* and *Morinda Lucida* on Pcv of Phenylhydrazin-Induced Anaemic Rats

Effects of methanol leaf extracts of *Pupalia lappacea, Iresine herbstii, Ficus capensis,* and *Morinda lucida* on PCV of phenylhydrazin-induced anaemic rats are shown in Table 1. Across all groups (A–E), there was a significant decrease in PCV after phenylhydrazine induction, confirming successful anaemia induction. The negative control group (A, induced but not treated) remained anaemic after the treatment phase, with no improvement in PCV. In all four extracts, higher doses (100 mg/kg and 1000 mg/kg) led to a greater increase in PCV post-treatment compared to lower doses (10 mg/kg). The highest dose (1000 mg/kg) of each extract resulted in the most significant improvement in PCV, similar to the standard drug's effect. The standard drug consistently restored PCV close to baseline values, showing the highest percentage increase (109.4–132.2%) across all plant extracts. The T-value measures the statistical significance of PCV recovery between after-induction and after-treatment values. Higher doses (100 mg/kg and 1000 mg/kg) show significant improvements ($P < 0.05$). The F-value and P-value showed that there are significant differences in PCV recovery among the different treatment groups ($P = 0.000$ for all extracts). *Morinda lucida* (1000 mg/kg) had the highest percentage increase (132.2%) in PCV while *Ficus capensis* (1000 mg/kg) showed a 59.5% increase in PCV, which was lower than the other extracts but still effective. *Pupalia lappacea* and *Iresine herbstii* at 1000 mg/kg also significantly improved PCV but were slightly less effective than *Morinda lucida*. At lower doses (10 mg/kg), the PCV increase was minimal, indicating dose-dependent therapeutic effects. All four methanol extracts showed dose-dependent effectiveness in improving PCV in anaemic rats.

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Table 1: Effects of the Methanol Leafy Extracts on Packed Cell Volume of Henylhydrazin-Induced Anaemic Rats

Samples	Groups (n=6 rats per Group)	Baseline (%)	After Induction (%)	After Treatment (%)	Mean Difference between after Induction And after Treatment	% Change between after Induction and after Treatment	T Value between after Induction and after Treatment
Pupalia lappacea	A (induced but not treated)	40.2±1.30	17.6±2.51	16.6±2.41	1.0	5.7↓	0.643
	B(10 mg/kg body weight)	39.4±1.67	22.2±1.79	27.8±1.64	-5.6	25.2↑	5.155***
	C(100 mg/kg body weight	38.8±2.78	24.4±3.36	30.2±3.96	-5.8	23.8↑	-2.496**
	D(1000 mg/kg body weight)	39.6±1.52	22.0±1.87	36.4±1.82	-14.4	65.5↑	-12.348*
	E (Astymin blood tonic-0.1 mg/kg body weight)	41.2±1.64	19.2±1.30	40.2±1.48	-21.0	109.4↑	-23.778***
	F-value			**76.124**			
	P-value			**0.000*** **			
Iresine herbstii	A (induced but not treated)	40.2±1.30	17.6±2.51	16.6±2.41	1.0	5.7↓	0.643
	B (10 mg/kg body weight)	38.2±2.86	24.0±1.58	32.2±2.28	-8.2	34.2↑	-.608***
	C (100 mg/kg body weight)	41.0±1.58	24.4±2.97	36.0±1.58	-11.6	47.5↑	7.716***
	D (1000 mg/kg body weight)	38.2±2.86	20.6±5.03	37.4±1.34	-16.8	81.6↑	7.216***
	E (Astymin blood tonic-0.1 mg/kg body weight)	41.2±1.64	19.2±1.30	40.2±1.48	-21.0	109.4↑	23.778***
	F-value			**118.672**			
	P-value			**0.000*** **			
Ficus capensis	A (induced but not treated)	40.2±1.30	17.6±2.51	16.6±2.41	1.0	5.7↓	0.643
	B (10 mg/kg body weight)	40.6±1.34	25.4±1.67	35.8±1.48	-10.4	40.9↑	10.400***
	C (100 mg/kg body weight)	40.4±1.14	26.8±2.28	39.0±1.58	-12.2	45.5↑	9.831***
	D (1000 mg/kg body weight)	41.0±1.00	24.2±1.48	38.6±2.97	-14.4	59.5↑	9.708***
	E (Astymin blood tonic-0.1 mg/kg body weight)	41.2±1.64	19.2±1.30	40.2±1.48	-21.0	109.4↑	23.778**
	F-value			**105.048**			
	P-value			**0.000*** **			
Morinda lucida	A (induced but not treated)	40.2±1.30	17.6±2.51	16.6±2.41	1.0	5.7↓	0.643
	B (10 mg/kg body weight)	40.6±1.67	19.0±4.00	33.8±2.28	-14.8	77.9↑	7.188***
	C (100 mg/kg bodyweight)	42.0±1.58	22.2±3.03	36.4±3.21	-14.2	64.0↑	7.190***
	D (1000 mg/kg body weight)	39.4±2.19	17.4±2.41	40.4±1.14	-23.0	132.2↑	19.301***

E (Astymin blood tonic- 0.1 mg/kg body weight)	41.2±1.64	19.2±1.30	40.2±1.48	-21.0	109.4↑	23.778***
F-value			**92.755**			
P-value			**0.000*** **			

↓ = Decrease ↑ = Increase *P<0.05 **P<0.01 ***P<0.001

Effects of the Methanol Leaf Extracts from *Pupalia lappacea, Iresine herbstii, Ficus capensis,* and *Morinda lucida* on Red Blood Cell Count of Phenylhydrazin-Induced Anaemic Rats

Table 2 presents the effects of methanol leaf extracts from *Pupalia lappacea, Iresine herbstii, Ficus capensis, and Morinda lucida* on red blood cell (RBC) count of phenylhydrazin-induced anaemic rats. In all groups, RBC counts dropped significantly after phenylhydrazine induction, confirming successful anaemia induction. The untreated groups (Group A) remained anaemic with further decline in RBC count. The RBC counts of the extract-treated groups (Groups B, C and D) increased significantly ($P < 0.05$) in a dose-dependent manner. The 1000 mg/kg BW group (Group D) in all extracts showed the highest increase in RBC count, sometimes even exceeding pre-induction levels while the lowest dose (10 mg/kg) had a lesser but still significant effect. *Morinda lucida* at 1000 mg/kg led to the highest RBC recovery (133.3% increase) while *Pupalia lappacea* had the least effect. All treatments showed highly significant effects ($p < 0.001$).

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Table 2: Effects of the Methanol Leafy Extracts on Red Blood Cell Count of Phenylhydrazin-Induced Anaemic Rats

Samples	Groups (n=6 rats per Group)	Baseline (C/µL)	After Induction (C/µL)	After Treatment (C/µL)	Mean difference between after Induction and after Treatment	% Change between after Induction and after Treatment	T Value between after Induction and after Treatment
Pupalia lappacea	A (induced but not treated)	10.5±0.27	5.9±0.59	4.4±0.16	1.5	25.4↓	5.348**
	B (10 mg/kg body weight)	10.6±0.60	4.7±0.56	7.3±0.57	- 2.6	55.3↑	7.285***
	C (100 mg/kg body weight)	10.6±0.54	5.2±0.60	8.6±0.57	-3.5	67.2↑	9.319***
	D (1000 mg/kg body weight)	10.6±0.36	5.2±0.69	9.5±0.63	-4.3	82.7↑	10.479***
	E (Astymin blood tonic- 0.1 mg/kg body weight)	10.4±0.23	5.9±0.52	9.6±0.38	-3.7	62.7↑	12.664***
	F-value			**112.006**			
	P-value			**0.000*** **			
Iresine herbstii	A (induced but not treated)	10.5±0.27	5.9±0.59	4.4±1.63	1.5	25.4↓	-5.348**
	B (10 mg/kg body weight)	10.4±0.44	5.4±1.05	9.8±0.43	-4.4	81.5↑	8.540***
	C (100 mg/kg body weight)	10.3±0.43	5.0±1.07	9.6±0.63	-4.6	92.0↑	8.357***
	D (1000 mg/kg body weight)	10.0±0.55	5.1±0.89	9.8±0.85	-4.7	92.2↑	8.673***
	E (Astymin blood tonic- 0.1 mg/kg body weight)	10.4±0.23	5.9±0.52	9.6±0.31	-3.7	62.7↑	12.664***
	F-value			**97.034**			
	P-value			**0.000*** **			
Ficus capensis	A (induced but not treated)	10.5±0.27	5.9±0.59	4.4±0.10	1.5	25.4↓	5.693***
	B (10 mg/kg body weight)	11.4±0.48	5.3±0.75	10.4±0.5	-5.1	96.2↑	12.060***
	C (100 mg/kg body weight)	10.7±0.55	5.2±0.68	10.3±0.43	-5.1	98.1↑	14.137***
	D (1000 mg/kg body weight)	10.6±0.40	5.3±0.68	10.5±0.20	-5.2	98.1↑	6.135***
	E (Astymin blood tonic- 0.1 mg/kg body weight)	10.3±0.20	5.9±0.52	9.6±0.38	-3.7	62.7↑	12.664***
	F-value			**203.882**			
	P-value			**0.000*** **			
Morinda lucida	A (induced but not treated)	10.5±0.27	5.9±0.59	4.4±0.16	1.5	25.4↓	5.348**
	B (10 mg/kg body weight)	10.4±0.25	4.8±0.73	8.3±0.49	-3.5	72.9↑	8.745***
	C (100 mg/kg body weight)	10.6±0.07	4.8±0.60	9.7±0.33	-4.9	102.1↑	-5.811***

D (1000 mg/kg body weight	10.5±0.11	4.5±0.21	10.5±0.44	-6	133.3↑	-7.573***
E (Astymin blood tonic- 0.1 mg/kg body weight)	10.4±0.23	5.9±0.52	9.6±0.38	-3.7	62.7↑	-2.664***
F-value			**188.866**			
P-value			**0.000*** **			

↓ = Decrease ↑ = Increase **P<0.01 ***P<0.001 C/µL = counts per microlitre

Effects of Methanol Leaf Extracts from *Pupalia lappacea, Iresine herbstii, Ficus capensis,* and *Morinda lucida* on Haemoglobin Level of Phenylhydrazin-Induced Anaemic Rats

The effects of the methanol leaf extracts of *Pupalia lappacea, Iresine herbstii, Ficus capensis,* and *Morinda lucida* on haemoglobin level of phenylhydrazin-induced anaemic rats are shown in Table 3. The untreated group (A) remained anaemic with continuous decrease in haemoglobin. *Pupalia lappacea* treated groups (B, C, D) exhibited dose-dependent increases in Hb level, with the highest dose (1000 mg/kg, Group D) showing a 93.9% improvement, comparable to the standard drug (E, 98.0%). *Iresine herbstii* treated groups (B, C, D) had remarkable improvements of 78.8%, 106.1% and 102.0% increase, respectively. *Ficus capensis* treatment resulted in Hb increases, with highest increase at 100 mg/kg (C, 71.0%). Interestingly, the 1000 mg/kg dose resulted in a slightly lower increase (65.6%) compared to 100 mg/kg. Standard drug produced the highest increase (98.0%) among this set. The *Morinda lucida* treated groups had significant Hb increase in low, moderate and high doses at 71.4%, 100.0%, and 163.0%, respectively. All treatment groups showed highly significant improvements ($p < 0.001$), except for Group D, which showed a slightly lower significance level ($p < 0.01$).

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Table 3: Effects of the Methanol Leafy Extracts on Haemoglobin Level of Phenylhydrazin-Induced Anaemic Rats

Samples	Groups (n=6 rats per group)	Baseline (g/dL)	After Induction (g/dL)	After Treatment (g/dL)	Mean Difference between after Induction and after Treatment	% Change between after Induction and after Treatment	T value between after Induction and after Treatment
Pupalia lappacea	A (induced but not treated)	10.4±0.36	5.0±0.51	4.5±0.69	0.5	10.0↓	1.313
	B (10 mg/kg body weight)	9.7±0.45	4.6±0.25	7.2±0.74	2.6	56.5↑	7.551***
	C (100 mg/kg body weight)	10.1±0.39	4.7±0.33	8.6±0.49	-3.9	83.0↑	14.418***
	D (1000 mg/kg body weight)	10.4±0.37	4.9±0.55	9.5±0.67	-4.6	93.9↑	11.684***
	E (Astymin blood tonic-0.1 mg/kg body weight)	10.4±0.30	5.0±0.25	9.9±0.62	-4.9	98.0↑	16.049***
	F-value			**68.493**			
	P-value			**0.000*** **			
Iresine herbstii	A (induced but not treated)	10.6±0.36	5.0±0.51	4.5±0.69	0.5	10.0↓	1.313
	B (10 mg/kg body weight)	10.3±0.22	5.2±0.34	9.3±0.35	-4.1	78.8↑	18.805***
	C (100 mg/kg body weight)	10.3±0.53	4.9±0.22	10.1±0.43	-5.2	106.1↑	23.935***
	D (1000 mg/kg body weight)	10.4±0.56	5.1±0.22	10.3±0.51	-5.2	102.0↑	21.066***
	E (Astymin blood tonic-0.1 mg/kg body weight)	10.4±3.00	5.0±0.25	9.9±0.62	-4.9	98.0↑	16.049***
	F-value			**100.747**			
	P-value			**0.000*** **			
Ficus capensis	A (induced but not treated)	10.4±0.36	5.0±0.51	4.5±0.69	0.5	10.0↓	1.313
	B (10 mg/kg body weight)	10.2±0.57	6.3±0.30	9.3±0.53	-3.0	47.6↑	10.896***
	C (100 mg/kg body weight)	10.6±0.40	6.2±0.38	10.6±0.27	-4.4	71.0↑	20.929***
	D (1000 mg/kg body weight)	10.6±0.30	6.1±0.30	10.1±0.27	-4.0	65.6↑	22.249***
	E (Astymin blood tonic-	10.4±0.30	5.0±0.25	9.9±0.62	-4.9	98.0↑	16.049***

	0.1 mg/kg body weight)						
	F-value			**112.967**			
	P-value			**0.000*** **			
Morinda lucida	A (induced but not treated)	10.4±0.36	5.0±0.51	4.5±0.69	0.5	10.0↓	1.313
	B (10 mg/kg body weight)	10.7±0.45	4.9±0.49	8.4±0.33	-3.5	71.4↑	13.516***
	C (100 mg/kg body weight)	10.8±0.51	4.8±0.64	9.6±0.30	-4.8	100.0↑	15.159***
	D (1000 mg/kg body weight)	10.6±0.30	4.6±0.63	12.1±4.06	-7.5	163.0↑	4.047**
	E (Astymin blood tonic-0.1 mg/kg body weight)	10.4±0.30	5.0±0.25	9.9±0.62	-4.9	98.0↑	16.049***
	F value			**11.669**			
	P value			**0.000*** **			

↓ = Decrease ↑ = Increase ***P<0.001 **P<0.01

FINDINGS

The significant reduction in PCV, RBC count, and Hb levels following phenylhydrazine administration confirmed the successful induction of anaemia in all groups. Phenylhydrazine is a well-known haemolytic agent that induces oxidative stress, leading to erythrocyte membrane damage and haemoglobin oxidation, ultimately resulting in haemolysis and anaemia (Grosso et al., 2020; Oyedeji et al., 2021). The untreated control group (A) remained anaemic throughout the study, indicating that spontaneous recovery was minimal within the experimental timeframe. The increase in PCV following treatment with the methanol leaf extracts suggests their potential haematopoietic effects. Khare (2007) reported that *Iresine herbstii*, commonly referred to as a blood booster or tonic in India, is traditionally consumed as an infusion or extract for its nutritional and medicinal properties, with no concerns regarding toxicity. Among the extracts, *Morinda lucida* (1000 mg/kg) exhibited the highest PCV increase (132.2%), comparable to the standard drug treatment. This aligns with previous studies that reported the erythropoietic potential of *Morinda lucida* in anaemic models (Adeoye et al., 2018). The positive effects of *Morinda lucida* leaf extract on PCV levels may be attributed to its abundance of blood-forming nutrients and microminerals such as protein, zinc, cobalt, and folate. These nutrients play vital roles in erythropoiesis for instance, iron and folate support DNA synthesis in erythroid precursors, while zinc and cobalt serve as cofactors for enzymes critical in red cell maturation and haemoglobin synthesis.

Similarly, *Iresine herbstii*, *Pupalia lappacea and Ficus capensis* at high doses also significantly improved PCV, although their effects were slightly lower than *Morinda lucida*. *Ficus capensis* leaves contains 14.60 mg/kg of copper and 29.30 mg/kg of zinc (Uzoekwe & Mohammed, 2015). Mgbemena et al. (2022) also found that the leaves contain 0.55 mg/g of alkaloids and 29.30 mg/kg of zinc, therefore an increased quantity could enhance its phytonutrient profile. Copper and zinc act as cofactors for antioxidant enzymes like superoxide dismutase (SOD), which protects erythrocyte membranes from oxidative damage. Marcin

(2014) noted that copper-zinc SOD plays a crucial role in homeostasis, serving as a cofactor in ceruloplasmin and contributing to antioxidant activities. This protective mechanism likely aids the survival of newly formed red blood cells. *Ficus capensis* showed the lowest PCV increase (59.5%) at the high dose (1000 mg/kg), indicating a relatively weaker effect compared to the other extracts. However, this increase was still statistically significant ($P < 0.001$), suggesting that despite being the least potent among the extracts, it may still confer a clinically meaningful improvement in PCV. The dose-dependent nature of these effects is consistent with findings from Olorunnisola et al. (2016), who reported similar trends in medicinal plant-based anaemia treatments.

The RBC count results further support the haematopoietic potential of these extracts. The 1000 mg/kg dose resulted in the highest RBC count increase, with *Morinda lucida* showing the most pronounced effect (133.3%). Interestingly, the percentage changes in red blood cell (RBC) counts for the three doses of *Pupalia lappacea* extract (55.3%, 67.2%, and 82.7%) exceeded that of the standard drug (62.7%). This may be due to the phytochemical composition of the extract, which includes flavonoids, alkaloids, and saponins. Flavonoids exhibit antioxidant properties that reduce oxidative stress in the bone marrow microenvironment, thereby supporting the proliferation and differentiation of erythroid progenitor cells. They also modulate cytokine signaling pathways such as erythropoietin (EPO) signaling by stabilizing hypoxia-inducible factor-1 alpha (HIF-1α), thereby promoting endogenous EPO production (Zhang et al., 2022; Kaplan, 2018). Alkaloids are known to stimulate bone marrow function by enhancing DNA and RNA synthesis in hematopoietic stem cells, leading to increased erythropoiesis (Zhang et al., 2020). Saponins can increase membrane permeability, which improves iron absorption and bioavailability. This is an essential factor for haemoglobin synthesis (Bissinger et al., 2014). These compounds, individually and synergistically, contribute to red cell regeneration and anaemia recovery (Ukwuani et al., 2019).

Celine et al. (2012) emphasized that the biochemical compounds in *Pupalia lappacea* promote healing processes in both animals and humans, with specific phytochemicals such as stigmasterol exhibiting haemostatic and anti-inflammatory activities. This finding is in line with earlier studies by Ajayi et al. (2017), which demonstrated the RBC-regenerating effects of *Morinda lucida* in experimental anaemia models. *Morinda lucida* produced the highest increase in hemoglobin levels (163.0% at 1000 mg/kg), significantly outperforming the standard drug (98.0%, $p < 0.01$). This supports the strong haematinic potential of *Morinda lucida*. The high haemoglobin restoration aligns with the findings of Anowi et al. (2022), who reported the ability of *Morinda lucida* to enhance erythropoiesis in anaemic conditions. This superior effect may be attributed to its high content of blood-forming vitamins and minerals such as vitamin B2, folate, iron, and zinc (Osuntokun et al., 2016). These micronutrients directly contribute to the synthesis of haem by serving as cofactors for enzymes such as aminolevulinic acid synthase and ferrochelatase, which are involved in the haem biosynthetic pathway.

Various parts of *Ficus capensis* have been documented for their medicinal properties. Its leaves and roots are traditionally used as blood tonics and anti-rheumatic agents, and for reducing fever and treating tuberculosis. Interestingly, *Ficus capensis* showed a slight reduction in Hb at 1000 mg/kg compared to 100 mg/kg, indicating a possible saturation effect or homeostatic regulation. This observation warrants further investigation into optimal dosage thresholds for maximum efficacy. The dose-dependent increase in Hb across all extracts is in agreement with

prior studies highlighting the role of phytochemicals in haemoglobin synthesis and erythropoiesis (Ezekwesili et al., 2020).

CONCLUSION AND RECOMMENDATIONS

Conclusion

The findings of this study suggest that these medicinal plants have significant erythropoietic activity and may serve as natural alternatives for managing anaemia, particularly in resource-limited settings where conventional therapies may be limited. To build on these findings, we recommend comprehensive phytochemical investigations to isolate and identify the bioactive compounds responsible for the haematopoietic effects. Specific methodologies such as liquid chromatography-mass spectrometry, gas chromatography-mass spectrometry, and high-performance liquid chromatography, in combination with bioassay-guided fractionation, would be suitable for characterizing the active constituents. In vitro bioassays, such as erythropoietin stimulation assays using bone marrow-derived stem cells or HepG2 cell lines, could help elucidate potential mechanisms of action.

Recommendation

Given the strong haematopoietic effect observed with *Morinda lucida*, we recommend that future studies explore its safety and efficacy through toxicity assessments and pilot clinical trials in humans. Such investigations would be essential to determine its potential for clinical application in anaemia management.

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Conflicts of Interest Declaration

The authors declare no conflict of interest.

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