



STANDARDIZED METHOD FOR EVALUATING PLANT EXTRACTS FOR ANEMIA TREATMENT IN PUBLIC HEALTH NUTRITION: USING A RAT MODEL OF CHEMICALLY-INDUCED ANEMIA

**HASSAN ABDULSALAM ADEWUYI¹; TIMILEYIN
JOSHUA OLUWADEPO²; JESS CEGBEYI
ZEPHANIA³; AYODEJI IBRAHEEM⁴; ABIOLA
CLEMENTINA AJIBOLA⁵; ALI KINGSLEY
CHIJIJOKE⁶; ADENIYI OLUSEGUN OYEYEMI⁷; &
TAIWO MATHIAS ALADESANMI⁸**

¹Department of Biochemistry, Federal University of Technology, Minna, PMB 65, Niger State, Nigeria.

²Department of Public Health, Texila American University, Georgetown, Guyana. ³Berith Specialist Hospital Suleja, Niger State, Nigeria. ⁴Department of Public Health, EHESP-Ecole des hautes etudes en santé publique. ⁵Center for Integrated Programs, FCT Abuja.

⁶Department of Biochemistry, University of Nigeria, Nsukka. ⁷Disease Prevention and Monitoring Department, eHealth Africa. ⁸Department of Human Nutrition, University of Ibadan, Nigeria

Corresponding Author:

timileyinoluwadepo@gmail.com

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Introduction

Anemia continues to rank among the most widespread nutritional disorders worldwide, posing a persistent challenge to global public health. It is estimated that over 1.6 billion people are affected globally, with the burden falling disproportionately on women of reproductive age, children, and populations in low- and middle-income countries (Chaparro and Suchdev, 2019). The condition is not merely a laboratory abnormality but a multifaceted health problem with far-reaching consequences. According to the World Health Organization, anemia is associated with impaired physical growth, delayed cognitive development, reduced immune competence, diminished work capacity, and increased risk of adverse pregnancy outcomes, including maternal and perinatal mortality (WHO, 2017). These impacts collectively undermine individual well-being and national socioeconomic

Abstract

Anemia remains a major global public health concern, underscoring the need for innovative nutritional and therapeutic strategies. Indigenous food plants such as *Carica papaya* (pawpaw) have attracted attention as potential remedies; however, their claimed benefits require rigorous scientific confirmation through well-designed experimental models prior to clinical consideration. This study was designed to formulate and demonstrate a validated, two-stage experimental framework for the systematic assessment of plant-based anti-anemic activity, with *C. papaya* leaf and seed extracts used as a representative model. Anemia was experimentally induced in Wistar rats using aluminum chloride (AlCl_3) administered orally at 0.5 mg/kg daily for 14 days. The evaluation protocol consisted of two consecutive

phases. In Stage 1, crude

Keywords: Nutritional anemia, Animal model validation, Public health nutrition, Traditional food plants, *Carica papaya*, Hematological screening, Preclinical evaluation.

methanol extracts were screened at graded doses of 100, 300, and 500 mg/kg. Stage 2 focused on the most active extract identified in Stage 1, from which alkaloid and flavonoid fractions were isolated and tested at 75 and 150 mg/kg. Key hematological indices red blood cell count (RBC), hemoglobin concentration (Hb), packed cell volume (PCV), and full blood count were assessed throughout the 14-day treatment period. Administration of AlCl_3 produced marked anemia, evidenced by a decline in RBC from $7.15 \times 10^{12}/\text{L}$ and a reduction in Hb from 13.65 to 8.55 g/dL in untreated

anemic controls. During Stage 1, methanol extracts of *C. papaya* leaves at 300 and 500 mg/kg demonstrated the strongest hematological recovery, significantly improving RBC, Hb, and PCV values toward normal ranges. In Stage 2, the leaf-derived alkaloid fraction at 150 mg/kg emerged as the most potent intervention, surpassing flavonoid fractions and exhibiting a clear dose-responsive effect. Overall, this work introduces a reliable and reproducible two-stage experimental approach for screening and characterizing plant-derived anti-anemic agents. The results highlight *C. papaya* leaf methanol extract, particularly its alkaloid fraction, as a promising candidate for anemia management and provide a standardized methodological framework for future studies in this area.

productivity. Iron supplementation and food fortification programs have long served as the cornerstone of anemia control strategies. While these approaches have demonstrated benefits, they are not without notable limitations. Oral iron therapy is often associated with poor absorption, low compliance due to gastrointestinal discomfort, and limited effectiveness in cases where anemia arises from causes other than iron deficiency, such as chronic inflammation, toxic exposure, or impaired erythropoiesis (Tolkien et al.,

2015). These challenges highlight the need to broaden anemia management strategies beyond conventional supplementation and to explore sustainable, food-based alternatives that can be locally sourced, culturally acceptable, and biologically effective.

Traditional food plants offer considerable promise in this regard, particularly in resource-constrained settings where access to pharmaceutical interventions may be limited. Across many cultures, plant-based remedies have been used for generations to manage blood-related disorders, often based on empirical knowledge passed down through practice (Yadav et al., 2017). Among such plants, *Carica papaya* (pawpaw) occupies a prominent place in traditional medicine and household nutrition. Its leaves and seeds have been used for diverse health purposes, including the management of fatigue, infections, and blood disorders (Saeed et al., 2014). Despite widespread traditional use, the scientific evidence supporting its anti-anemic potential remains fragmented, underscoring the importance of structured experimental validation.

Recent methodological advancements (2020–2024) emphasize the need for standardized, multi-stage screening protocols to bridge ethnopharmacological knowledge and evidence-based validation, moving beyond descriptive studies to mechanistic and fraction-based analyses (Heinrich et al., 2020). Contemporary preclinical studies on plant-based interventions for anemia underscore this need, demonstrating efficacy while often highlighting the remaining gap between crude extract testing and the identification of active principles (Biswas et al., 2021; Eze et al., 2023).

Preclinical studies using well-defined animal models are an essential step in translating traditional claims into evidence-based nutritional or therapeutic recommendations. Reliable animal models allow for controlled investigation of disease mechanisms and treatment responses before progressing to human studies. Chemical induction of anemia provides a reproducible and mechanistically informative approach for such evaluations (Mahieu et al., 2000). Aluminum chloride (AlCl_3) is a well-recognized hematotoxic agent that induces anemia through several interrelated pathways, including oxidative damage to red blood cells, interference with iron metabolism, and suppression of bone marrow erythropoietic activity (Gómez et al., 1997; Yousef, 2004). This complex mode of action closely reflects real-world pathological processes, making AlCl_3 -induced anemia a relevant model for assessing plant-derived interventions with antioxidant, hematinic, or erythropoiesis-stimulating properties.

One of the persistent methodological challenges in plant-based nutrition research is the difficulty of linking observed biological effects to specific bioactive constituents. Many studies rely solely on crude extracts, which can obscure the contributions of individual phytochemical classes and limit reproducibility (Heinrich et al., 2018). Without a structured strategy, promising findings may remain inconclusive or fail to progress toward practical application. To address this gap, the present study introduces a sequential, two-stage *in vivo* evaluation protocol tailored to public health nutrition research. This approach enables initial screening of crude plant extracts, followed by targeted assessment of purified phytochemical fractions responsible for observed anti-anemic effects. By integrating traditional knowledge with systematic experimental

design, this framework provides a clear and efficient pathway for identifying and validating plant-based interventions for anemia management.

MATERIALS AND METHODS

Experimental Site

The study was carried out at the Animal House Facility of the Department of Biochemistry, Federal University of Technology, Minna, Nigeria. All experimental procedures and laboratory analyses were conducted within the department's research laboratory, which is equipped for animal handling and hematological investigations.

Ethical Approval and Animal Care

Ethical clearance for this study was obtained from the Institutional Animal Ethics Committee of the Federal University of Technology, Minna (*Reference: FUTMin/IAEC/2023/BC-012*). All experimental procedures complied strictly with internationally accepted guidelines for the care and use of laboratory animals as outlined by the National Research Council (2011). A total of seventy young adult Wistar rats weighing between 125 and 160 g were sourced from the National Veterinary Research Institute, Vom, Nigeria. The animals were allowed to acclimatize for a period of two weeks under standard laboratory conditions, including a temperature of $22 \pm 3^{\circ}\text{C}$, relative humidity of $50 \pm 5\%$, and a 12-hour light and 12-hour dark cycle. Throughout the acclimatization and experimental periods, the rats had unrestricted access to standard pelletized feed and clean drinking water.

Preparation of Plant Materials

Fresh leaves and seeds of *Carica papaya* were prepared following previously established procedures (Hassan et al., 2024). The plant materials were properly identified and authenticated before processing. After washing to remove surface contaminants, the samples were air-dried under shade to preserve heat-sensitive constituents and subsequently ground into fine powder. The powdered materials were subjected to methanol extraction to obtain crude extracts. Methanol was selected as the extraction solvent due to its polarity, which allows for efficient extraction of a broad spectrum of medium- to high-polarity phytoconstituents, including alkaloids, flavonoids, and phenolics, which are often associated with bioactive properties. Portions of the crude extracts were then further processed using standard fractionation techniques to isolate alkaloid and flavonoid fractions for subsequent biological evaluation.

Induction of Experimental Anemia

Experimental anemia was induced using aluminum chloride hexahydrate ($\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$). The compound was freshly prepared each day in distilled water and administered orally at a dose of 0.5 mg/kg body weight. The induction protocol lasted for 14 consecutive days. Treatment with plant extracts, fractions, or reference drug commenced 10 minutes after AlCl_3 administration each day, a timing chosen to simulate a therapeutic intervention rather than a prophylactic effect.

Experimental Design: Two-Stage Evaluation Protocol

Stage 1: Screening of Crude Extracts

In the first phase of the study, thirty-five rats were randomly assigned into nine experimental groups, with five animals per group. Group 1 served as the normal control and received distilled water only. Group 2 functioned as the anemic control and received AlCl_3 without treatment. Group 3 served as the positive control and was treated with AlCl_3 followed by ferrous sulfate at a dose of 3 mg/kg. Groups 4, 5, and 6 received AlCl_3 in combination with *C. papaya* leaf methanol extract at doses of 100, 300, and 500 mg/kg, respectively. Groups 7, 8, and 9 were treated with AlCl_3 alongside *C. papaya* seed methanol extract at corresponding doses of 100, 300, and 500 mg/kg.

The dosage range of 100, 300, and 500 mg/kg for crude extracts was selected based on common effective ranges reported in prior preclinical phytopharmacology studies for similar plant materials, allowing for the observation of potential dose-dependent effects.

Stage 2: Evaluation of Bioactive Fractions

Based on the outcomes of the crude extract screening, a second phase was conducted to assess the activity of specific phytochemical fractions. A fresh set of thirty-five rats was used and distributed into eleven groups, with three to five animals per group depending on availability. The first three groups served as the normal, anemic, and positive controls, identical to those in Stage 1. Groups 4 and 5 received AlCl_3 along with the seed alkaloid fraction at doses of 75 and 150 mg/kg, respectively, while Groups 6 and 7 were administered the seed flavonoid fraction at the same dose levels. Groups 8 and 9 received the leaf-derived alkaloid fraction at 75 and 150 mg/kg, whereas Groups 10 and 11 were treated with the leaf flavonoid fraction at identical doses.

Monitoring and Hematological Assessment

Body weight measurements were taken on days 0, 3, 7, 10, and 14 of the experimental period to monitor general health and treatment-related effects. Animals were observed daily for signs of distress, changes in feeding behavior, or abnormal physical activity. On day 15, all rats were humanely sacrificed under light ether anesthesia. Blood samples were collected via cardiac puncture into ethylenediaminetetraacetic acid (EDTA) tubes and immediately analyzed for hematological parameters using an automated hematology analyzer (Sysmex KX-21N).

Statistical Analysis

All data were expressed as mean $\pm$ standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA), followed by Tukey's post-hoc test to identify differences between groups. Analyses were conducted using GraphPad Prism software Version 21 GraphPad Software, San Diego, CA, USA, and statistical significance was accepted at $p < 0.05$.

RESULTS

Validation of the Anemia Model

Administration of aluminum chloride produced clear and consistent hematological alterations characteristic of anemia. Rats in the untreated anemic group exhibited pronounced declines in all major erythrocyte indices when compared with the normal control group. Red blood cell count fell by 29.4%, decreasing from $7.15 \times 10^{12}/L$, while hemoglobin concentration dropped by 37.4%, from 13.65 to 8.55 g/dL. A similar trend was observed for packed cell volume, which was reduced by 38.4%, declining from 43.0% in healthy controls to 26.5% in anemic animals. These substantial reductions confirmed the successful establishment of experimental anemia. In contrast, rats treated with ferrous sulfate showed marked recovery across all measured parameters, thereby confirming both the reliability of the model and its sensitivity to a standard anti-anemic intervention.

Effect of Crude Extracts: Stage 1 Evaluation

Screening of the crude methanol extracts revealed a clear difference in efficacy between plant parts. The leaf extract exhibited a stronger anti-anemic effect than the seed extract across the tested dose range. At doses of 300 and 500 mg/kg, the leaf extract significantly ($p < 0.05$) improved red blood cell counts to 6.45 and $6.82 \times 10^{12}/L$, respectively, compared with $5.05 \times 10^{12}/L$ in the untreated anemic group (Figures 1A). Correspondingly, hemoglobin levels increased to 11.8 g/dL and 12.3 g/dL at these doses (Figure 1B), approaching the value observed in the normal control group (13.65 g/dL) (Figure 1C). Improvements in packed cell volume followed a similar pattern. In contrast, the seed extract demonstrated only modest activity, with noticeable improvement observed primarily at the highest dose of 500 mg/kg, and even then, the magnitude of response was less pronounced than that of the leaf extract.

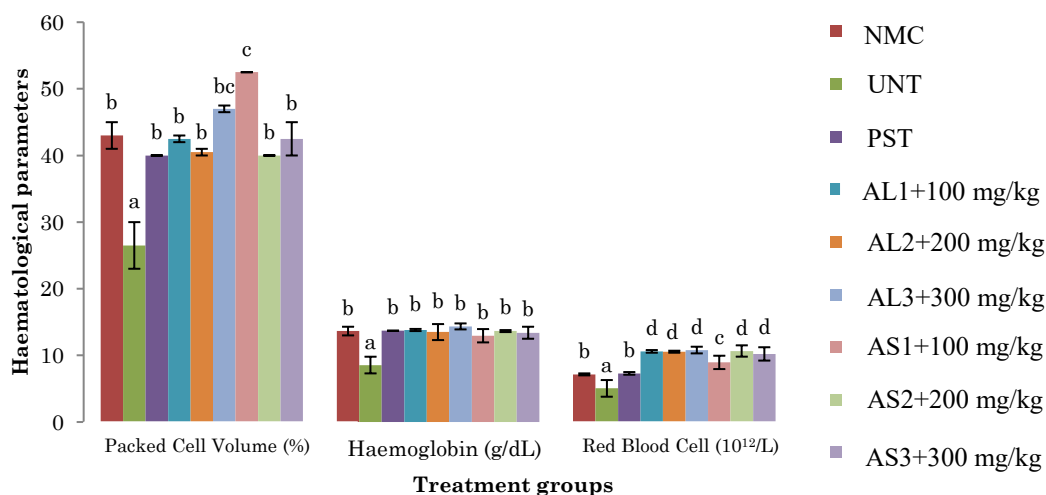


Figure 1A. Effect of Leaves and Seeds Extractsof *C. papaya* on Packed Cell Volume (PCV), Haemoglobin (Hgb) and Red Blood Cells (RBC) in $AlCl_3$ -induced Anaemic Rats

Values are presented in Mean $\pm$ Standard Error of three replicate determinations. Values with different alphabets on the bars are significantly different ($p < 0.05$).

Keys;

NMC: Normal control rats received 1 mL distilled water; UNT; Untreated rats received 0.5 mg/kg bw AlCl_3 and were not treated; PST; Positive standard group of rats received 0.5 mg/kg bw AlCl_3 and treated with 3 mg/kg bw FeSO_4 ; AL1; Group of rats received 0.5 mg/kg bw AlCl_3 and treated with 100 mg/kg bw *C.papaya* leaves extract; AL2; Group of rats received 0.5 mg/kg bw AlCl_3 and treated with 200 mg/kg bw *C.papaya* leaves extract; AL3; Group of rats received 0.5 mg/kg bw AlCl_3 and treated with 300 mg/kg bw *C.papaya* leaves extract; ACL1; Group of rats received 0.5 mg/kg bw AlCl_3 and treated with 100 mg/kg bw *C.papaya* seeds extract; ACL2; Group of rats received 0.5 mg/kg bw AlCl_3 and treated with 200 mg/kg bw *C.papaya* seeds extract; ACL3; Group of rats received 0.5 mg/kg bw AlCl_3 and treated with 300 mg/kg bw *C.papaya* seeds extract.

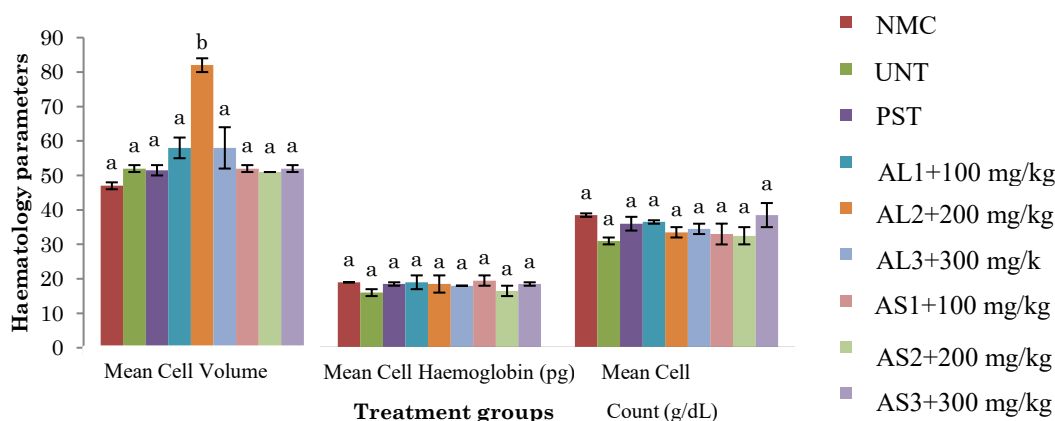


Figure 2A. Effect of Leaves and Seeds Extracts of *C. papaya* on Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), and Mean Cell Haemoglobin Counts (MCHC) in AlCl_3 -induced Anaemic Rats

Values are presented in Mean $\pm$ Standard Error of three replicate determinations. Values with different alphabets on the bars are significantly different ($p < 0.05$).

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NMC: Normal control rats received 1 mL distilled water; UNT; Untreated rats received 0.5 mg/kg bw AlCl_3 and were not treated; PST; Positive standard group of rats received 0.5 mg/kg bw AlCl_3 and treated with 3 mg/kg bw FeSO_4 ; AL1; Group of rats received 0.5 mg/kg bw AlCl_3 and treated with 100 mg/kg bw *C.papaya* leaves extract; AL2; Group of rats received 0.5 mg/kg bw AlCl_3 and treated with 200 mg/kg bw *C.papaya* leaves extract; AL3; Group of rats received 0.5 mg/kg bw AlCl_3 and treated with 300 mg/kg bw *C.papaya* leaves extract; ACL1; Group of rats received 0.5 mg/kg bw AlCl_3 and treated with 100 mg/kg bw *C.papaya* seeds extract; ACL2; Group of rats received 0.5 mg/kg bw AlCl_3 and treated with 200 mg/kg bw *C.papaya* seeds extract; ACL3; Group of rats received 0.5 mg/kg bw AlCl_3 and treated with 300 mg/kg bw *C.papaya* seeds extract.

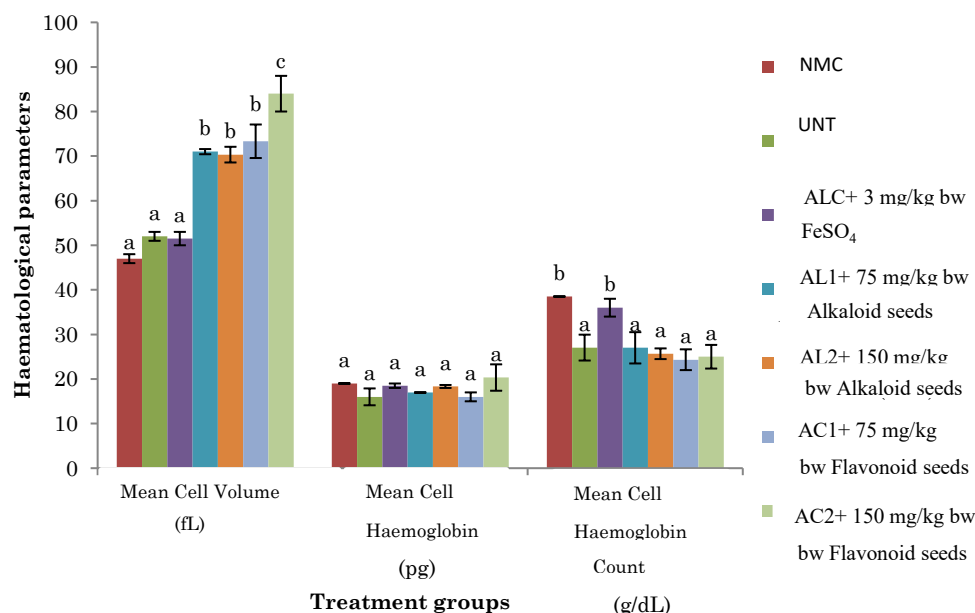


Figure 2C. Effect of Alkaloid and Flavonoid Fractions of *C. papaya* Seeds Extracts on Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), and Mean Cell Haemoglobin Counts (MCHC) in AlCl₃-induced Anaemic Rats

Values are presented in Mean $\pm$ Standard Error of three replicate determinations. Values with different alphabets on the bars are significantly different ($p < 0.05$).

Keys;

NMC: Normal control rats received 1 mL distilled water; UNT; Group of rats that received 0.5 mg/kg bw AlCl₃ and were not treated; ALC; Positive standard group of rats that received 0.5 mg/kg bw AlCl₃ and treated with 3 mg/kg bw FeSO₄; AL1; Group of rats that received 0.5 mg/kg bw AlCl₃ and treated with 75 mg/kg bw alkaloid seeds extract; AL2; Group of rats that received 0.5 mg/kg bw AlCl₃ and treated with 150 mg/kg bw alkaloid seeds extract; AC1; Group of rats that received 0.5 mg/kg bw AlCl₃ and treated with 75 mg/kg bw flavonoid seeds extract; AC2; Group of rats that received 0.5 mg/kg bw AlCl₃ and treated with 150 mg/kg bw flavonoid seedsextract.

Effects of Purified Phytochemical Fractions: Stage 2 Evaluation

Further fractionation of the most active extract revealed that the alkaloid fraction derived from *C. papaya* leaves was the principal contributor to the observed anti-anemic activity. At a dose of 150 mg/kg, this fraction produced the most pronounced restoration of hematological indices, with red blood cell count rising to $6.95 \times 10^{12}/L$ (Figure 1A), hemoglobin concentration reaching 12.8 g/dL (Figure 1B and 1C), and packed cell volume increasing to 40.2%. These values not only exceeded those obtained with the crude leaf extract but were also comparable to the effects observed in the ferrous sulfate-treated group. The flavonoid fraction from the leaves produced moderate improvements, whereas

both alkaloid and flavonoid fractions obtained from the seeds showed minimal corrective effects on anemia-related parameters.

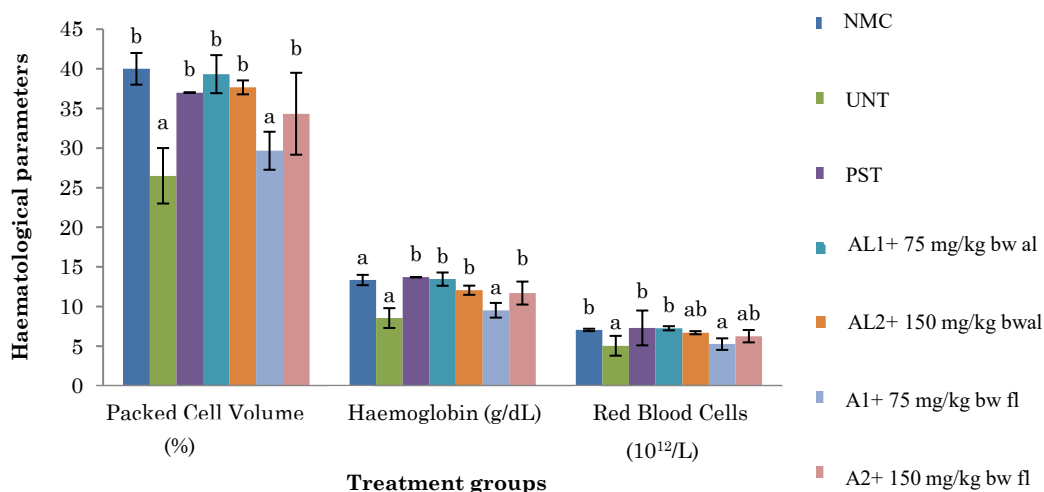


Figure 1B. Effect of Alkaloid and Flavonoid Fractions of *C. papaya* Leaves on Packed Cell Volume (PCV), Haemoglobin (Hgb) and Red Blood Cells (RBC) in AlCl₃-induced Anaemic Rats

Values are presented in Mean $\pm$ Standard Error of three replicate determinations. Values with different alphabets on the bars are significantly different ($p < 0.05$).

Keys;

NMC: Normal control rats received 1 mL distilled water; UNT; Group of rats that received 0.5 mg/kg bw AlCl₃ and were not treated; ALC; Positive standard group of rats that recieved 0.5 mg/kg bw AlCl₃ and treated with 3 mg/kg bw FeSO₄; AL1; Group of rats that recieved 0.5 mg/kg bw AlCl₃ and treated with 75 mg/kg bw alkaloid leaves extract; AL2; Group of rats that recieved 0.5 mg/kg bw AlCl₃ and treated with 150 mg/kg bw alkaloid leaves extract; AC1; Group of rats that recieved 0.5 mg/kg bw AlCl₃ and treated with 75 mg/kg bw flavonoid leaves extract; AC2; Group of rats that recieved 0.5 mg/kg bw AlCl₃ and treated with 150 mg/kg bw flavonoid leaves extract.

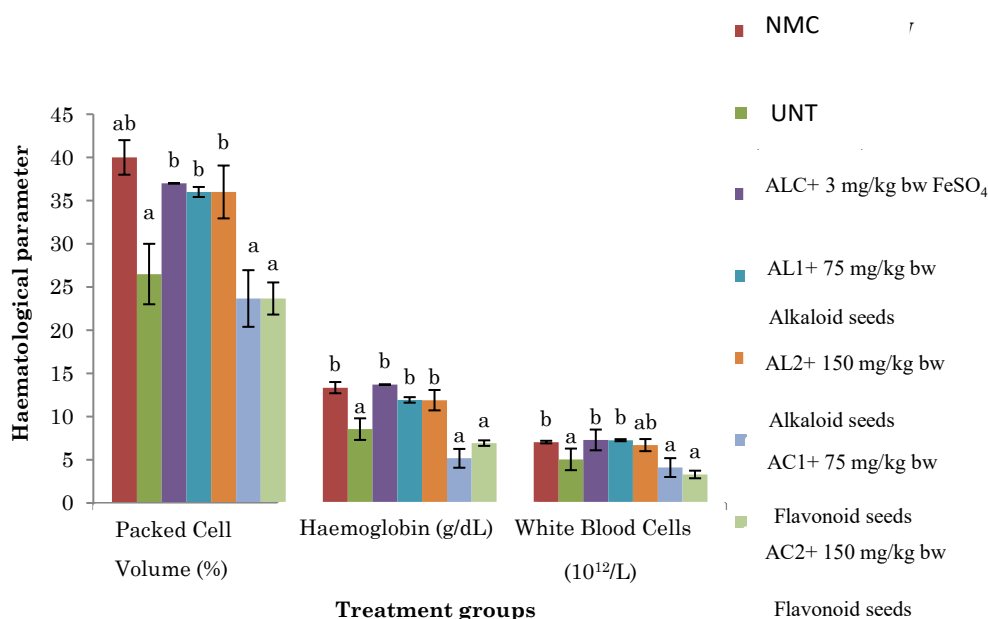


Figure 2B. Effect of Alkaloid and Flavonoid of *C. papaya* Seeds Extract on Red Blood Cells (RBC), Haemoglobin (Hgb) and White Blood Cells (WBC) in AlCl₃-induced Anaemic Rats

Values are presented in Mean $\pm$ Standard Error of three replicate determinations. Values with different alphabets on the bars are significantly different ($p < 0.05$).

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NMC: Normal control rats received 1 mL distilled water; UNT; Group of rats that received 0.5 mg/kg bw AlCl₃ and were not treated; ALC; Positive standard group of rats that received 0.5 mg/kg bw AlCl₃ and treated with 3 mg/kg bw FeSO₄; AL1; Group of rats that received 0.5 mg/kg bw AlCl₃ and treated with 75 mg/kg bw alkaloid seeds extract; AL2; Group of rats that received 0.5 mg/kg bw AlCl₃ and treated with 150 mg/kg bw alkaloid seeds extract; AC1; Group of rats that received 0.5 mg/kg bw AlCl₃ and treated with 75 mg/kg bw flavonoid seeds extract; AC2; Group of rats that received 0.5 mg/kg bw AlCl₃ and treated with 150 mg/kg bw flavonoid seedsextract.

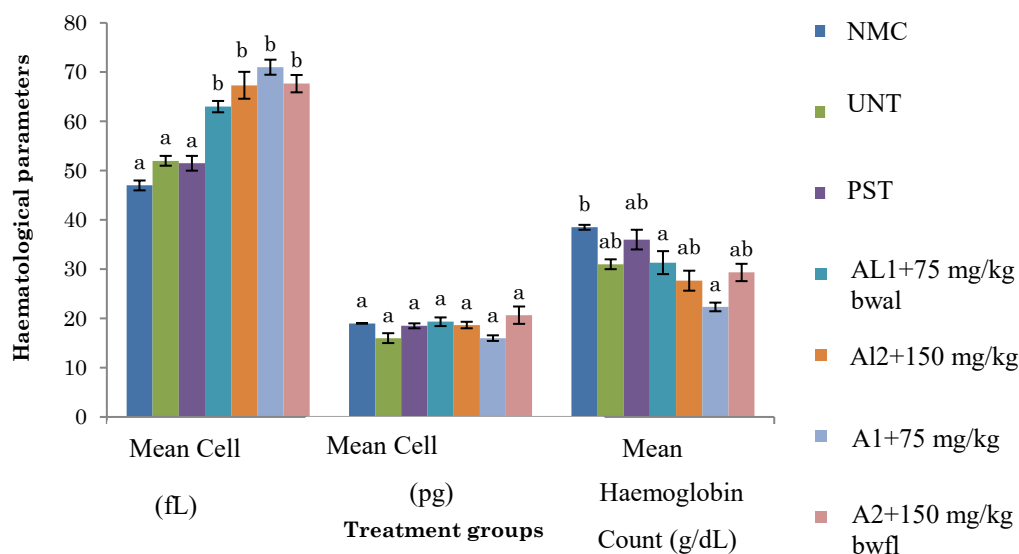


Figure 2C. Effect of Alkaloid and Flavonoid Fractions of *C. papaya* Leaves Extracts on Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), and Mean Cell Haemoglobin Counts (MCHC) in AlCl_3 -induced Anaemic Rats

Values are presented as mean $\pm$ Standard Error of three replicate determinations. Values with different alphabets on the bars are significantly different ($p < 0.05$)

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NMC: Normal control rats received 1 mL distilled water; UNT; Group of rats that received 0.5 mg/kg bw AlCl_3 and were not treated; ALC; Positive standard group of rats that received 0.5 mg/kg bw AlCl_3 and treated with 3 mg/kg bw FeSO_4 ; AL1; Group of rats that received 0.5 mg/kg bw AlCl_3 and treated with 75 mg/kg bw alkaloid leaves extract; AL2; Group of rats that received 0.5 mg/kg bw AlCl_3 and treated with 150 mg/kg bw alkaloid leaves extract; AC1; Group of rats that received 0.5 mg/kg bw AlCl_3 and treated with 75 mg/kg bw flavonoid leaves extract; AC2; Group of rats that received 0.5 mg/kg bw AlCl_3 and treated with 150 mg/kg bw flavonoid leaves extract.

Extended Hematological Findings

In addition to improvements in primary erythrocyte indices, treatment with the effective extracts and fractions influenced other components of the hematological profile. Aluminum chloride exposure resulted in a reduction in white blood cell counts, indicative of leukopenia, which was partially or fully reversed by the more active treatments. Analysis of red cell indices, including mean corpuscular volume, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration, suggested a predominantly normocytic anemia pattern that normalized following treatment. Platelet counts remained within physiological ranges across all experimental groups, indicating that neither the induction protocol nor the plant treatments adversely affected thrombopoiesis.

DISCUSSION

The present study provides a structured and scientifically grounded approach for assessing plant-based interventions for anemia, a condition that continues to pose a major challenge in public health nutrition. By adopting a two-stage experimental strategy, this work addresses a common gap in phytomedicine research: the disconnect between traditional use of medicinal plants and evidence-based validation. The stepwise progression from crude extract screening to targeted evaluation of bioactive fractions offers a practical and reproducible framework for identifying effective plant-derived anti-anemic agents.

The aluminum chloride-induced anemia model employed in this study proved to be both reliable and relevant. It produced consistent and marked hematological alterations that resemble important features of anemia observed in humans. The multifactorial nature of $AlCl_3$ toxicity, including oxidative stress-mediated erythrocyte damage, disruption of iron homeostasis, and suppression of bone marrow erythropoiesis, makes this model particularly suitable for evaluating plant extracts with complex and potentially complementary mechanisms of action. Given that most medicinal plants contain multiple phytochemicals, this model allows for a realistic assessment of their therapeutic potential. The superior activity of the leaf-derived alkaloid fraction invites mechanistic speculation. Alkaloids from various medicinal plants have been reported to stimulate erythropoiesis by modulating erythropoietin production or enhancing the responsiveness of erythroid progenitor cells in the bone marrow. Furthermore, their recognized antioxidant properties could protect developing and circulating erythrocytes from oxidative damage induced by aluminum toxicity, thereby reducing hemolysis and improving RBC survival. Future studies focusing on bone marrow histopathology, serum erythropoietin levels, and antioxidant enzyme activities are warranted to elucidate the precise pathways involved. A key finding of this study was the superior anti-anemic activity of *Carica papaya* leaf extract compared with the seed extract. This observation aligns with ethnobotanical practices in several communities where papaya leaves, rather than seeds, are traditionally used to manage blood-related disorders. Beyond confirming traditional claims, the study advances current knowledge by identifying the alkaloid fraction of the leaf extract as the principal contributor to the observed hematological improvements. This result is particularly important, as it shifts the focus from the use of crude preparations to the possibility of developing more refined, alkaloid-enriched formulations with improved consistency and efficacy.

From a public health nutrition standpoint, these findings carry important implications. First, they provide scientific backing for the traditional use of papaya leaves in anemia management, supporting their potential inclusion in food-based or nutraceutical interventions. Second, the marked differences observed between plant parts highlight the necessity of systematic evaluation rather than assuming uniform therapeutic effects across all components of a plant. Third, the identification of specific active fractions creates opportunities for standardization and quality control, which are essential for translating experimental findings into safe and effective community-level interventions.

The pronounced restoration of red blood cell count, hemoglobin concentration, and packed cell volume observed with the leaf alkaloid fraction is particularly noteworthy. The near-normalization of these parameters suggests an efficacy comparable to conventional iron therapy, although the underlying mechanisms may differ. This is especially relevant for forms of anemia that are not solely attributable to iron deficiency, as well as for individuals who experience adverse effects or poor compliance with standard iron supplements. The results therefore point to the potential role of plant-derived alkaloids as complementary or alternative agents in anemia management.

CONCLUSION

This study introduces and validates a systematic, two-stage protocol for the evaluation of plant-derived anti-anemic agents within a public health nutrition context. By combining initial screening of crude extracts with targeted assessment of purified phytochemical fractions, the approach provides an efficient and scientifically robust method for identifying both effective plant materials and their active components. Application of this protocol to *Carica papaya* clearly demonstrates that the leaf methanol extract, particularly its alkaloid fraction, possesses significant anti-anemic activity.

The findings contribute to the growing body of evidence supporting the scientific validation of traditional dietary practices and medicinal plant use. Moreover, the proposed protocol offers a practical framework that can be applied to other traditional food plants, especially in resource-limited settings where anemia remains highly prevalent. By balancing methodological rigor with real-world applicability, this study supports the integration of evidence-based plant interventions into broader anemia control strategies.

Recommendations

- i. Adoption of the proposed two-stage evaluation protocol for the systematic assessment of traditional plants used in anemia management.
- ii. Further investigation of the *C. papaya* leaf alkaloid fraction through extended studies focusing on long-term safety, toxicity, and sustained efficacy.
- iii. Exploration of potential synergistic effects between identified bioactive fractions and conventional anti-anemic therapies.
- iv. Conduct of community-based studies to evaluate acceptability, feasibility, and practical implementation of papaya leaf-based preparations.
- v. Development of standardized processing and formulation methods to ensure consistent bioactive compound content and reproducible therapeutic outcomes.

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Conflict of Interest

The authors declare no conflict of interest.

Authors' Contributions

All authors read and approved the final manuscript.

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