

HEMATOLOGICAL EFFECTS OF *CHROMOLAENA ODORATA* ON PHENYL HYDRAZINE INDUCED ANEMIC RATS.

ABSTRACT

Aim: This study aim to investigate the phytochemical profiles of the leaves of *Chromolaena odorata* and evaluate the effects of its ethanolic extracts on hematological parameters in phenylhydrazine-induced anemia in albino rats.

Study Design: A controlled laboratory experiment was conducted to analyze the hematological effects of *Chromolaena odorata* extracts on induced anemia.

Place and Duration of Study: Department of Biochemistry, Federal University of Technology, Owerri, Nigeria, from June 2016 to August 2016.

Methodology: Twelve albino male rats (weight range: 80-170 g) were divided into four groups of three. Group 1 received standard feed and water (control). Group 2 was injected with 40 mg/kg phenylhydrazine to induce anemia. Groups 3 and 4 received the same dose of phenylhydrazine and were subsequently treated with 150 mg/kg and 300 mg/kg of *Chromolaena odorata* ethanolic extract, respectively, for eight days. Hematological parameters, including red blood cell count, hemoglobin concentration, and platelet count, were measured and analyzed.

Results: The hematological parameters were raised after the oral administration of the ethanol leaf extract of *Chromolaena odorata*. The red blood cell count, hemoglobin, hematocrit and platelet count was 5.75 ± 0.19 , 11.83 ± 0.123 , 36.24 ± 0.25 and 562.33 ± 2.52 respectively, with those of the induce rats and treated rats raised to 8.62 ± 0.11 , 21.65 ± 0.50 , 61.00 ± 0.42 and 587.50 ± 3.54 respectively while that of the control remained low. There was also increase in mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and red cell distribution width.

Conclusion: The findings shows that the ethanolic extract of *Chromolaena odorata* has potential therapeutic effects in alleviating hemolytic anemia by improving hematological parameters. Further studies are warranted to explore its mechanisms and broader applications in treating anemia.

Key words:(*Chromolaena Odorata*, Hematological Parameters, Anemia, Phytochemicals And Phenyl Hydrazine).

INTRODUCTION

Anemia remains a significant health challenge in many tropical regions including Nigeria due to high incidence of malaria and other parasitic infections which contribute to decreased hemoglobin levels [1]. Studies had shown that 43% of children and 33% of non-pregnant women globally were anemic, with African and South Asia having the highest incident [2]. Anemia can be defined as the reduction of the total number of red blood cells in blood or reduction of their quantity according to blood volume. Anemia can be caused by low, insufficient or anomalous production of red blood cells, inappropriate red blood cells loss or destruction [3]. Its impact on health, physical and mental productivity negatively affect the quality of life and culminate in profound economic losses for individual and countries where anemia is highly prevalent [4].

Therapeutic use of plants is as old as mankind. Most medicinal plants are traditionally used all over the world to alleviate anemia. The connection between man and the search for drugs in nature dates from far past [5]. Plants have been utilized for their therapeutic benefits for centuries, with various cultures harnessing the healing power of nature to treat ailments and promote well-being [6]. Ever since ancient times people look for drugs in nature. The beginning of the medicinal plant use were instinctive as the case with animal [5]. The use of medicinal plants as medicine predates written human history. Many of the herbs and species used by humans to season food also yield useful medicinal compounds [7]. Angiosperms (flowering plants) were the original source of most plant medicines [8]. Many of the common weeds that populate human settlements, such as nettle, dandelion and chickweed have medicinal properties. In view of the fact that at the time there was not sufficient information either concerning the reasons for the illnesses or concerning which plant and how it could be utilized as a cure, everything was based on experience. In time, the reasons for the usage of specific medicinal plants for treatment of certain diseases were being discovered; thus, the medicinal plants' usage gradually abandoned the empiric framework and became founded on explicatory facts. Until the advent of iatrochemistry in 16th century, plants had been the source of treatment and prophylaxis [9]. The advantages of herbal medicines over orthodox drugs include minimal or no side effects on the organic functioning of the body, consistent potency and the fact that they are well absorbed and distributed in the area of infection [10]. Phytochemicals are non-nutritive part of plant; they are naturally occurring chemical compounds that have therapeutic potentials aside their macro and micro-nutrients. It is known that most of the rural population rely largely on herbal remedies and there are so many beneficial

advantages related and some plants had been reported all over the world as being rich source of therapeutic agents for healing of diseases such as anemia [11].

Chromolaena odorata is a rapidly growing perennial herb with multi stemmed shrubs and grows up to 2.5m in open areas. It is sometimes grown as a medical or ornamental plant. It often used as a traditional medicine in Indonesia, Thailand, Malaysia, and part of Africa including Nigeria [12]. In Nigeria *Chromolaena odorata* is commonly called independent plant or Elizabeth plant [13]. *Chromolaena odorata* is also known as 'Independence' or 'Awolowo'. In Malaysia, the herb is known as 'Pokok kapal terbang', 'Pokok jerman', 'rumpun Jepun' or 'rumpun siam'. It thrives in most soils and is a prolific weed found in abundance on open wasteland and around roadsides. It is used as an antibacterial, antispasmodic, antiprotozoal, antitrypanosomal, antifungal, antihypertensive, anti-inflammatory, astringent, diuretic and hepatropic agent [14]. *Chromolaena odorata* leaves decoction have been reportedly used in folk medicines for wound healing and stoppage of bleeding. For some time now, *Chromolaena odorata* was just known for its medicinal properties but recent studies has implicated the plants in biotechnology where it has been used in phytoremediation of organic and inorganic contaminants [15]. Despite numerous literatures on the haemostatic and antimicrobial activities of *Chromolaena odorata*, not much information is available on the effect of the leaves extracts on hematological parameters. This study aims to address this gap by investigating the effects of ethanolic leaf extract of *Chromolaena odorata* on hematological parameters in phenyl hydrazine-induced anemic rats. Knowledge of the phytochemical content and composition of the plants used in ethno-medicine may open a new world of manipulation of pure extracts of these plants in other to discover new drugs that will be more effective in improving hematological parameters. The key measurements will include platelet count, packed cell volume, hemoglobin level, and red blood cell count. These parameters are essential for assessing the impact of the extract on anemia and evaluating its potential as a therapeutic agent.

MATERIALS AND METHODS

2.1 Sample Collection and Identification

Leaves of *Chromolaena odorata* were collected from Aboh Mbaise and Ezinihitte Mbaise Local Government Areas of Imo State, Nigeria, in June 2016. Plant identification and authentication were performed by Mr. F. Iwueze, a plant taxonomist from the Department of Forestry and Wildlife Technology, School of Agricultural and Agricultural Technology, Federal University of Technology, Owerri, Nigeria.

2.2 Preparation of Plant Materials and Extracts

The procedure began by removing any unhealthy leaves from the sample. Subsequently, the leaves underwent a thorough washing process by tap water and rinsed with distilled water. To facilitate proper grinding, the leaves were air-dried under shade for duration of two weeks. Further drying was then carried out using a hot air oven at 50°C for 24 hours. This ensured that the leaves were adequately dried and prepared for grinding. The grinding process itself was conducted using a high-speed grinding machine, specifically an industrial 1000A high-speed grinder. Finally, 245 grams of each leaf were precisely weighed for subsequent usage.

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For the extract preparation, 245 grams of *Chromolaena odorata* also known as independent or awolowo leaf powder was soaked in 400 ml absolute ethanol in a sterile conical flask. The mixture was shaken vigorously every 4 hours for 72 hours at ambient temperature. The extract was filtered through muslin cloth and Whatman No.1 filter paper, concentrated to dryness in a water bath, weighed, and stored in a sterile container at 4°C in a refrigerator until required for use.

2.3 Preparation of Phenyl-hydrazine

Phenyl-hydrazine solution was prepared by combining phenyl-hydrazine (manufactured by Sigma-Aldrich, Batch Number: PHZ789001) with distilled water v/v and 2-propanol in a ratio of 1:5:5. This entailed mixing 1 part of phenyl hydrazine with 5 parts of distilled water v/v and 5 parts of 2-propanol.

2.4 Experimental Design

Twelve healthy albino male rats, weighing between 80 to 170 grams were obtained from Relief Market, Owerri. They were housed in stainless steel cages with free access to water and food, and acclimatized for

one week prior to the experiment. The rats were divided into four groups of three rats each and treated as follows:

Group 1 (Normal control)

The rats in this group; were fed with feed vital finisher and water *ad libitum* throughout the period of experiment, with no other treatment.

Group 2 (Induced control)

The rats in this group, were made anemic by daily intraperitoneal of 2,4-dinitrophenyl hydrazine (PHZ) at 40 mg/kg for eight days during which they were fed with feed (vital finisher) and water *ad libitum* with no other treatment.

Group 3 (Induced treated-T1)

The rats in this group were made anemic by intraperitoneal administration of 40 mg/kg of 2,4-dinitro phenyl hydrazine for eight days at 2days interval. The rats were also fed with feed vital finisher and water *ad libitum*. They were treated with 150 mg/kgb.wt of the freshly prepared *Chromolaena odorata* leaf extract dissolved in olive oil for eight days.

Group 4 (Induced treated-T2)

The rats in this group were made anemic by intraperitoneal administration of 40 mg/kg of 2,4-dinitro phenyl hydrazine for eight days at 2days interval. The rats were also fed with feed vital finisher and water *ad libitum*. They were treated with 300 mg/kgb.wt of the freshly prepared *Chromolaena odorata* leaf extract for eight days together with the toxicant.

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2.5 Hematological Analysis

Hematological parameters were analyzed using an automated hematological analyzer (Model: Sysmex KX-21N, Sysmex Corporation, Kobe, Japan). The analyzer used hydrodynamic focusing DC detection methods for Red Blood Cell and Platelet counts, the Non-cyanide Sodium Lauryl Sulphate (SLS) method for Hemoglobin levels, and the Cumulative Pulse Height Detection (CPHD) Method for Packed Cell Volume (PCV). Blood samples with EDTA were mixed gently, probed with the analyzer, and results were printed immediately.

2.6 Phytochemical Screening of Plant Extracts

Phytochemical screening was conducted based on [16], on the powdered samples to detect bioactive compounds such as alkaloids, saponins, flavonoids, tannins, cyanogenic glycosides, cardiac glycosides, steroids and phenol.

2.6.1 Qualitative Analysis

Test for Alkaloids (Mayer's Test): Two 2 ml of the extracts were dissolved individually in 1% dilute hydrochloric acid and filtered. The filtrates were treated with few drops of Mayer's reagent (potassium mercuric iodide). Formation of a yellow cream precipitate indicated the presence of alkaloids.

Test for Saponins (Froth Test): Twenty 20 mg of each sample was boiled in 20 ml of distilled water in a water bath for 5 minutes and filtered. Ten 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for froth formation. 3 drops of olive oil was mixed with froth, shaken vigorously and observed for emulsion development.

Test for Flavonoids: The 50 mg of each of the sample was suspended in 100 ml of distilled water to get the filtrate. 5 ml of dilute ammonia was added to 10 ml of the filtrate followed by few drops of concentrated tetraoxosulphate (vi) (H_2SO_4). The presence of flavonoids was confirmed by yellow coloration

Test for Tannins: The 50 mg of each of the sample was boiled in 20 ml distilled water and filtered. A few drops of 0.1% $FeCl_3$ was added in the filtrate and observed for color change. Brownish green or blue-black coloration was taken as evidence for the presence of tannin

Test for Cyanogenic Glycosides: The 5 grams of each of the sample was added to 50 ml distilled water in a conical flask and allowed to stand overnight. To 1 ml of the sample filtrate in a corked test tube, 4ml of alkaline picrate was added and incubated in a water bath for 5 minutes. The presence of color change from yellow to reddish brown after 5 minutes incubation in a water bath indicated the presence of cyanide

Test for Phenol: The 5 grams of each of the sample was added to 50ml distilled water in a conical flask and allowed to stand overnight. To 1 ml of the sample filtrate in a corked test tube, 4 ml of alkaline picrate

was added and incubated in a water bath for 5 minutes. The presence of color change from yellow to reddish brown after 5 minutes incubation in a water bath indicated the presence of cyanide

Test for Steroids: The 200 mg of each of the dried samples boiled in 10ml of chloroform was filtered; a 2ml filtrate was added to 2 ml of acetic anhydride and concentrated H₂SO₄. Blue green ring indicates the presence of steroids and red color indicated the presence of terpenoids

Test for Cardiac Glycosides: Five 5 ml (mg/ml in ethanol) of each of the sample was mixed with 2 ml of glacial acetic acid having/containing one drop of ferric chloride (FeCl₃) solution. To the mixture obtained 1 ml of concentrated H₂SO₄ was added to form a layer. The presence of a brown ring at the interface is characteristic of cardiac glycosides

2.6.2 Quantitative Analysis

Flavonoid Determination

The 1 gram of each sample was weighed with 50ml beaker and 40 ml of 2 M HCl was added to each beaker. The beaker and its content was boiled for about 3 minutes on a hot plate, it was then cooled and filtered using Whatman No.1 filter paper. Flavonoid was precipitated from the filtrate by addition of excess drops of ethyl acetate solution. The precipitate was recovered from an already weighed oven dried filter paper. The precipitate and filter paper was dried in an oven at 105°C until constant weight of the filter paper gave flavonoid content.

% Flavonoid = weight of flavonoid/ weight of sample x 100/1.

Phenol Determination

Total phenol was determined by the Folin Denis spectrophotometric method. 1 gram of the sample was extracted by 50 ml of absolute ethanol in a 50 ml beaker. The mixture was stirred occasionally for up to 30 minutes at room temperature and then filtered using Whatman No.1 filter paper. 1 ml of each plant extract was treated with equal volume of Folin Denis reagent followed by the addition of 2 ml of 5% sodium carbonate solution and 5 ml of distilled water. The solution was allowed to stay for 5minutes. Standard phenol solutions was prepared and diluted accordingly. The absorbance of the sample was measured at 560nm using a spectrophotometer.

% Phenol = $\frac{W_2 - W_1}{W_3} \times 100/1$
Where W₁ = weight of empty beaker
W₂ = weight of beaker and sample
W₃ = weight of the sample used (g)

Cyanide Determination By Alkaline Picrate Colorimetric Method

The 1g of each sample was dispersed in 50ml of distilled water and the solution was incubated for 18hours and then filtered. 1 ml of the filtrate was pipette into 20 ml test tube and 4 ml of alkaline picrate solution was added to it. The mixture was boiled for 8minutes on a water bath to allow for full color development. The mixture was the cooled and absorbance was read at 490 nm. Cyanide standard concentration was prepared, diluted and treated accordingly. Each standard concentration absorbance was obtained at the same wavelength.

Alkaloid Determination

The alkaline precipitation gravimetric method was used. 1 gram of each sample was dispersed into 30 ml of 10% acetic acid in ethanol solution. It was allowed to stay for 1 hour. The mixture was filtered using Whatman No.1 filter paper. Alkaloid was precipitated from the filtrate by addition of excess drops of ammonium solution. The precipitate was recovered using an oven dried weighed filter paper. The precipitate and filter paper was oven dried at 105°C to a constant weight. The difference in weight gave alkaloid content of the sample.

$\% \text{ Alkaloid} = \text{weight of alkaloid} / \text{weight of sample} \times 100/1$

Tannin Determination

Tannin content of the samples was determined by the Folin Denis spectrophotometric method. 1g of the sample was dispersed in 50 ml of water using a volumetric flask. The mixture was shaken for 30 minutes at room temperature and filtered using Whatman filter paper. The residue was washed further with water until 50 ml filtrate was obtained. An aliquot of the extract 1 ml was mixed with equal volume of Folin Denis reagent in a 50 ml volumetric flask. 2 ml saturated solution of Na_2CO_3 was added to it. The mixture was diluted to the 50 ml mark and allowed to incubate for 20 minutes at room temperature. After incubation, the absorbance of the standard and samples were measured at 750nm using a spectrophotometer. A standard tannin solution was prepared with tannic acid and diluted to the desired concentration.

$\text{Tannin in mg/100} = X-Z/Y-Z = \text{Extract-blank/Standard} - \text{blank}$

Where X= Absorbance of 5ml extract

Y = Tannin solution

Z = Blank

Phytate Determination

2 grams of each sample was weighed using a weighing balance into 250 ml conical flask. 100 ml of 2% hydrochloric acid was added using a volumetric flask to soak each sample in the conical flask for 3 hours. This was then filtered using Whatman filter paper. 50 ml of each filtrate was placed in 250 ml conical flask and 107 ml distilled water was added in each case to give proper acidity. 10 ml of 0.3% ammonium thiocyanate (NH₄SCN) solution was added into each solution. This was titrated with standard iron (III) chloride solution which contained 0.00195g Fe/ml. The end point was slightly brownish yellow which persisted for 45minutes.

2.6.3 Statistical Analysis

All values were expressed as Means \pm SEM. Statistically difference between groups were determined using one way Analysis of variance (ANOVA) followed by Least significant difference test in significant cases using SPSS version 20 software. A P value of ≤ 0.05 was considered statistically significant.

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RESULTS

Results of qualitative phytochemical screening of ethanol leaf extract of *Chromolaena odorata*

Table 1: Shows the qualitative phytochemical screening of ethanol leaf extracts of *Chromolaena odorata*.

EXTRACTS	CHROMOLAENA ODORATA

Alkaloids	+
Saponins	+
Tannins	+
Cyanogenic glycosides	+
Cardiac glycosides	+
Steroids	+
Phenols	+
Flavonoids	+

Note: '+' indicates presence (positive)

'-' indicates absence (negative)

From table 1, the phytochemical analysis revealed the presence of saponins, tannins, alkaloids, flavonoids, phenols, steroids, cardiac glycosides and carcinogenic glycosides in *Chromolaena odorata*.

Results of quantitative phytochemical screening of ethanol leaf extract of *Chromolaena odorata*.

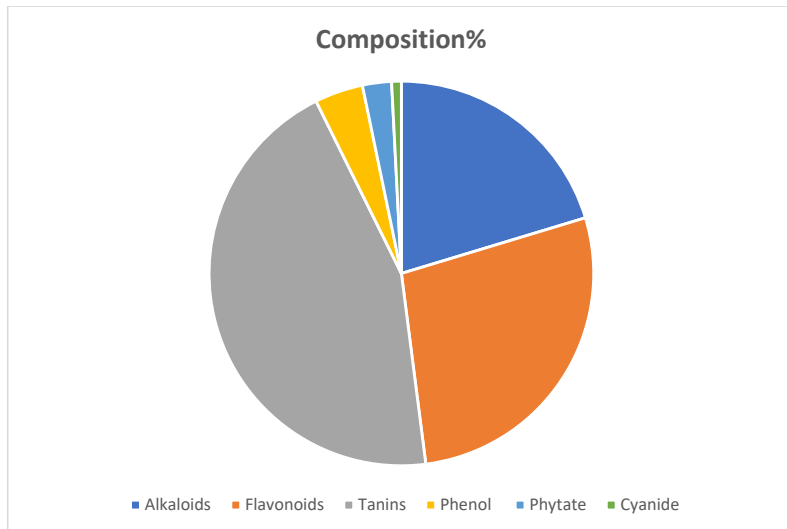


Fig. 1. This pie chart representing quantitative phytochemical screening of ethanol leaf extract of *Chromolaena odorata*. Results are expressed in mean \pm standard deviation of triplet determination.

The above pie chart shows the percentage composition of six phytochemicals of interest. Tannin have the highest concentration of 5.5% followed by Flavonoids with 3.4% followed by Alkaloids with 2.5% followed by phenol with 0.5 % followed by phytate with 0.3% and cyanide with 0.1%.

Table 2: Weight of animals

Normal control	Phenyl hydrazine	150mg/kg extract	300mg/kg extract
Group 1	Group 2	Group 3 T1	Group 4 T2
85.1	162.6	103.2	108.9
85.6	159.1	91.6	114.9
39.9	123.6	96.6	107.6
85.35	148.43	97.13	110.47

Table 3: Hematological Parameters of PHZ-Induced Hemolytic Rats

Parameters	Normal Control	PHZ	150mg/kg(T1)	300mg/kg(T2)
RBC	6.53±0.02 ^b	5.75±0.19 ^a	5.61±0.15 ^a	8.62±0.11 ^c
Hgb	14.43±0.32 ^b	11.83±0.21 ^a	11.87±0.15 ^a	21.65±0.50 ^c
HCT	42.77±0.15 ^c	36.24±0.25 ^b	35.43±0.42 ^a	61.00±0.42 ^d
MCV	64.60±0.10 ^c	63.07±0.40 ^b	62.20±0.20 ^a	68.70±0.28 ^d
MCH	21.73±0.15 ^c	20.53±0.29 ^b	18.10±0.10 ^a	24.15±0.21 ^d
MCHC	33.73±0.15 ^b	32.60±0.10 ^a	33.43±0.40 ^b	35.00±0.14 ^c
RDW	19.97±0.90 ^a	21.20±0.10 ^b	19.43±0.15 ^a	25.50±0.71 ^c
PLT	581.33±2.31 ^c	562.33±2.52 ^b	394.33±2.52 ^a	587.50±3.54 ^d

Values are shown as Mean ±Standard Deviation. Values with different superscripts within rows are significantly different.

The results of the hematological parameters are presented in Table 3. The result shows that there was an increase in hematological parameters of the rats as shown in Table 3. Daily intraperitoneal injection of 40 kg/mg 2,4-dinitro phenyl hydrazine for 8 days caused a significant decrease in group 2, while the red blood cell count decreased from 6.53±0.02 to 5.75±0.19, hemoglobin concentration and platelet concentration decreased from 14.43±0.32 and 581.33±2.31 to 11.83±0.21 and 562.33±2.52 respectively. There was also a decrease in hematocrit level, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and red cell distribution width.

The hematological parameters were raised after the oral administration of the ethanol leaf extract of *Chromolaena odorata*. The red blood cell count, hemoglobin, hematocrit and platelet count was 5.75±0.19, 11.83±0.123, 36.24±0.25 and 562.33±2.52 respectively, with those of the induce rats and treated rats raised to 8.62±0.11, 21.65±0.50, 61.00±0.42 and 587.50±3.54 respectively while that of the control remained low. There was also increase in mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and red cell distribution width.

DISCUSSION

This study investigated the phytochemical profiles of the leaves of *Chromolaena odorata*, Phytochemical screening revealed the presence of flavonoids, saponins, tannins, phenols, cyanogenic glycosides, alkaloids, and steroids in the extracts, this observation is in consonance with earlier report by [17] and [18]. The study further **investigate and evaluated** the effects of ethanolic extracts of *Chromolaena odorata* on hematological parameters in phenyl hydrazine-induced anemia in albino rats and their implication on human health. Phytochemicals and the ethanol extract of *Chromolaena odorata* (Independence plant) was used to treat phenyl hydrazine induced albino rats. The use of medicinal plants plays a vital role in combating diseases in developing parts of the world where poverty and drug resistance limit access to and effectiveness of synthetic drugs for chemotherapy [19].

A significant increase in the hematological parameters was observed in group 4 albino rats when compared with group 1 (control). This result pattern indicates that some of the phytochemical constituents of the ethanolic extracts of *Chromolaena odorata* may have stimulating effect on the bone marrow or thrombocyte production and hemoglobin synthesis. This observed effect may be as a result of tannin and phenol content.

As shown in table 3, the decrease in the hematological parameters in group 2 could be as a result of breakdown of red blood cells caused by 2,4-dinitro phenyl hydrazine. This study has shown that the ethanol leaf extract of *Chromolaena odorata* caused an increase in packed cell volume, red blood cell, platelet count, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration. This research also shows that the ethanolic leaf extract can serve as a cure for anemia as reported by some researchers. Specified and suitable concentration of the ethanolic extracts leads to positive effects on the hematological parameters investigated. The result of this study strongly suggest the possible role of *Chromolaena odorata* leave extract might have in treatment- induced erythropoietic activity. *Chromolaena odorata* could be incorporated into the pharmaceutical products intended to boost erythropoietic activities.

CONCLUSION

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The above result shows that *Chromolaena odorata* contain phytochemicals with potential phytochemical values that can be harnessed for economic development. The result of this research indicated that 300 mg/kg ethanolic leaf extract of *Chromolaena odorata* could elevate the packed cell volume, red blood cells, platelet count, mean corpuscular volume, mean corpuscular hemoglobin, red blood cell distribution width and mean corpuscular hemoglobin concentration more in the rats induced with phenyl hydrazine, hence oral administration of the extract could cure hemolytic anemia.

This research and its results give justification to the use of the leaves of the above plant in trado-medical practices for the cure of human ailments and anemia. Leaves of *Chromolaena odorata* can therefore be used as a potential source of new drugs.

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SUMMARY

- i. The study investigate and evaluated the effects of ethanolic extracts of **Chromolaena odorata** on hematological parameters in phenyl hydrazine-induced anemia in albino rats.
- ii. Ethanol leaf extract of *Chromolaena odorata* caused an increase in packed cell volume, red blood cell, platelet count, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration.
- iii. The Leaves of *Chromolaena odorata* can therefore be used as a potential source of new drugs.

REFERENCES

1. Toma I, Victory NC, Kabir Y. The effect of aqueous leaf extract of fluted pumpkin on some hematological parameters and liver enzymes in 2,4-dinitrophenylhydrazine-induced anemic rats. *Academic Journals. 2015; 9(7):95-99.
2. Stevens GA, Finucane MM, De-Regil LM, Paciorek CJ, Flaxman SR, Branca F, et al. Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and nonpregnant women for 1995–2011: A systematic analysis of population-representative data. Lancet Glob Health. 2013; 1(1):e16–e25. [https://doi.org/10.1016/S2214-109X\(13\)](https://doi.org/10.1016/S2214-109X(13)).
3. Dacie V, Lewis M. Practical haematology. Churchill Livingstone, London. 2004:49-59.

4. Diallo A, Gbeassor M, Vovor A, Eklu-Gadegbeku K, Aklikokou K, Agbonon A, et al. Effect of *Tectona grandis* on phenylhydrazine-induced anemia in rats. *Fitoterapia*. 2008; 79:332–336. <https://doi.org/10.1016/j.fitote.2008.02.005>.
5. Biljana BP. Historical review of medical plants. *Pharmacogn Rev*. 2012; 6:1-5.
6. Obazelu PA, Gaius-igboanugwo. Effect of a bi-herbal formula extract on ETS variant-6 and nuclear factor erythroid-2 genes expression in phenyl-hydrazine-induced anaemia in albino Wistar rats. *Journal of Medicine and Biomedical Research*. 2024; 23(1):117-127.
7. Tapsell LC, Hemphill I, Cobiac L. Health benefit of herbs and spices: The past, the present, and the future. *Australian Journal of Medical Research*. 2006; 185(4):4-24.
8. Stepp JR. The role of weeds as sources of pharmaceuticals. *American Journal of Ethnopharmacology*. 2004; 92(23):163-166.
9. Kelly K. History of medicine. New York: Facts on File; 2009:29-50.
10. Cheji R. The MacDonald encyclopaedia of medicinal plants. MacDonald and Press Limited, London. 1988; 6(5):54-59.
11. Awaga KL, Sowounou KE, Koudouvo K, Kutshik RJ, Longdet IY, Alemika. Evaluation of antianemic potential compounds of the leaf of a plant in Togo flora, *Lannea kerstingii*. *Journal of Pharmacognosy and Phytochemistry*. 2023; 12(4):127-130.
12. Heiss EH, Tran TV, Zimmermann K, Schwaiger S, Vouk C, Mayehofer B, et al. Identification of Chromomoric Acid C-1 as Nrf2 activator in *Chromolaena odorata*. *International Journal of Natural Products*. 2014; 34:7-6.
13. Omotayo MA, Avungbeto O, Sokefun OO, Eleyowo OO. Antibacterial activity of *Crossocephalum crepidioides* (fireweed) and *Chromolaena odorata* (siam weed) hot aqueous leaf extract. *International Journal of Pharmacy and Biological Sciences*. 2015; 5:114-122.
14. Akinmoladun AC, Ibukun EO, Dan-Ologe IA. Phytochemical constituents and antioxidant properties of extract from the leaves of *Chromolaena odorata*. *Scientific Research Essay*. 2007; 2(6):191-194.
15. Singh S, Thorat V, Kaushik CP, Raj K, D'Souza SF. Potential of *Chromolaena odorata* for phytoremediation of ¹³⁷Cs from solution and low-level nuclear waste. *Journal of Hazardous Materials*. 2009; 162:743-745.

16. Roopashree TS, Raman D, Shoba RH, Narendra C. Antibacterial activity of antisoriatic herbs, *Cassia flora, Momordica charantia, and Calendula officinalis. International Journal of Applied Research in Natural Products. 2008; 1(3):20-28.

17. Afolabi CA, Ibukun EO, Dan-Oluge JA. Phytochemical constituents and antioxidant properties of extract from the leaf of Chromolaena odorata. Scientific Research Essay*. 2007; 2:191-194.

18. Inyang CU, Adegoke AA. Antibacterial properties of preliminary phytochemical screening of Chromolaena odorata (siam weed or sapysa weed). Nigerian Journal of Microbiology. 2008; 22:1652-1659.

19. Girish HV, Satish S. Antibacterial activity of important medicinal plants on human pathogenic bacteria: A comparative analysis. World Applied Sciences Journal. 2008; 5(3):267-271.

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