



**A COMPARATIVE EVALUATION OF THE HAEMATOPOIETIC POTENTIALS OF
ETHANOLIC EXTRACTS OF *TALINUM TRIANGULARE*, *TELFAIRIA OCCIDENTALIS*
AND *AMARANTHUS HYBRIDUS* ON 2,4-DINITROPHENYLHYDRAZINE (DNPH)
INDUCED ANAEMIC WISTAR RATS**

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ABSTRACT

Anaemia is global public health problem with prevalence higher among low-income countries. The prohibitive cost of treatment has continued to fuel interest in the screening of medicinal plants for their anti-anemic potentials. This study is aimed at comparing the efficacies of *Talinum triangulare*, *Telfairia occidentalis* and *Amaranthus hybridus* on 2,4-Dinitrophenylhydrazine (DNPH) induced anaemic Wistar rats. The study was conducted using 45 male adult Wistar rats. Anaemia was induced using a 10 days oral administration of DNPH. The animals were treated using 30 and 60mg/kg ethanolic leaf extracts and a standard anti-anaemic drug. Haematological parameters were analyzed using standard automated methods. Results from this study indicate that treatment with ethanolic leaf extracts of *T. Triangulare*, *T. Occidentalis* and *A. Hybridus* all caused significant increase in the mean values of PCV, HGB, RBC, MCH, MCHC and MCV compared with the untreated anaemic rats ($P < 0.05$). *A. Hybridus* caused the highest percentage increase in PCV(59.7%), HGB(48.4%), MCH(18.7%), MCV(32.6%), followed by *T. Occidentalis* and *T. Triangulare* in a decreasing order. Also, *T. Triangulare* and *A. Hybridus* caused a significant reduction in the values of WBC compared to the untreated animals ($p < 0.05$) while the ethanolic extract of *T. Occidentalis* caused a significant increase in WBC compared to the untreated animals ($p < 0.05$). Only *eA. Hybridus* significantly raised platelet count compared with the untreated anaemic animals ($p < 0.05$). The present study has further validated the anti-anaemic potentials of the individual extracts of *T. Triangulare*, *T. Occidentalis* and *A. Hybridus* while showing their various degrees of efficacy.

KEYWORDS: Anaemia, Haematology, *Talinum triangulare*, *Telfairia occidentalis*, *Amaranthus hybridus*, 2,4-Dinitrophenylhydrazine.

INTRODUCTION

Anaemia is a blood disorder that is commonly attributed to nutritional deficiencies. It is a global public health problem, affecting both developing and developed countries with major consequences for human health and development.^[1, 2] Anaemia is characterized by the reduction in the population of healthy red blood cells which carry oxygen to the body's tissues.^[3,4] It can be temporary or long term, ranging from mild to severe. The prevalence of anaemia is five times higher in low-

income countries when compared to high income countries and is inversely related with economic status.^[5,6] Nutritional deficiencies, haemorrhage, parasitic contaminations and acquired disorders are some of the causes of anaemia.^[1, 4, 6] Treatment options for anaemia naturally starts with a healthy, well varied diet with focus on food with adequate iron, copper, zinc, folic acid, Vitamin B-12 and protein.^[7-9] Other options for the treatment of anaemia include the use of synthetic iron supplements and multi-vitamins as well as blood

transfusion.^[4, 9] In poor and developing countries where access to anti-anaemic drugs is a challenge or the cost is prohibitive, the use of herbs and natural vegetables alone or in combination with anti-anaemic drugs in the treatment of anaemia has continued to gain traction.^[10-12]

Leafy vegetables serve as indispensable constituents of the human diet, supplying the body with proteins, vitamins and minerals.^[13, 14] Several leaves and vegetables have been screened for their hematopoietic potentials especially in the amelioration and treatment of anaemia.^[12, 15-18] The objective is always get the lowest effective dose which will produce maximum efficiency with the least side effects.^[19] *Talinum triangulare*, *Telfairia occidentalis* and *Amaranthus hybridus* are among the abundant and widely consumed leafy vegetables in West Africa.

Talinum triangulare commonly known as water leaf is a perennial deciduous herb which has woody stems and succulent leaves from the family of Portulacaceae.^[14, 20] In Nigeria, it is consumed as a vegetable normally as a sauce constituent.^[14] It contains important nutrients such as proteins, vitamins and β -carotene as well as essential minerals like potassium, magnesium and calcium.^[14, 20, 21] Medicinal uses of water leaf include treatment for diarrhea and other gastrointestinal disturbances, inflammation, obesity and cardiovascular diseases.^[14, 20, 22] Documented reports have shown *Talinum triangulare* to possess hematopoietic potentials^[23-27], antioxidant properties^[28-31], hypoglycaemic and hypolipidaemic activities^[32, 33] and anti-ulcer potentials.^[34, 35]

Telfairia occidentalis, commonly known as Fluted Pumpkin is another staple vegetable that is commonly found in Nigeria and many other West and Central African regions.^[36, 37] It is a vegetable with luxuriant green leaves from the family of *Cucurbitaceae* that is mostly cultivated for both culinary and medicinal uses.^[36] It is rich in antioxidants, proteins, fiber, minerals like calcium, iron, sodium as well as vitamins B1, B3 and C.^[36-38] It is widely used in the folk remedy of diabetes mellitus, hypertension, inflammations and bacterial infections.^[36, 37] Reports have shown *Telfairia occidentalis* to possess hematopoietic potentials^[39-43], antioxidant and hepatoprotective properties^[44-46], antiparasitic and antimicrobial properties^[47], antidiabetic activities^[48-50], anti-inflammatory and analgesic activity^[51, 52] and ability to enhance male fertility.^[53, 54]

Amaranthus hybridus is an evergreen monoecious herb from the family of *Amaranthaceae* commonly known as pigweed or amaranth. It is commonly found growing as weed in cultivated fields and are characterized by green leafy vegetables with its abundant flowers.^[55, 56] They are consumed alone or with other leafy vegetables as soup or other delicacies.^[57] *Amaranthus hybridus* is rich in protein, carbohydrate and fibre, as well as sodium, potassium, magnesium, iron and phosphorus.^[56, 58] It is

also rich in vitamins A and C, nitrates and oxalates.^[59] Its medicinal uses include treatment for indigestion, toothache, boils, burns and snake bites.^[55] Several reports have demonstrated its hematopoietic^[60], immunomodulatory^[61], antioxidant and hepatoprotective^[62], antimicrobial^[63] and anti-carcinogenic potentials.^[64]

No studies have attempted to compare the hematopoietic effects of these vegetables under the same dosage and experimental conditions. In the light of their hematopoietic effects and their common traditional use in the treatment of a wide variety of ailments, the present study is therefore aimed comparing the haematopoietic effects of *Talinum triangulare*, *Telfairia occidentalis* and *Amaranthus hybridus* on 2,4-Dinitrophenylhydrazine (DNPH) induced anaemic Wistar rat models.

MATERIALS AND METHOD

Plant Material and Preparation of Extract

Fresh leaves of *Talinum triangulare*, *Telfairia occidentalis* and *Amaranthus hybridus*, were procured from the popular Choba market, Port Harcourt, Rivers State, Nigeria. They were duly identified and authenticated at the Department of Plant Science and Biotechnology, University of Port Harcourt. Leaves were air dried for two weeks, the dried leaves were ground into coarse powder and used for ethanolic extraction and phytochemical screening according to methods described by Odebiyi and Sofowora.^[65]

Induction of anaemia

Anaemia was induced using an oral administration of 2,4-Dinitrophenylhydrazine (DNPH) (Sigma-Aldrich, Germany) for 10 days at 30mg/kg.^[66, 67] Anaemic rats were treated with ethanolic extracts of *T. Triangulare*, *T. Occidentalis* and *A. Hybridus* as well as standard drug Astymin (Fidson Healthcare PLC, Nigeria).

Research Animals and Research Design

Forty-five (45) male Wistar rats weighing 180-200g were used for this study. The animals were procured and housed in standard cages at the Animal house of the Department of Human Physiology, University of Port Harcourt. The rats were allowed three weeks of acclimatization before the start of the study under standard laboratory conditions: Temperature at 25 - 29°C, 55 - 65% relative humidity under natural light/dark natural cycle. During the period of acclimatization, the animals were fed a balanced commercial rat chow ad libitum.

The research animals were randomly divided into nine groups (Groups 1-9) consisting of five rats each and treated as follows.

Group 1: Control group [Distilled water]

Group 2: Negative control; [Dinitrophenyl Hydrazine only]

Group 3: Positive control: Dinitrophenyl Hydrazine + Astymin

Group 4: Dinitrophenyl Hydrazine + 30mg/kg *T. Triangulare*
 Group 5: Dinitrophenyl Hydrazine + 60/kg *T. Triangulare*
 Group 6: Dinitrophenyl Hydrazine + 30mg/kg *T. Occidentalis*
 Group 7: Dinitrophenyl Hydrazine + 60mg/kg *T. Occidentalis*
 Group 8: Dinitrophenyl Hydrazine + 30mg/kg *A. Hybridus*

Group 9: Dinitrophenyl Hydrazine + 60mg/kg *A. Hybridus*
 Oral treatment using ethanolic extracts of *T. Triangulare*, *T. Occidentalis* and *A. hybridus* and standard drug (Astymin) lasted for 28 days. The animals were anaesthetized using cervical dislocation and blood was collected via cardiac puncture. Blood was analyzed for hematological parameters using standard automated methods.

RESULTS AND DISCUSSION

Table 1: Result of qualitative phytochemical analysis of ethanolic extract of *Talinum triangulare*, *Telfairia occidentalis* and *Amaranthus hybridus*.

Phytochemicals	Test	<i>Talinum triangulare</i>	<i>Telfairia occidentalis</i>	<i>Amaranthus hybridus</i>
Alkaloids	<i>Dragendorff's Test</i>	Negative	Negative	Negative
	<i>Meyer's Test</i>	Negative	Negative	Negative
	<i>Hager's Test</i>	Positive	Positive	Positive
Flavonoids	<i>Shinoda Test</i>	Negative	Negative	Negative
	<i>Alkali Test</i>	Positive	Positive	Negative
Tannins	<i>Fec_{l3} Test</i>	Positive	Positive	Positive
	<i>Phlobatannins Test</i>	Negative	Negative	Negative
Anthraquinone	<i>Free Anthraquinone</i>	Negative	Negative	Negative
	<i>Combined Anthraquinone</i>	Negative	Negative	Negative
Triterpenoid /Steroids	<i>Liebermann-Buchard test</i>	Positive	Positive	Positive
	<i>Salkowski Test</i>	Negative	Negative	Negative
Carbohydrates	<i>Molisch Test</i>	Positive	Positive	Positive
	<i>Fehling's Test</i>	Positive	Positive	Positive
Cardenolide	<i>Keller Killani Test</i>	Negative	Positive	Positive
	<i>Kedde Test</i>	Positive	Positive	Positive
Cyanogenic glycosides		Negative	Negative	Negative
Saponins	<i>Frothing Test</i>	Negative	Negative	Negative
	<i>Emulsion Test</i>	Positive	Positive	Negative

Table 2: Red cell count and red cell indices of anaemic rats treated with ethanolic extracts of *T. Triangulare*, *T. Occidentalis* and *A. Hybridus*.

Research Groups	PCV (%)	HGB (g/dl)	RBC ($\times 10^{12}/L$)	MCH (pg)	MCHC (g/dl)	MCV (fl)
Control	35.18 $\pm$ 0.34	11.70 $\pm$ 0.14	6.46 $\pm$ 0.11	18.13 $\pm$ 0.28	33.26 $\pm$ 0.37	54.48 $\pm$ 0.43
Negative Control	27.58 $\pm$ 0.47	8.68 $\pm$ 0.22	5.28 $\pm$ 0.66	16.43 $\pm$ 0.29	31.45 $\pm$ 0.39	50.25 $\pm$ 0.43
Positive Control	39.54 ^a $\pm$ 0.98	12.08 ^a $\pm$ 0.07	6.16 ^a $\pm$ 0.11	19.61 $\pm$ 0.31	30.57 $\pm$ 0.44	64.24 ^a $\pm$ 1.45
<i>T. triangulare</i> 30mg/kg	36.76 ^a $\pm$ 2.00	12.57 ^a $\pm$ 0.59	6.89 ^{ab} $\pm$ 0.30	18.23 ^{ab} $\pm$ 0.30	34.30 ^b $\pm$ 1.00	53.32 ^{ab} $\pm$ 1.70
<i>T. triangulare</i> 60mg/kg	34.86 ^{ab} $\pm$ 1.93	11.75 ^a $\pm$ 0.24	6.67 ^{ab} $\pm$ 0.17	17.61 ^{ab} $\pm$ 0.15	33.99 ^b $\pm$ 1.41	52.10 ^{ab} $\pm$ 1.79
<i>T. occidentalis</i> 30mg/kg	37.00 ^a $\pm$ 1.38	11.88 ^a $\pm$ 0.53	6.40 ^a $\pm$ 0.09	18.55 ^a $\pm$ 0.60	32.10 $\pm$ 0.58	57.79 ^{ab} $\pm$ 1.52
<i>T. occidentalis</i> 60mg/kg	34.26 ^{ab} $\pm$ 1.35	12.46 ^a $\pm$ 0.32	6.38 ^a $\pm$ 0.29	19.51 ^a $\pm$ 0.32	36.71 ^a $\pm$ 2.34	53.90 ^b $\pm$ 2.99
<i>A. hybridus</i> 30mg/kg	37.52 ^a $\pm$ 0.88	12.50 ^a $\pm$ 0.51	6.76 ^{ab} $\pm$ 0.26	18.50 ^a $\pm$ 0.26	33.32 $\pm$ 0.91	55.59 ^b $\pm$ 1.06
<i>A. Hybridus</i> 60mg/kg	44.04 ^{ab} $\pm$ 0.73	12.88 ^a $\pm$ 0.47	6.62 ^a $\pm$ 0.15	19.50 ^a $\pm$ 0.84	29.23 $\pm$ 0.76	66.65 ^a $\pm$ 1.92

Results are given as mean $\pm$ standard error of mean.

^aSignificantly different compared to negative control.

^bSignificantly different compared to positive control.

Table 3: White blood cell and platelet count of anaemic rats treated with ethanolic extracts of *T. Triangulare*, *T. Occidentalis* and *A. Hybridus*.

Research Groups	WBC ($\times 10^9/L$)	Neutrophil ($\times 10^9/L$)	Lymphocyte ($\times 10^9/L$)	Monocyte ($\times 10^9/L$)	Eosinophil ($\times 10^9/L$)	Basophil ($\times 10^9/L$)	Platelet ($\times 10^9/L$)
Control	9.29 $\pm$ 1.69	3.74 $\pm$ 0.60	5.04 $\pm$ 0.72	0.16 $\pm$ 0.05	0.35 $\pm$ 0.02	0.03 $\pm$ 0.03	809.20 $\pm$ 51.32
Negative Control	15.07 $\pm$ 0.43	3.91 $\pm$ 0.33	9.69 $\pm$ 0.62	0.08 $\pm$ 0.04	0.28 $\pm$ 0.03 ²	0.08 $\pm$ 0.01	733.60 $\pm$ 62.78
Positive Control	14.87 $\pm$ 0.58	4.21 $\pm$ 0.30	9.52 $\pm$ 0.75	0.05 $\pm$ 0.012	0.13 $\pm$ 0.01	0.76 $\pm$ 0.01	815.00 $\pm$ 92.51
<i>T. Triangulare</i> 30mg/kg	6.69 ^{ab} $\pm$ 0.96	2.20 ^{ab} $\pm$ 0.45	5.49 ^{ab} $\pm$ 0.94	0.21 $\pm$ 0.01 ^{a,b}	0.39 ^b $\pm$ 0.04	0.04 $\pm$ 0.01	658.20 $\pm$ 40.81
<i>T. Triangulare</i> 60mg/kg	12.47 $\pm$ 2.13	2.59 ^{ab} $\pm$ 0.27	8.50 $\pm$ 0.56	0.17 ^{ab} $\pm$ 0.03	0.21 $\pm$ 0.02	0.09 ^a $\pm$ 0.0	747.60 $\pm$ 50.16
<i>T. Occidentalis</i> 30mg/kg	15.50 $\pm$ 1.63	5.95 ^{ab} $\pm$ 0.50	8.47 $\pm$ 1.35	0.21 $\pm$ 0.08 ^{1,2}	0.33 ^b $\pm$ 0.06	0.10 $\pm$ 0.02	794.20 $\pm$ 85.77
<i>T. Occidentalis</i> 60mg/kg	25.24 ^{ab} $\pm$ 2.40	6.57 ^{ab} $\pm$ 0.62	14.57 ^{ab} $\pm$ 0.7	0.21 ^{ab} $\pm$ 0.06	0.63 ^{ab} $\pm$ 0.07	0.21 ^a $\pm$ 0.06	787.00 $\pm$ 90.09
<i>A. Hybridus</i> 30mg/kg	11.69 $\pm$ 1.16	4.31 $\pm$ 0.34	7.41 $\pm$ 0.91	0.09 $\pm$ 0.03	0.28 ^b $\pm$ 0.05	0.05 ^a $\pm$ 0.02	943.80 ^a $\pm$ 35.62
<i>A. Hybridus</i> 60mg/kg	8.14 ^{ab} $\pm$ 0.38	2.39 ^{ab} $\pm$ 0.10	5.52 ^{ab} $\pm$ 0.27	0.10 $\pm$ 0.02	0.04 ^{ab} $\pm$ 0.01	0.04 ^{ab} $\pm$ 0.01	939.60 ^a $\pm$ 31.99

Results are given as mean $\pm$ standard error of mean

^aSignificantly different compared to negative control

^bSignificantly different compared to positive control

Table 4. Percentage changes of hematological parameters of treated anemic rats compared to the untreated anaemic rats.

Parameters	<i>T. Triangulare</i> 30mg/kg	<i>T. Triangulare</i> 60mg/kg	<i>T. Occidentalis</i> 30mg/kg	<i>T. Occidentalis</i> 60mg/kg	<i>A. Hybridus</i> 30mg/kg	<i>A. Hybridus</i> 60mg/kg
PCV (%)	33.3%	26.4%	34.86%	24.0%	36.0%	59.7%
HGB (g/dl)	44.8%	35.4%	36.9%	43.5%	44.0%	48.4%
RBC ($\times 10^{12}/L$)	30.5%	26.3%	21.2%	20.8%	28.0%	25.4%
MCH (pg)	11.0%	7.2%	12.6%	18.7%	12.6%	18.7%
MCHC (g/dl)	9.1%	8.1%	2.1%	16.7%	5.9%	-7.1%
MCV (fl)	6.1%	3.7%	15.0%	7.3%	10.6%	32.6%
WBC ($\times 10^9/L$)	-55.6%	-20.0%	-2.9%	67.5%	-22.4%	-46.0%
Neutrophil ($\times 10^9/L$)	-43.7%	-33.8%	-52.2%	-68.0%	10.2%	-38.9%
Lymphocyte ($\times 10^9/L$)	-43.3%	-12.3%	-12.6%	50.4%	-23.5%	-43.0%
Monocyte ($\times 10^9/L$)	162.5%	112.5%	162.5%	162.5%	12.5%	25.0%
Eosinophil ($\times 10^9/L$)	-25.0%	17.9%	39.3%	125.0%	0.0%	-85.0%
Basophil ($\times 10^9/L$)	-50.0%	12.5%	25.0%	162.5%	-37.5%	-50.0%
Platelets ($\times 10^9/L$)	-10.3%	1.9%	8.3%	7.3%	28.7%	28.1%

RESULTS

Table 1 show the qualitative analysis of ethanolic leaf extract of *T. Triangulare*, *T. Occidentalis* and *A. Hybridus*. The result indicate that all the extracts contain alkaloids, flavonoids, tannins, triterpenoid/steroids. Free and combined anthraquinones were not detected in any of the extracts. Also, saponins were not detected on *A. Hybridus*. These phytochemical results are similar to results from other studies.^[46,68,69]

Table 2 show the effects of various ethanolic leaf extract on red blood cell count and red cell indices of Wistar rats. The results indicate that the mean values for PCV, HGB, RBC, MCH, MCHC and MCV were found to be significantly higher among rats treated with ethanolic extracts of *T. Triangulare*, *T. Occidentalis* and *A. Hybridus* (groups 3-9) compared to the untreated DNPH

induced anaemic Wistar rat (group 2) ($p < 0.05$). When compared to the standard drug, the mean value for PCV was found to be higher among the animals treated with 60mg/kg of *A. Hybridus* (Group9) ($p < 0.05$), the mean RBC count was higher in animals treated with *T. Triangulare* (groups 3&4) ($p < 0.05$) as well as among rats treated with 30mg/kg of *A. Hybridus* (group 7) ($p < 0.05$). The mean values for MCH and MCHC were also found to significantly higher in rats treated with 30mg/kg and 60mg/kg of *T. Triangulare* (groups 3&4) when compared to the standard drug ($p < 0.05$).

Table 3 shows the effect of ethanolic leave extract of *T. Triangulare*, *T. Occidentalis* and *A. Hybridus* on WBC and Platelets counts. The results show that the mean values for WBC were found to be significantly lower in rats treated with 30mg of *T. Triangulare* (group 4)

($p < 0.05$) and 60mg/kg of *A. Hybridus* (group 9) ($p < 0.05$). However, the mean values for WBC was found to be significantly higher among rats treated with 60mg/kg of *T. Occidentalis* (group 7). The same pattern was also observed when compared to a standard drug with lower WBC counts observed for rats in groups 4 and 9 and a significantly higher mean values for group 7 ($p < 0.05$). The mean values for neutrophil count was found to be significantly lower in animals treated with ethanolic extracts of *T. Triangulare* and *A. Hybridus* ($p < 0.05$) and significantly higher among rats treated with *T. Occidentalis* when compared to the untreated anaemic rats ($p < 0.05$). Ethanolic extracts of *T. Triangulare* and *A. Hybridus* also caused a significant decrease in the lymphocyte count when compared to untreated anaemic rats and the animals treated with a standard drug ($p < 0.05$). However, *T. Occidentalis* caused a significant when compared with the untreated animals ($p < 0.05$). The monocyte and eosinophil counts among animals treated with *T. Triangulare* and *T. Occidentalis* were observed to be significantly higher when compared to the untreated anaemic rats ($p < 0.05$). The basophil count was observed to be significantly lower in animals treated with *T. Occidentalis* and *A. Hybridus* when compared to a standard drug ($p < 0.05$), however, animals treated with 60mg/kg of *T. Occidentalis* had a significantly raised mean value when compared to the untreated anaemic rats. Finally, a significantly raised platelet count was observed for animals treated with *A. Hybridus* compared with the untreated animals ($p < 0.05$).

Table 4 shows the percentage changes of haematological parameters of treated anaemic rats compared to the untreated rats. The result indicates that ethanolic extracts of *A. Hybridus* caused highest percentage increase in PCV(59.7%), HGB(48.4%), MCH(18.7%), MCV (32.6%), neutrophil(10.2%) and platelets counts (28.7%) when compared to the untreated anaemic animals. Similarly, *T. Triangulare* caused highest percentage increase in RBC (30.5%). *T. Occidentalis* caused highest percentage increase in WBC(67.5%), lymphocyte (50.4%), monocytes (162.5%), Eosinophil (125%) and Basophil (162.5%). Conversely, *A. Hybridus* caused highest percentage decrease in WBC (46%) and eosinophil(85%) and jointly with *T. Occidentalis* has the highest percentage decrease in neutrophil (43%) and basophil(50%). *A. Hybridus* caused the highest percentage increase in platelet counts (28%) while only *T. Occidentalis* at 60mg/kg led to the only decrease at 10%.

DISCUSSION

The present study attempts to compare the effects ethanolic leaf extracts of *T. Triangulare*, *T. Occidentalis* and *A. Hybridus* on haematological parameters of DNPH-induced anaemia using Wistar rat models. Oral and dermal administration DNPH has been known to cause haemolytic anemia.^[12,60,66,67] by causing oxidative stress within erythrocytes leading to rapid precipitation of haemoglobin and alteration in iron metabolism.^[70]

Results from this study indicate that treatment with ethanolic leaf extracts of *T. Triangulare*, *T. Occidentalis* and *A. Hybridus* all caused significant increase in the mean values for PCV, HGB, RBC, MCH, MCHC and MCV compared with the untreated anaemic rats ($P < 0.05$). This goes to confirm the already established haematopoietic potentials of the various leaf extracts.^[23-27,39-43,60] However, the extracts showed varied degrees of enhancement of hematopoiesis with the various hematological parameters. While treatment with extracts of *A. Hybridus* caused the highest percentage increase in PCV(59.7%), HGB(48.4%), MCH(18.7%), MCV(32.6%), followed by *T. Occidentalis* and *T. Triangulare* in a decreasing order. The similar chemical composition of these extracts^[14,21, 36,37,55,56,58,69,71,72] give them their hematopoietic potential as the presence of iron, zinc and copper are necessary for the formation of erythropoietin which stimulates the formation of red blood cells.^[73,74] The observed higher percentage increase in the PCV, HGB, RBC, MCH, MCHC and MCV in animals treated with *A. Hybridus* could be attributed to its iron content which is major factor in red cell production. Reports have shown higher content of iron in *A. Hybridus* compared to *T. Occidentalis* and *T. Triangulare*.^[72,75]

Also, flavonoids as contained in the leaf extracts.^[46,68,69], acting as antioxidants has been shown to exhibit high membrane stabilizing capability. By stabilizing the membrane of red blood cells, they reduce their destruction and subsequently enhance their population in circulation.^[76,77] Red blood cell membranes though rich in unsaturated fat are regularly exposed to oxygen and hence are more susceptible to oxidative damage. Though the exact mechanism by which flavonoid prevent red blood cell haemolysis is yet to be determined, it is thought that flavonoids possibly react with intracellular oxidants and prevent hemoglobin oxidation.^[78] Marginally higher content of antioxidants such as flavonoids and β -carotene have been reported in *A. Hybridus* compared to *T. Occidentalis* and *T. Triangulare*.^[71]

The leaf extracts showed varying effects on the WBC of the animals. Ethanolic leaf extract of *T. Triangulare* and *A. Hybridus* caused a significant reduction in the values of WBC compared to the untreated animals ($p < 0.05$) while the ethanolic extract of *T. Occidentalis* caused a significant increase compare to the untreated animals ($p < 0.05$). Reports have associated DNPH with the activation of immune response.^[70,79] This is clearly evident in the significant increase in the mean value of WBC of the untreated DNPH induced anaemic rats when compared to the normal control ($p < 0.05$). White blood cells function mainly to defend the body against pathogens as well as the removal of toxins and other waste products.^[80] Clinically, higher WBC is widely used as an indicator of systemic inflammation.^[81] The observed significant reduction in the values of WBC among the animals treated with ethanolic leaf extract of

T. Triangulare and *A. Hybridus* could be attributed to their ability possibly ameliorate excessive immune system activation or inflammation caused by DNPH. Alkaloids as contained in the leaf extracts have been shown to exhibit potent anti-inflammatory activities.^[82-84] In the same way, the various extracts caused variations in the levels white cell differential counts in different ways. *T. Triangulare* and *A. Hybridus* caused reduction in the mean values of neutrophils, lymphocyte and monocytes counts compared to the untreated anaemic animals, while *T. Occidentalis* caused more elevation in these values. Circulating neutrophils and eosinophils are able to recognize and fight bacteria and other pathogens, basophils are mobilized to sites of injury while lymphocytes play specific roles in body defense and immunity.^[80] This suggests that these leaf extract may in some way, play a possible role in immuno-modulation.

Only ethanolic extracts of *Amaranthus A. Hybridus* significantly raised platelet count compared with the untreated anaemic animals ($p < 0.05$). Platelets play a major role not only in the initiation of coagulation cascades associated with damage to blood vessel, but they also function in other pathophysiological processes like including inflammation and antimicrobial activities.^[85,86] The raised platelet levels suggest that *A. Hybridus* may play a role in blood coagulation.

In conclusion, the present study has further validated the anti-anaemic potentials of the individual extracts of *T. Triangulare*, *T. Occidentalis* and *A. Hybridus* while showing varied degrees of efficacy. It will be interesting to see what happens when these extracts are combined in other to harness their different haematopoietic and possible immuno-modulatory potentials.

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