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Evaluation of haematinic activity of *Tapinanthus globiferus* (A. Rich.) van Tiegh leaf extract and fractions in phenylhydrazine-induced anaemic rats

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Abstract
Anaemia is a leading cause of morbidity and mortality in both males and females of all ages. Anaemia was induced by intraperitoneal (i.p.) administration of phenylhydrazine (60 mg/kg) for a period of 3 days. Anaemic rats were allotted to five (5) groups; group I served as the negative control, group II served as the positive control received Astyfer syrup (0.15 mg/kg p.o) , groups III-V served as the test groups that received *Tapinanthus globiferus* methanol extract, ethyl acetate and *n*-hexane fractions (100, 200 and 400 mg/kg, p.o.), respectively. Packed cell volume (PCV), haemoglobin concentration (HB) and red blood cell count (RBC) were analysed as indices of anaemia at 7-day intervals for 21 days. The mean cell volume (MCV) and mean cell haemoglobin concentration (MCHC) were calculated accordingly. Leaf extract and fractions of *Tapinanthus globiferus* significantly ($P<0.001$) increased the PCV, the HB concentration and the RBC, which had been originally decreased by phenylhydrazine ($P<0.001$) administration within one week of treatment. The extract and fractions were compared with rats administered with the positive and vehicle-treated group. The results suggested that *Tapinanthus globiferus* leaves have haematinic activities.

Key words: Haematinic activity; *Tapinanthus globiferus;* Haemolytic anaemia; Phenylhydrazine.

INTRODUCTION

Anaemia is the most prevalent red blood cell problem, affecting people of all ages and associated with a variety of disorders such as nutritional inadequacies, genetic or acquired abnormalities, parasitic infections, haemorrhage of all causes, and drug toxicity. The elderly pregnant women, neonates and infants are at higher risk. Anaemia is one of the most common and serious public health and nutritional issues worldwide [1, 2]. Anaemia is a common nutritional condition among the

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young population, and it is caused primarily by iron deficiency. Iron deficiency arrest cell cycle and induces the death of cells [3, 4]. Anaemia is defined by the World Health Organization (WHO) as a condition in which the number of red blood cells (oxygen carrying capability) is insufficient to meet the body's physiologic needs [5]. Anaemia affects approximately two billion individuals worldwide, or one-third of the adult population; half of these are women of reproductive-age (15-49 years) [6, 7]. According to World Bank data from 2016, 33% of reproductive-age women globally were anaemic [8]. As of 2016, almost 35.4% of reproductive-age women in poor and middle-income nations were anaemic. During the same timeframe, however, the prevalence of anaemia in Sub-Saharan African countries grew to 39% [7]. Women are among the most vulnerable demographics, owing to menstruation and pregnancy and childbirth-related blood loss. Anaemia in women of reproductive age can be caused by both nutritional and non-nutritional factors [9]. Anaemia has a high impact, particularly among pregnant women, because it raises the risk of miscarriage, intrauterine fetal death, premature delivery, low birth weight, and mortality [10]. Anaemia affects 43% of children under the age of five worldwide, with Sub Saharan African (SSA) countries having a greater prevalence. Despite the deployment of control programs such as iron supplementation, deworming, and the distribution of insecticide-treated bed nets, anaemia remains a serious global concern in child health, particularly in Sub-Saharan Africa [11]. While it may be difficult to distinguish the effects of anaemia (low hemoglobin (Hb)) from those of its underlying biological mechanisms (e.g., nutritional deficiencies, chronic infections, haemoglobinopathies), anaemia has been independently associated with overall increased mortality, lower cognitive performance, and, in severe cases, lower aerobic exercise capacity and heart failure in children [12, 13]. The pediatric brain requires more oxygen, therefore it is especially vulnerable to the effects of severe anaemia [11] iron deficiency (ID) is regarded to be the most common cause of anaemia , although other dietary deficiencies, such as those in vitamins A, B_{12}, B_6, C, D, and E, folate, riboflavin, copper, and zinc, can also cause anaemia [14, 15]. Vitamins A, B_6, B_{12}, folic acid, and riboflavin are among the nutrients needed for proper RBC formation. RBCs may be protected by other nutrients with antioxidant characteristics, such as vitamins C and E. Trace metals such as copper and zinc are found in the architecture of enzymes that regulate iron metabolism. It is critical to focus on reducing the prevalence of anaemia among reproductive-age women in order to improve positive pregnancy outcome, women's and children's health, academic achievement, productivity, and general development [7]. Anaemia reduction is one of the Sustainable Development Goals (SDGs) and World Health Assembly Nutrition Targets for 2025 [16, 17].

Despite the fact that mainstream therapies are utilized all over the world, the bulk of health problems in Sub-Saharan Africa, including anaemia , are treated using a decoction of fresh leaves, barks, and herbs [18], because of their availability, affordability and ethnobotanical considerations. Many conventional medications have negative side effects. They include iatrogenic adverse effects, which are directly related to the therapy and include nausea, vomiting, and black stools [19]. As a result, novel haematinic medicines with fewer side effects are being sought all around the world as an alternative therapy for anaemia.

Tapinanthus globiferus (Loranthaceae) is a widely widespread species of Viscum album that is usually referred to as Mistletoe. It is also known in Nigerian languages as Kauchi (in Hausa), Afomo onisanani (in Yoruba), and Apari / Awushie (in Igbo) [20].

T. globiferus is a semi-parasitic flowering plant that grows on deciduous host trees such as *Azadirachta indica* (neem) and *Albizia lebbeck* and can negatively impact their host's growth and fruiting. *Tapinanthus* species is used as a medicinal plant to treat metabolic, chronic, and other conditions. It has been scientifically proven to have antibacterial, antioxidant, anti-diabetic, antihypertensive, anticancer, and immunomodulatory properties, as well as wound healing properties. [21]. These actions were linked to variations in bioactive chemicals discovered in the plant and the host tree [22]. The current study investigated the haematinic properties of *T. globiferus* leaf extract in albino rats with anaemia triggered by phenylhydrazine (PHZ).

EXPERIMENTAL METHODS

Collection and processing of plant material. The leaves of *T. globiferus* were collected from fresh plant harvested from a wild in Udenu, Enugu State, Nigeria. The plant was authenticated by a taxonomist Dr. Afred A. Ozioko of the International Centre for Ethnomedicine and Drug Development (InterCEDD) in Nsukka, Enugu State, Nigeria, from which a voucher specimen (InterCEDD 301) was lodged. The leaves were cleaned with tap water and shade-dried for two weeks. Prior to extraction, the dried leaves were powdered with a miller (Lab mill, serial no. 4745, Christy and Norris Ltd, England), weighed, stored in an airtight container with clear labeling, and kept at room temperature.

Experimental animals. Albino rats of both sex weighing between 120 and 250g were purchased from the Enugu State University of Science and Technology's animal home in Agbani, Enugu state. They were housed in propylene cages with husk padding that were changed daily to ensure experimental cleanliness. Acclimatization took place for one week with a 12-hour dark-light cycle, a humidity of 50%, and a temperature of 25°C 15%. The animals were provided with standard Laboratory pellets and water *ad libitum*. The protocols followed the criteria for the proper care and use of animals established by Enugu State University of Science and Technology, Agbani, Enugu state.

Phytochemical screening. Phytochemical screening of the extract and fractions was performed using established procedures [23].

Standard drug. Astyfer syrup obtained via a local representative of Fidson Healthcare PLC Nigeria was used as standard therapy.

Preparation of methanol extract. Based on a modified procedure, 906.08 g of finely powdered leaves were macerated in 4.5 L of analytical-grade methanol for 72 hours [24]. The mixture was mixed on a regular basis and filtered twice, first with muslin cloth and then with Whatman No 1 filter papers. The resulting filtrate was concentrated using a rotary vacuum to obtain the methanol extract. The concentrated extract (MEcr) was weighed, labeled, and stored in a refrigerator at 4^{o}C before use.

Fractionation. The methanol crude extract (MEcr) was subjected to fractionation in a silica gel (70–230 nm mesh, Merck Germany) column using n-hexane, ethyl acetate and methanol solvents. The fractions were concentrated to obtain the *n*-hexane fraction (HF), ethyl acetate fraction (EF) and methanol fraction (MF).

Acute oral toxicity studies. Acute toxicity of MEcr of *T. globiferus* was performed using twelve adult rats of both sex [25]. Twelve mature rats of either sex, weighing between 60 and 140 kg, were employed in the test and had unrestricted access to food and clean water. In the first phase, nine (9) rats were used. The animals were allotted to three (3) groups of three (3) animals per group. Following the appropriate administration of intrabuccal dosages of 10, 100, and 1000 mg/kg of MEcr

of *T. globiferus* to each group, the animals were given free access to food and water and were observed for signs of mortality for a full day. Any mortality was recorded and the results obtained were used in phase two of the experiment. In the second phase, three rats were divided into three groups of a rat each and where administered 1900, 2600 and 5000 mg/kg of MEcr of *T. globiferus* respectively. The rats had unrestricted access to food and water, and then they were monitored over 24 hours for mortality.

Induction of anaemia. Hematological baseline values were established prior to the initiation of anaemia induction. Rats were given an intraperitoneal (i.p.) injection of 60 mg/kg phenyl hydrazine for three (3) days in order to induce anaemia [26]. When the rats' levels of hemoglobin (Hb) and red blood cells (RBC) dropped to at least 30% of their initial values, they were deemed anaemic. Group 1 (10 mg/kg Vehicle treated), group 2 (0.15 mg/kg Astyfer syrup), group 3 (methanol extract), group 4 (ethyl-acetate) and group 5 (n-Hexane) were given in three doses: 100, 200 and 400 mg/kg daily. Every seven days for the next 21 days, the PCV and HB concentrations were measured using a hematocrit reader, and the RBC count was assessed under a microscope before induction of anaemia, and subsequently at 7 days intervals over 21 days. The mean corpuscular hemoglobin (MCH), and mean corpuscular volume (MCV) were calculated using standard formula [31].

The MCV was calculated using the formula,
MCV= (PCV % X 10)/RBC ----------1

The MCH was calculated using the formula,
MCH = [HB (g per 100ml) X10]/RBC-------2

Haematological analysis. Before induction of anaemia, approximately 2 milliliters of blood were withdrawn from each animal using the retro orbital bleeding technique and placed in an EDTA vial. The parameters measured were the packed cell volume (PCV), red blood cell (RBC) based on [27] method and hemoglobin (Hb) based on cynomet Hb method [28].

Elemental analysis of *Tapinanthus globiferus* leaves. The elemental composition of the leaves of *T. globiferus* was determined using Atomic Absorption spectrophotometry (AAS) Varian AA 280 [29, 30].

Vitamin profile test of *Tapinanthus globiferus.* Vitamin A, C and E were evaluated as described by [31].

Amino acid profile. The amino acid profile of *T. globiferus* leaves were determined as described by [20]

Data analysis. The data obtained were analyzed using GraphPad Prism version 8.0.1 (www.graphpad.com, San Diego, California). Two-way analysis of variance was used to examine the data, and Turkey's post hoc test was used to identify group differences. The results were provided as mean ± standard deviation (SD). The threshold for significance was $p < 0.05$.

RESULTS

Percentage yield. Table 1 shows the yield of the methanol extract and the fractions of N-hexane and ethyl acetate with values **of** 84.35 g, 9.2 g, and 7.4 g respectively. The yield gotten from the leaves of *T. globiferus* after macerating 906.08 g of finely powdered leaves was 84.35 g. The percentage yield was calculated by dividing the amount of extract obtained by the amount of crude drug that was extracted and multiplying the result by 100. This gave a percentage yield of 9.31%.

Acute oral toxicity study. No mortality was recorded during the first and second phase of study.

Phytochemical analysis. Preliminary phytochemical screening carried out on the crude extract and fractions of *T. globiferus*

leaves revealed the presence of secondary metabolites such as alkaloids, flavonoids, saponins as summarized in the Table 2.

Effect of the *T. globiferus* extracts on the phenylhydrazine-induced anaemic rats. The extracts at dose 100, 200 and 400 mg/kg showed a haematinic effect but its haematinic effect was not dose dependent. The positive control and the various fractions of *T. globiferus* showed significant haematinic effect at $p < 0.05$ when compared to the negative control. Amongst the crude extract and fractions of *T. globiferus*, methanol extract (200 mg/kg) and *n*-hexane (100 mg/kg) had the highest haematinic activity (Table 6).

Effect of *T. globiferus* extract on the packed cell volume (PCV) of phenyl hydrazine induced anaemic rats. Table 6 shows the effect of *T. globiferus* on the PCV. The PCV of rats in different groups were comparable during the pre-treatment period. For instance, PCV ranged between 38.00±3.06 to 44.00±1.15. Administration of rats with phenyl hydrazine caused a significant ($p < 0.001$) decrease in PCV from negative control when compared to positive control on the 7th day. Treatment of the animals with *T. globiferus* reversed these anomalies in the blood to different degrees as shown in table 7. There is a non-dose dependent significant ($p < 0.001$) increase in PCV during the treatment period with the maximum effects observed at 200 mg/kg methanol extract (44.90±2.00) on the 21th day.

Haemoglobin concentration. Table 7 shows the effect of *T. globiferus* extract on the haemoglobin (Hb). The Hb of rats in different groups were comparable during the pre-treatment period. For instance, Hb ranged between 12.67±1.02 to 14.67±0.38. Administration of rats with phenyl hydrazine caused a significant ($p < 0.001$) decrease in Hb from negative control when compared to positive control from 7th to 21st day as shown in table 8. Treatment of the animals with *T. globiferus* extract reversed these anomalies in the blood to different degrees. There was significant ($p < 0.001$) increase in Hb during the treatment period with the maximum effects observed at 100 mg/kg n-hexane fraction (15.78±0.59) on the 14th day. The effect was not dose dependent.

Red blood cell count. Administration of rats with phenyl hydrazine caused a significant ($p < 0.001$) decrease in RBC table 8. There is no dose dependent significant ($p < 0.001$) increase in RBC during the treatment period with the maximum effects observed at 400 mg/kg methanol (5.07±0.41) and 200 mg/kg N-hexane (5.00±0.36) on the 14th day.

Mean corpuscular volume. Table 9 shows the effect of *T. globiferus* extract on the MCV. The MCV of rats in different groups were comparable during the pre-treatment period. Administration of rats with phenyl hydrazine caused a significant ($p < 0.001$) decrease in MCV in negative when compared to the positive control. Treatment of the animals with *T. globiferus* extract reversed these anomalies in the blood to different degrees. There is a no dose dependent significant ($p < 0.001$) increase in MCV during the treatment period with the maximum effects observed at 100mg/kg n-hexane fraction (94.16±5.93) on the 7th day.

Mean corpuscular haemoglobin. Table 10 shows the effect of *T. globiferus* extract and fractions on the MCH. Administration of rats with phenyl hydrazine caused a significant ($p < 0.05$) decrease in MCH in negative control when compared to the positive control. Treatment of the animals with *T. globiferus* extract and fractions reversed these anomalies in the blood to different degrees. There is a no dose dependent significant ($p < 0.05$) increase in MCH during the treatment period with the maximum effects observed at 100 mg/kg n-hexane fraction (31.39±1.98) on the 7th day.

Table 1: The yield of extract and fractions of *Tapinanthus globiferus*

	Initial weight (g)	Final weight (g)	Percentage yield (%)
Crude methanol extract	906.08	84.35	9.31
Ethyl-acetate fraction	20	7.4	37
n-Hexane fraction	20	9.2	46

Table 2: Phytochemical analysis of extract and fractions of *Tapinanthus globiferus* leaves

Phytoconstituents	Methanol crude	n-Hexane fraction	Ethyl-acetate fraction
Alkaloids	+	+	+
Flavonoids	+	+	+
Terpenoids	+	+	+
Tannins	+	+	+
Saponins	+	+	+
Cardiac glycosides	-	-	-
Steroids	+	+	+
Anthraquinone	-	-	-
Reducing sugars	-	-	-
Carbohydrates	-	-	-

+ = present - = absent

Table 3: Elemental profile of *Tapinanthus globiferus* leaf methanol extract.

Elements	Concentration (ppm)
Calcium (Ca)	21350.00
Iron (Fe)	8220.00
Magnesium (Mg)	4320.00
Potassium (K)	13200.00
Sodium (Na)	817.00
Phosphorus (P)	1210.00
Copper (Cu)	15.00
Zinc (Zn)	55.11
Lead (Pb)	142.60
Manganese (Mn)	44.39

Table 4: Vitamin profile of methanol extract of *Tapinanthus globiferus* is shown below:

Vitamins	Result
Vitamin A	+
Vitamin C	++
Vitamin E	++

+ = present - = absent

Table 5: Amino acid content of methanol extract of *Tapinanthus globiferus* leaf

Amino acids (g/100g protein)	Concentration (%)	Amino acids (g/100g protein)	Concentration (%)
Leucine	8.61	Glycine	9.46
Lysine	3.54	Alanine	4.23
Isoleucine	3.06	Arginine	6.10
Phenylalanine	4.50	Cysteine	1.04
Tryptophan	1.14	Proline	4.02
Methionine	1.42	Serine	4.27
Histidine	1.91	Aspartate	8.43
Threonine	5.12	Glutamate	10.34
Valine	3.93		

Table 6: Effect of extract and fractions of *Tapinanthus globiferus* on PCV of phenylhydrazine induced anaemic rats

Treatment	Packed cell volume (%)				
	Before induction	(0) days	7th day post administration	14th day post administration	21st day post administration
Normal saline (NC)	41.83±1.33	24.33±0.88	22.33±1.23***	20.80±1.50	20.60±1.12
Astyfer syrup 0.15 mg/kg (PC)	40.00±1.63	24.83±1.92	38.00±0.82	41.83±0.98	43.83±1.11
Methanol Extract 100 mg/kg	42.67±1.33	27.00±1.53	41.33±1.33***	43.67±0.33***	43.54±0.23***
Methanol Extract 200 mg/kg	39.33±2.19	22.33±1.45	43.33±0.67***###	44.67±1.76***	44.90±2.00***
Methanol Extract 400 mg/kg	42.00±1.53	25.33±4.33	39.00±2.08***	41.67±2.33***	43.33±2.91***
Ethyl acetate fraction 100 mg/kg	38.00±3.06	23.33±2.60	34.33±0.88	37.67±0.33	38.00±0.10
Ethyl acetate fraction 200 mg/kg	41.67±2.03	31.00±1.15***###	36.67±1.45	42.67±1.86##	44.00±0.58
Ethyl acetate fraction 400 mg/kg	42.00±2.52	24.33±1.45	37.00±2.52	42.67±1.33	43.33±0.67###
n-Hexane fraction 100 mg/kg	39.00±1.54	24.33±0.67	41.00±1.73	45.33±1.33***	44.67±0.67***
n-Hexane fraction 200 mg/kg	44.00±1.15	23.33±±1.86	41.00±3.79	44.33±2.33***	43.67±1.86***
n-Hexane fraction 400 mg/kg	40.67±0.67	21.33±2.02	34.33±2.03#	43.00±1.53***	43.00±1.50***

Results are presented as means ± standard deviation, n = 5, *, ** and ***: significantly different from the NC at $p < 0.05$, 0.01 and 0.001 respectively. #, ## and ###: significantly different from the PC at $p < 0.05$, 0.01 and 0.001 respectively. Two-way, ANOVA followed by Turkey HSD. NC is negative control, PC is positive control

Table 7: Effect of extract and fractions of the *Tapinanthus globiferus* on the haemoglobin concentration of phenylhydrazine induced anaemic rats

Treatment	Hemoglobin concentration (g/dl)				
	Before induction	(0) days	7th day post administration	14th day post administration	21st day post administration
Normal saline (NC)	13.94±0.44	8.08±0.29	7.44±0.41***	6.93±0.50***	6.87±0.37***
Astyfer syrup 0.15 mg/kg (PC)	13.34±0.54	8.25±0.63	12.67±0.27	13.94±0.33	14.61±0.37
Methanol Extract 100 mg/kg	14.22±0.45	8.97±0.52	13.78±0.45	14.46±0.11***	14.56±0.21***
Methanol Extract 200 mg/kg	13.11±0.73	7.40±0.49	14.45±0.22***	14.89±0.59***	14.89±0.59***
Methanol Extract 400 mg/kg	14.00±0.51	8.43±1.44	13.00±0.69***	13.22±0.29***	14.45±0.97***
Ethyl acetate fraction 100 mg/kg	12.67±1.02	7.73±0.87	11.44±0.29***#	12.56±0.11***#	12.67±0.10***#
Ethyl acetate fraction 200 mg/kg	13.89±0.67	10.30±0.40***###	12.22±0.49	14.22±0.62***	14.67±0.19***
Ethyl acetate fraction 400 mg/kg	14.00±0.84	8.10±0.49	12.33±0.84	14.22±0.45***	14.45±0.22***
n-Hexane fraction 100 mg/kg	13.00±0.39	8.07±0.23	13.67±0.58***#	15.78±0.59***#	14.89±0.22***
n-Hexane fraction 200 mg/kg	14.67±0.38	7.77±0.62	13.67±1.26	14.78±0.78***	14.55±0.62***
n-Hexane fraction 400 mg/kg	13.55±0.22	7.10±0.66	11.44±0.68	14.33±0.51***	14.38±0.61***

Results are presented as means ± standard deviation, n = 5. *, ** and ***: significantly different from the NC at $p < 0.05$, 0.01 and 0.001 respectively. #, ## and ###: significantly different from the PC at $p < 0.05$, 0.01 and 0.001 respectively. Two-way, ANOVA followed by Turkey HSD. NC is negative control, PC is positive control

Table 8: Effect of the extract and fractions of the *Tapinanthus globiferus* on the red blood cell count of phenylhydrazine induced anaemic rats

Treatment	Red blood cell count ($\times 10^{12}$/L)				
	Before induction	(0) days	7th day post administration	14th day post administration	21st day post administration
Normal saline (NC)	4.62±0.10	3.18±0.08	3.05±0.22***	2.98±0.32***	2.92±0.23***
Astyfer syrup 0.15 mg/kg (PC)	4.58±0.13	3.12±0.28	3.97±0.10	4.55±0.13	4.62±0.09
Methanol Extract 100 mg/kg	4.73±0.18	3.50±0.12	4.50±0.15***#	4.90±0.21***	4.77±0.09***
Methanol Extract 200 mg/kg	4.43±0.33	3.03±0.19	4.63±0.20***###	4.80±0.12***	4.80±0.06***
Methanol Extract 400 mg/kg	4.63±0.12	3.40±0.35	4.40±0.20***	5.07±0.41***#	4.77±0.12***
Ethyl acetate fraction 100 mg/kg	4.33±0.13	3.03±0.26	4.27±0.07***	4.17±0.12***	4.17±0.12***
Ethyl acetate fraction 200 mg/kg	4.70±0.20	3.80±0.10**##	4.50±0.11***#	4.73±0.07***	4.80±0.10***
Ethyl acetate fraction 400 mg/kg	4.57±0.23	3.20±0.20	4.17±0.32***	4.93±0.27***	4.93±0.27***
n-Hexane fraction 100 mg/kg	4.43±0.18	3.10±0.12	4.37±0.02***	4.87±0.03***	4.87±0.07***
n-Hexane fraction 200 mg/kg	5.03±0.23	3.13±0.23	4.77±0.52***###	5.00±0.36***	4.93±0.30***
n-Hexane fraction 400 mg/kg	4.50±0.30	3.07±0.20	4.27±0.34***	4.77±0.27***	4.77±0.37***

Results are presented as means ± standard deviation, n = 5, *, ** and ***: significantly different from the NC at $p < 0.05$, 0.01 and 0.001 respectively. $^{\#}$, $^{\#\#}$ and $^{\#\#\#}$: significantly different from the PC at $p < 0.05$, 0.01 and 0.001 respectively. Two-way, ANOVA followed by Turkey HSD. NC is negative control, PC is positive control

Table 9: Effect of extract and fractions of the *Tapinanthus globiferus* on mean corpuscular volume of phenylhydrazine induced anaemic rats

Treatment	Mean Corpuscular Volume (femtoliter)				
	Before induction	(0)days	7th day post administration	14th day post administration	21st day post administration
Normal saline (NC)	90.00±1.44	76.36±1.45	73.91±3.01***	71.09±4.25***	71.31±3.32***
Astyfer syrup 0.15 mg/kg (PC)	86.83±3.09	80.45±2.81	96.36±1.18	92.08±1.94	94.96±1.72
Methanol Extract 100 mg/kg	89.67±0.88	77.03±2.07	91.87±0.62***	89.48±4.33***	91.69±2.28**
Methanol Extract 200 mg/kg	88.67±2.03	73.60±1.30	93.77±2.94***	93.28±5.53***	93.14±4.54***
Methanol Extract 400 mg/kg	88.00±1.73	73.43±5.25	90.10±4.16***	84.02±11.31$^{***\#}$	91.72±6.60***
Ethyl acetate fraction 100 mg/kg	86.67±4.41	76.57±1.99	80.52±2.62$^{***\#\#\#}$	90.59±3.31***	91.35±2.58***
Ethyl acetate fraction 200 mg/kg	90.00±1.00	81.53±1.56	81.42±1.94$^{***\#\#\#}$	90.07±2.71***	91.67±1.20***
Ethyl acetate fraction 400 mg/kg	91.33±0.88	76.10±2.01	90.17±0.57***	86.73±2.29***	88.25±3.72***
n-Hexane fraction 100 mg/kg	88.00±3.79	78.53±1.47	94.16±5.93***	93.14±2.47***	91.83±2.26***
n-Hexane fraction 200 mg/kg	87.33±1.86	74.40±1.03	86.35±1.71$^{***\#\#\#}$	88.93±2.75***	88.76±2.92***
n-Hexane 400 mg/kg fraction	90.00±2.52	69.27±2.14$^{\#}$	80.84±3.05$^{***\#\#\#}$	90.59±4.28***	90.59±4.28***

Results are presented as means ± standard deviation, n = 5, *, ** and ***: significantly different from the NC at $p < 0.05$, 0.01 and 0.001 respectively. $^{\#}$, $^{\#\#}$ and $^{\#\#\#}$: significantly different from the PC at $p < 0.05$, 0.01 and 0.001 respectively. Two-way, ANOVA followed by Turkey HSD. NC is negative control, PC is positive control.

Table 10: Effect of extract and fractions of the *Tapinanthus globiferus* on the mean corpuscular haemoglobin of phenylhydrazine induced anaemic rats

Treatment	Mean corpuscular haemoglobin (picograms/cell)				
	Before induction	(0) day	7th day post administration	14th day post administration	21st day post administration
Normal saline (NC)	29.50±0.50	25.37±0.49	24.64±1.00*	23.69±1.42*	23.76±1.11**
Astyfer syrup 0.15 mg/kg (PC)	28.50±1.02	26.75±0.96	31.95±0.39	30.69±0.65	31.65±0.57
Methanol Extract 100 mg/kg	29.67±0.33	25.60±0.72	30.52±0.29	29.82±1.44*	30.56±0.76*
Methanol Extract 200 mg/kg	29.00±0.58	24.40±0.46	31.25±0.98	31.09±1.84	31.05±1.51*
Methanol Extract 400 mg/kg	29.67±0.33	24.43±1.76	30.03±1.39	28.00±3.77	30.57±2.20*
Ethyl acetate fraction 100 mg/kg	28.33±1.45	25.37±0.69	26.84±0.88	30.20±1.10*	30.45±0.86
Ethyl acetate fraction 200 mg/kg	29.00±0.58	27.07±0.57	27.13±0.40	30.02±0.90	30.22±0.71
Ethyl acetate fraction 400 mg/kg	30.33±0.33	25.30±0.68	30.05±0.19	28.91±0.76	29.41±1.24
n-Hexane fraction 100 mg/kg	29.00±1.15	26.03±0.44	31.39±1.98*	31.05±0.82*	30.61±0.75*
n-Hexane fraction 200 mg/kg	28.67±0.67	24.77±0.34	28.78±0.57	29.65±0.92	29.59±0.97
n-Hexane fraction 400 mg/kg	29.67±0.67	23.07±0.68	26.94±1.02	30.19±1.42	30.19±1.42

Results are presented as means ± standard deviation, n = 5, *, ** and ***: significantly different from the NC at $p < 0.05$, 0.01 and 0.001 respectively. #, ## and ###: significantly different from the PC at $p < 0.05$, 0.01 and 0.001 respectively. Two-way, ANOVA followed by Turkey HSD. NC is negative control, PC is positive control.

DISCUSSION

Phenylhydrazine (PHZ) and its derivatives were originally employed as antipyretics, studies have shown that they have deleterious effects on blood cells and can cause irreversible cellular damage [26]. Some of the adverse effects of phenylhydrazine include hemolytic anaemia, hypoxia, and inflammation [32], spleen, kidney, and liver damage [33]. The drug's capacity to trigger hemolysis is determined by how it interacts with RBCs. This reaction generates hydrogen peroxide and destroys the hemoglobin pigment by generating oxidized derivatives and hydrazine free radicals [34]. Because of its interaction with the plasma membrane, PHZ produces reactive oxygen species (ROS) in addition to lipid peroxidation and protein

oxidation. Hemolytic anaemia occurs when spectrin in the membrane cytoskeleton is damaged by oxidation [32]. The production of harmful free radicals during hydrazine microsomal oxidation has been linked to the liver toxicity of hydrazine derivatives.

Phenylhydrazine (PHZ), a strong oxidizing chemical, is widely used to induce hemolysis in animal models. Many illnesses, including hematological abnormalities, have oxidative stress as a major contributing factor in their origin, as is well known [35]. Hemolytic anaemia is caused by a variety of harmful cellular processes, including membrane lipid peroxidation and protein oxidation, which erode the integrity of RBC membranes and are caused by the autoxidation of PHZ [36].

Treatment of PHZ-induced anaemic rats with the reference hematinic, Astyfer syrup resulted in a significant increase ($P < 0.001$) in the number of RBCs and Hb concentration compared with the vehicle-treated PHZ-induced anaemic rats. Astyfer syrup contains vitamins B1, B2, B3, B6 and B12, zinc, folic acid and iron. Hb is formed by the combination of globin chains with iron, which together form the nucleus of the iron-porphyrin haem ring. The B complex vitamins serve as building blocks for the production of cofactors necessary for protein synthesis and hematopoiesis [37, 38]. This may have accounted for the increase in number of RBCs and Hb concentration shown by the rats fed Astyfer syup. Treatment of PHZ-induced anaemic rats with the extract and fractions showed the significant increase ($P < 0.001$) in the number of PCV, HB, RBCs, MCV and ($P < 0.05$) MCH concentration over the experimental period as shown in Tables 6, 7, 8, 9 and 10 respectively. Amino acids such as threonine, valine, leucine, isoleucine, tryptophan, lysine, phenylalanine, methionine, histidine, and arginine are essential for plasma protein production as shown in Table 5. The phytochemicals in the plant extracts may be responsible for the rise in RBC count [39-41], which could counteract the negative consequences of PHZ-induced anaemia. Numerous studies have demonstrated that *T. globiferus* protects against oxidative stress-related diseases[42, 43]. The ability of plant extract to scavenge free radicals is attributed to a broad range of antioxidants of the polyphenol class [44] as shown in Table 2.

T. globiferus is a plant known for its antioxidant properties, which may have decreased hemolysis and enhanced oxygen transport to the organs by reducing the damaging effects of PHZ on the erythrocyte membrane. Rats treated with PHZ had reduced levels of PCV, RBC, HB, MCV, and MCHC. Following a 21-day course of treatment with an oral *T. globiferus* leaf extract and fractions, the effects of PHZ-induced anaemia were restored to baseline levels. The treatment resulted in a significant increase in the level of RBC and HB ($p<0.001$). Comparable to the normal control, the improvement observed following therapy with *T. globiferus* extract and fractions were none dose dependent. Given that the two primary variables in this model are dose and time, it has been demonstrated that different reactions to the administration of PHZ are feasible. Not all researchers used the same quantities to induce anaemia —some used 40 mg/kg once, while others used bigger doses—60 mg/kg for varying lengths of time. This could be the reason for the observed disparities in immunological response, together with species differences [28]. It is often recognized that *T. globiferus* leaves are a good source of minerals (iron, copper, zinc), vitamins (A, C and E), and other beneficial components [20, 30] as shown in Table 3 and Table 4. These results are consistent with reports on extracts of the plants *Solanum torvum* and *Tectona grandisin* PHZ-induced anaemia in rats [45, 46]. Iron deficiency limits the synthesis of hemoglobin, the oxygen transporter, so iron is the main treatment for anaemia. Anaemia and iron metabolism disruption are known side

effects of PHZ [47]. Zinc supplementation in animals is likely to have increased erythropoiesis, which in turn increased hemoglobin production and red blood cell formation [48, 49]. *T. globiferus* as good source of magnesium and zinc as shown in Table 3. it has been demonstrated that the necessary elements magnesium and zinc have strong antioxidant activity and are useful in preventing oxidative stress in cells by strengthening internal antioxidant defense systems[50]. The observed anti-oxidant and hematinic activities of *T. globiferus* extract and fractions in this study may be attributed to the presence of these metal elements.

Conclusion. The extract and fractions of *T. globiferus* leaves exhibited a marked hematinic activity. This gives scientific evidence to the folkloric claims of its use in the management of anemic disorder. More mechanistic research is required to determine the exact mechanisms by which the extract and fractions displayed the observed hematinic activities.

REFERENCES

1. Natekar P, Deshmukh C, Limaye D, Ramanathan V, Pawar A. A micro review of a nutritional public health challenge: iron deficiency anaemia in India. Clinical Epidemiology and Global Health. 2022;14:100992.

2. Milman N. Anaemia —still a major health problem in many parts of the world! Annals of hematology. 2011;90:369-77.

3. Musallam KM, Taher AT. Iron deficiency beyond erythropoiesis: should we be concerned? Current medical research and opinion. 2018;34(1):81-93.

4. Prasanth R. Prevalence of anaemia in both developing and developed countries around the world. World J Anaemia . 2017;1(2):40-3.

5. Mosieri C, Chandler D, Reed DS, Craig MK, Hyatali F, Kallurkar A, et al. Managing preoperative anaemia: evolving concepts and strategies for improving patient outcomes. Best Practice & Research Clinical Anaesthesiology. 2020;34(2):183-97.

6. Tesfaye TS, Tessema F, Jarso H. Prevalence of anaemia and associated factors among "apparently healthy" urban and rural residents in Ethiopia: a comparative cross-sectional study. Journal of Blood Medicine. 2020:89-96.

7. Zegeye B, Anyiam FE, Ahinkorah BO, Ameyaw EK, Budu E, Seidu A-A, et al. Prevalence of anaemia and its associated factors among married women in 19 sub-Saharan African countries. Archives of Public Health. 2021;79(1):1-12.

8. Sosa-Moreno A, Reinoso-Gonzalez S, Mendez MA. Anaemia in women of reproductive age in Ecuador: Data from a national survey. PloS one. 2020;15(9):e0239585.

9. Wu Y, Ye H, Liu J, Ma Q, Yuan Y, Pang Q, et al. Prevalence of anaemia and sociodemographic characteristics among pregnant and non-pregnant women in southwest China: a longitudinal observational study. BMC pregnancy and childbirth. 2020;20:1-10.

10. Stephen G, Mgongo M, Hussein Hashim T, Katanga J, Stray-Pedersen B, Msuya SE. Anaemia in Pregnancy: Prevalence, Risk Factors, and Adverse Perinatal Outcomes in Northern Tanzania. Anaemia . 2018;2018:1846280.

11. Moschovis PP, Wiens MO, Arlington L, Antsygina O, Hayden D, Dzik W, et al. Individual, maternal and household risk factors for anaemia among young children in sub-Saharan Africa: a cross-sectional study. BMJ open. 2018;8(5):e019654.

12. Pivina L, Semenova Y, Doşa MD, Dauletyarova M, Bjørklund G. Iron deficiency, cognitive functions, and neurobehavioral disorders in children. Journal of Molecular Neuroscience. 2019;68:1-10.

13. Ebner N, Jankowska EA, Ponikowski P, Lainscak M, Elsner S, Sliziuk V, et al. The impact of iron deficiency and anaemia on exercise capacity and outcomes in patients with chronic heart failure. Results from the Studies Investigating Co-morbidities Aggravating Heart Failure. International journal of cardiology. 2016;205:6-12.

14. Godswill AG, Somtochukwu IV, Ikechukwu AO, Kate EC. Health benefits of micronutrients (vitamins and minerals) and their associated deficiency diseases: A systematic review. International Journal of Food Sciences. 2020;3(1):1-32.

15. Chaparro CM, Suchdev PS. Anaemia epidemiology, pathophysiology, and etiology in low- and middle-income countries. Annals of the new York Academy of Sciences. 2019;1450(1):15-31.

16. Stevens GA, Paciorek CJ, Flores-Urrutia MC, Borghi E, Namaste S, Wirth JP, et al. National, regional, and global estimates of anaemia by severity in women and children for 2000–19: a pooled analysis of population-representative data. The Lancet Global Health. 2022;10(5):e627-e39.

17. Al-Jawaldeh A, Taktouk M, Doggui R, Abdollahi Z, Achakzai B, Aguenaou H, et al. Are countries of the eastern mediterranean region on track towards meeting the world health assembly target for anaemia ? A review of evidence. International Journal of Environmental Research and Public Health. 2021;18(5):2449.

18. Schultz F, Anywar G, Wack B, Quave CL, Garbe L-A. Ethnobotanical study of selected medicinal plants traditionally used in the rural Greater Mpigi region of Uganda. Journal of Ethnopharmacology. 2020;256:112742.

19. Devlin EJ, Denson LA, Whitford HS. Cancer treatment side effects: a meta-analysis of the relationship between response expectancies and experience. Journal of pain and symptom management. 2017;54(2):245-58. e2.

20. Oseni F, Ibrahim S, Ahmad H, Abubakar M, Abbas S. Phytochemicals in methanolic leaf extracts of Tapinanthus globiferous from Azadirachta indica and Albizia lebbeck: A comparative analysis of some pharmacological potentials. Bayero Journal of Pure and Applied Sciences. 2022;13(1):223-9.

21. Szurpnicka A, Kowalczuk A, Szterk A. Biological activity of mistletoe: in vitro and in vivo studies and mechanisms of action. Archives of Pharmacal Research. 2020;43:593-629.

22. Singh BN, Saha C, Galun D, Upreti DK, Bayry J, Kaveri SV. European Viscum album: a potent phytotherapeutic agent with multifarious phytochemicals, pharmacological properties and clinical evidence. RSC advances. 2016;6(28):23837-57.

23. Harborne A. Phytochemical methods a guide to modern techniques of plant analysis: springer science & business media; 1998.

24. Ekalu A, Habila JD. Flavonoids: isolation, characterization, and health benefits. Beni-Suef University Journal of Basic and Applied Sciences. 2020;9(1):1-14.

25. Lorke D. A new approach to practical acute toxicity testing. Archives of toxicology. 1983;54:275-87.

26. Pandey K, Meena AK, Jain A, Singh R. Molecular mechanism of phenylhydrazine induced haematotoxicity: a review. Ame J Phytomed Clin Therapeut. 2014;2(3):390-4.

27. Ihedioha JI, Daniel-Igwe G. Development of an experimental albino rat (Rattus norvegicus) model for studies on sub-acute blood loss anaemia. Animal Research International. 2014;11(1):1889–98-–98.

28. Okolo KO. Protective effects of hydro-ethanolic leaf extract based formulation of Mucuna pruriens (Fabaceae) on phenylhydrazine induced hemolytic anaemia and metabo-hematological alterations in rats. Comparative Clinical Pathology. 2021;30(5):765-74.

29. Okerulu I, Onyema C, Onwukeme V, Ezeh C. Assessment of phytochemicals, proximate and elemental composition of Pterocarpus soyauxii (Oha) leaves. American journal of analytical chemistry. 2017;8(06):406.

30. Jeremiah C, Katsayal U, Nuhu A, Nuhu H. Pharmacognostic and elemental analysis of the leaves of Tapinanthus globiferus (A. Rich). Tiegh Res J Pharmacogn. 2019;6(1):11-8.

31. Rutkowski M, Grzegorczyk K. Adverse effects of antioxidative vitamins. International journal of occupational medicine and environmental health. 2012;25(2):105-21.

32. Allahmoradi M, Alimohammadi S, Cheraghi H. Protective effect of cynara scolymus l. on blood biochemical parameters and liver histopathological changes in phenylhydrazine-induced hemolytic anaemia in rats. Pharmaceutical and Biomedical Research. 2019;5(4):53-62.

33. Ekweogu CN, Ude VC, Nwankpa P, Emmanuel O, Ugbogu EA. Ameliorative effect of aqueous leaf extract of Solanum aethiopicum on phenylhydrazine-induced anaemia and toxicity in rats. Toxicological research. 2020;36:227-38.

34. Aloke C, Emelike CU, Obasi NA, Ogbu PN, Edeogu CO, Uzomba CG, et al. HPLC profiling and studies on Copaifera salikounda methanol leaf extract on phenylhydrazine-induced hematotoxicity and oxidative stress in rats. Arabian Journal of Chemistry. 2021;14(12):103428.

35. Cecchini R, Cecchini AL. SARS-CoV-2 infection pathogenesis is related to oxidative stress as a response to aggression. Medical hypotheses. 2020;143:110102.

36. Banerjee A, Dey T, Ghosh AK, Mishra S, Bandyopadhyay D, Chattopadhyay A. Insights into the ameliorative effect of oleic acid in rejuvenating phenylhydrazine induced oxidative stress mediated

morpho-functionally dismantled erythrocytes. Toxicology Reports. 2020;7:1551-63.

37. Godoy-Parejo C, Deng C, Zhang Y, Liu W, Chen G. Roles of vitamins in stem cells. Cellular and Molecular Life Sciences. 2020;77(9):1771-91.

38. Oliveira DC, Nogueira-Pedro A, Santos EW, Hastreiter A, Silva GB, Borelli P, et al. A review of select minerals influencing the haematopoietic process. Nutrition Research Reviews. 2018;31(2):267-80.

39. Checkouri E, Reignier F, Robert-Da Silva C, Meilhac O. Evaluation of polyphenol content and antioxidant capacity of aqueous extracts from eight medicinal plants from reunion island: Protection against oxidative stress in red blood cells and preadipocytes. Antioxidants. 2020;9(10):959.

40. Mbanaso E, Nwankwo A, Ijioma SN, Emmanuel O, Ugbogu EA, Nwagbara N, et al. Haematoprotective and red blood cell membrane stabilizing effects of Justicia carnae leaf extracts in sodium nitrate-treated rats. Journal of basic and clinical physiology and pharmacology. 2020;32(1):20190275.

41. Igbinaduwa PO, Kabari KM, Chikwue TC. Phytochemical and Anti-Anaemic Properties of Ethanol Leaf Extract of Justicia carneaVahl (Acantheceae). Nigerian Journal of Pharmaceutical and Applied Science Research. 2019;8(2):55-61.

42. Nworah F, Chukwumma I, Osuji D, Ezeako E. Assessment of the Aqueous Methanol Extract and Ethyl Acetate Fraction of Tapinanthus globiferus Leaves and their Effects on Ulceration and Leukocyte Mobilization in Wistar Albino Rats. 2021.

43. Abubakar K, Yunus A, Abubakar M, Ugwah-Oguejiofor J, Muhammad A. Antioxidant and antikindling effect of Tapinanthus globiferus growing on Ficus glumosa in pentylenetetrazole induced kindled rats. Afr J Biotechnol. 2018;17(4):73-80.

44. Kumar S, Lemos M, Sharma M, Shriram V. Free radicals and antioxidants. Adv Appl Sci Res. 2011;2(1):129-35.

45. Agbor GA, Oben JE, Ngogang JY. Haematinic activity of Hibiscus cannabinus. African journal of Biotechnology. 2005;4(8):833-7.

46. Yamoah A, Adosraku RK, Amenu JD, Baah MK, Abaye DA. Evaluation of the haematinic activities of extracts of Justicia secunda Vahl leaves in red blood cells of laboratory rats. Journal of Biosciences and Medicines. 2020;8(3):48-57.

47. Abd-El-Fattah ME, Dessouki AA, Abdelnaeim NS, Emam BM. Protective effect of Beta vulgaris roots supplementation on anemic phenylhydrazine-intoxicated rats. Environmental Science and Pollution Research. 2021;28:65731-42.

48. Jeng S-S, Chen Y-H. Association of Zinc with Anaemia. Nutrients. 2022;14(22):4918.

49. Myint ZW, Oo TH, Thein KZ, Tun AM, Saeed H. Copper deficiency anaemia. Annals of hematology. 2018;97:1527-34.

50. Umarudeen AM, Chika A. Phytochemical, elemental and in-vitro anti-oxidant activity screening of crude methanol Tapinanthus globiferus leaf extract. Int Arch Med Sci. 2019;1:29-34.