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Antioxidant and Anti-Anemic Effects of Ethanol Leaf Extracts of *Mucuna poggei* and *Telfairia occidentalis* in Phenyl-Hydrazine-Induced Anemia in Wistar Albino Rats

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Abstract

Introduction With the use of recent therapeutic data, several methods for finding medications from untapped natural materials continue to advance research and development. The antioxidant and antianemic effects of ethanol leaf extracts of *Telfairia occidentalis* and *Mucuna poggei* in phenylhydrazine-induced anemia in Wister albino rats were investigated.

Method A total of 54 male albino rats were randomly assigned to nine experimental groups ($n = 6$). Anemia was induced in groups 2 to 9 with 10 mg/kg body weight of phenylhydrazine. Group 1 (normal control) rats were administered normal saline only. Group 2 (anemic control) rats were untreated. Group 3 (standard controls) rats were treated with a standard multivitamin, groups 4, 5, and 6 rats were treated with varying doses of *M. poggei* leaf extract respectively, while groups 7, 8, and 9 rats were treated with similar doses of *T. occidentalis* leaf extract by oral intubation for 21 days.

Keywords
- *Mucuna poggei*
- *Telfairia occidentalis*
- phenylhydrazine
- antioxidant
- oxidative stress

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Results Earlier, the gas chromatography-mass spectrometry analysis indicated eighteen (18) and twenty-nine (29) active compounds present in *M. poggei and T. occidentalis*, respectively. More so, *T. occidentalis* and *M. poggei* were studied for their inhibitory potentials against 2,2-diphenyl-1 picrylhydrazyl-free radicals, nitric oxide, phosphomolybdenum, and ferric-reducing antioxidant power. *M. poggei and T. occidentalis* also showed the following trend of minerals Mg > Ca > Fe > Na > Zn > Mn > K and Mg > Fe > Ca > Na > K > Mn > Zn, respectively. The results also showed that the packed cell volume, red blood cells, and hemoglobin significantly ($p < 0.05$) decreased in anemic control rats compared with normal controls and were significantly ($p < 0.05$) increased on treatment with the leaf extracts. Catalase, superoxide dismutase, glutathione peroxidase activities, and glutathione levels significantly ($p < 0.05$) decreased in anemic control, compared with normal. However, these significantly ($p < 0.05$) increased on treatment with the leaf extracts in a dose-independent manner. Malondialdehyde levels took the reverse trend.

Conclusion This study indicated that leaf extracts of *M. poggei* and *T. occidentalis* can be used in the management of anemia and oxidative stress-related complications

Introduction

Since ancient times, people have used plants—whether they are herbs, shrubs, or trees—in various forms to cure and manage various illnesses and ailments.[1] According to the World Health Organization (WHO),[2] medicinal plants are plants that contain compounds which can be utilized therapeutically or as building blocks for the production of effective pharmaceuticals.[3]

One of these plants, *Mucuna poggei*, has morphological traits that deter both human and animals from approaching it. Its botanical classification was earlier given by Obioma et al.[3] The majority of rural residents in various areas of Nigeria, particularly the Igbo-speaking population, have turned to oral administration of the unprocessed aqueous extract of *M. poggei* as the most affordable source of multivitamins to raise blood levels.[3]

The tropical vine *Telfairia occidentalis*, also known as the "fluted pumpkin" in Ugu-Igbo, is planted in West and Central Africa for its edible seeds and as a leaf vegetable. Due to its high concentration of blood-enriching minerals, the leaf is used locally as a blood booster.[4,5]

The WHO claims that when your hemoglobin (Hb) or red blood cell (RBC) or pack cell volume (PCV) count are below normal, a disease known as anemia can develop.[6] These need to depend on several factors.[2] Over 30% of the world's population, particularly youngsters, suffer from anemia, which has had major economic repercussions and created barriers to national growth.[7] Anemia is more common in developing nations than in wealthy ones.[8] Children's normal development is impacted by anemia, which is a serious health issue for young children in underdeveloped nations[9]

In experimental settings, phenylhydrazine (PHZ) has been used to cause hemolytic anemia in animal models. One of the experimental models for examining the hematinic effects of medicines is PHZ-induced anemia.[10] When PHZ auto-oxidizes, reactive oxygen species (ROS) and PHZ-derived radicals are produced, which can result in a range of harmful cellular reactions, including hemolytic anemia.[11] The antioxidant and antianemic properties of ethanol leaf extracts of *M. poggei* and *T. occidentalis* were examined in this study in PHZ-induced anemia in Wister albino rats.

Materials and Methods

Materials

Chemicals and reagents used were of analytical grade, while animals used were 54 healthy adult male albino rats obtained from Daniel Okoro farms in Abakaliki, Ebonyi State, Nigeria. A 2-week period before the start of the experiment was given to the animals to acclimate to the laboratory setting. Throughout the stabilization period, they were housed in cages and provided a regular pellet meal along with water.

Methods

Collection, Preparation, and Extraction of Leave Samples

M. poggei and *T. occidentalis* fresh, matured leaves were gathered from a nearby farm in Okpoto, Ishielu local government area, Ebonyi state. The leaves were cleaned and allowed to air dry for 3 weeks at room temperature. Dry samples were processed into powder using a mechanical grinder after 3 weeks. For later usage, the powder was sieved using a sieve with a mesh size of 1 mm before being placed in polythene bags. The combined leaves were steeped for 48 hours in absolute ethanol at a 2:1 sample to solvent ratio according to the method of Maurice[12] Using a sieve cloth and Whatman No. 2 filter paper (Cat. No. 1001 125) with a pore size of 125 mm, each extracted solution was removed. By distilling the solvent from the filtrates, they were concentrated, and they were then dried out on a water bath at 45°C.

Then, the samples were then stored in refrigerator for subsequent usage.

Gas Chromatography-Mass Spectrometry (GC-MS) of Extracts

The Agilent GC-MS (Model No. 19091S-933) equipped with an HP-1MS capillary column (0.25 mm i.d. 30 m specified length 0.25 mL film thickness) and an auto sampler was used to analyze the chemical composition of the extracts. The aforementioned assay conditions were kept constant: Initial temperature and time were 70°C for 2 minutes; temperature was raised to 350°C at a rate of 20°C/min and held for 20 minutes. The total ion chromatogram was used to calculate percentages.

Mineral Analyses

The minerals analysis was performed in accordance with Csuros and Csuros instructions.[13] Using an atomic absorption spectrophotometer, the aqueous leaf extracts of *M. poggei* and *T. occidentalis* were examined separately for the presence of the minerals iron, zinc, copper, manganese, magnesium, potassium, sodium, calcium, and phosphorus.

In Vitro *Antioxidant Activity of Mucuna poggei* and *Telfairia occidentalis*

In vitro antioxidant scavenging activity of the extracts on 2,2-diphenyl-1-picrylhydrazyl (DPPH), nitric oxide, phosphomolybdenum reduction, and ferric-reducing antioxidant power (FRAP) were assayed using standard methods. DPPH-free radicals was assayed using the Kedare and Singh technique.[14] Nitric oxide scavenging activity was done in accordance with what Jagetia and Baliga described.[15] Phosphomolybdenum reduction assay was evaluated by the phosphomolybdenum method as described by Prieto et al,[16] and FRAP was assayed using a slightly modified version of Güder and Korkmaz.[17]

Experimental Design

A total of 54 male albino rats, weighing between 170 and 210 g, were divided into nine experimental groups of 1 to 9 ($n = 6$). PHZ was used to produce anemia in groups 2 to 9 by intraperitoneally delivering a daily dose of 10 mg/kg body weight for 4 days in a row.[18] Rats in group 1 (normal controls) received only normal saline injections. Group 2 (anemic control) rats were induced with anemia and left without treatment. Group 3 (standard control) rats were treated with standard multivitamin. Groups 4 to 6 were treated with 400, 200, and 100 mg/kg body weight of *M. poggei* ethanol leaf extract, respectively, while groups 7 to 9 were treated with 400, 200, and 100 mg/kg body weight of *T. occidentalis* ethanol leaf extract, respectively, by oral intubation. The animals in all groups were allowed free access to feed and water and the treatment lasted for 21 days.

Induction of Anemia

Anemia was induced by the modified method of Ogunrinola et al.[19] Anemia was induced in groups 2 to 9 with PHZ by administering a daily dose of 10 mg/kg body weight for 4 consecutive days, intraperitoneally making a total dose of 40 mg/kg body weight. Anemia was indicated by 40% reduction in packed cell volume (PCV), Hb, and RBC counts values of all the anemia-induced rat groups from the baseline values 4 days after administration of PHZ. On the day of establishment of anemia (day 5), the weight, PCV, Hb, RBC concentrations, and other hematological indices were taken in all the groups. After the final treatment, the meal was discontinued after the first 21 days. With unlimited access to water, the rats starved for the entire night. They were then given a chloroform anesthesia before being sacrificed. Using sterilized syringes and needles, 3 mL of whole blood was taken from each rat's heart during cardiac puncture. There were two portions of blood. To conduct the hematological assay, 1 mL of blood was drawn and placed into ethylenediaminetetraacetic acid sample vials with labels. The last 2 mL were transferred into clean tubes and left to clot for roughly two hours. After that the clot-filled blood was spun at 3,000 rpm (rpm) for 15 minutes to separate the serum from the clot-forming cells. The serum was examined using biochemical methods. To separate the serum for biochemical analysis, sterile syringes and needles were employed. These procedures were approved by the Department of Biochemistry Ethical Committee on Research, Innovation, and Institutional Ethical Committee (EBSU/ET/18/001).

Hematological Analysis

Manufactured in China 2020, the BE10 Automated Haematology Analyzer Midray was used to calculate the hematological parameters. Through the heart puncture, blood samples were obtained. On day 5 to confirm anemia, PCV, Hb, RBC concentrations, and other hematological indices were measured in all the groups. On day 21, the effect of treatment with extracts and the industry-standard multivitamin (HS-12) on these parameters was assessed. These tests served as indicators of the two plants' ethanol leaf extracts' antianemic properties.

Evaluation of Oxidative Stress Indicators

Lipid peroxidation was assayed using the method of Buege and Aust method in calculating the amount of thiobarbituric acid reactive substances (TBARS).[20] Antioxidant activity was assayed as follows; the Aebi technique was used to measure the catalase activity.[21] While the method published by Li., in 2012 for measuring superoxide dismutase (SOD) activity was used in measuring SOD.[22] The method used for the reduced glutathione test (GSH) was based on a concept first introduced by Ellman in 1959 and then updated by Hu in 1994.[23] The method used to measure glutathione peroxidase (GPX) activity was as modified by Hu and described by Ellman in the measurement of sulfhydryl groups.[24]

Statistical Analysis

The outcomes were presented as mean and standard deviation. The Turkey post-hoc test was used to perform a one-way analysis of variance on the data. Software called Graph Pad Prism 7 was used to analyze the data. p-Values under 0.05 ($p < 0.05$) were regarded as statistically significant.

Table 1 Chemical profile of *Mucuna poggei* obtained from gas chromatography-mass spectrometry (GCMS) analysis

SN	RT	TC (%)	Library ID (NISTILL)	Molecular formula	Quality (%)
1	13.096	1.23	p-Menth-8(10)-en-9-ol, cis-	$C_{10}H_{18}O$	38
2	13.428	12.93	Phytol, acetate	$C_{22}H_{42}O_2$	80
3	13.600	2.15	1,4-Eicosadiene	$C_{20}H_{38}$	70
4	13.725	3.69	1,13-Tetradecadiene	$C_{14}H_{26}$	64
5	14.017	0.53	2(1H)-Naphthalenone	$C_{10}H_8O$	64
6	14.343	0.28	5,6,6-Trimethyl-5-(3-oxobut-1-enyl)-1-oxaspiro[2.5]octan-4-one	$C_{14}H_{20}O_3$	44
7	14.458	0.69	Hexadecanoic acid	$C_{16}H_{32}O_2$	98
8	14.561	0.69	2,3-Dimethoxy-5-methyl-6-dekaisoprenyl-chinon	$C_{59}H_{90}O_4$	76
9	14.698	1.60	Cyclohexanecarboxamide	$C_7H_{13}NO$	35
10	15.110	1.34	4-Decenoic acid	$C_{10}H_{18}O_2$	38
11	15.539	6.81	(+)-Isomenthol	$C_{10}H_{20}O$	47
12	15.700	25.37	Ethyl 9,12,15-octadecatrienoate	$C_{20}H_{34}O_2$	99
13	15.848	08.37	Octadecanoic acid	$C_{18}H_{36}O_2$	99
14	17.439	1.35	6-Nonenal	$C_9H_{16}O$	30
15	17.748	2.45	Nonanoic acid	$C_9H_{18}O_2$	38
16	18.051	12.80	Cholest-2-ene-2-methanol	$C_{28}H_{48}O$	43
17	18.211	3.55	4-Oxatricyclo[20.8.0.0(7,16)]triac onta-1(20),7(16)-diene	$C_{29}H_{50}O$	45
18	19.379	1.04	9,19-Cyclolanost-24-en-3-ol	$C_{30}H_{50}O$	62

Abbreviations: RT, retention time; TC, total concentration.

Results

Chemical profile of *Telfairia occidentalis* and *Mucuna poggei* Obtained from GCMS

The GCMS analysis for the studied plants *M. poggei* and *T. occidentalis* is shown in ►**Tables 1** and **2**. Eighteen (18) and twenty-nine (29) active compounds were discovered in *M. poggei and T. occidentalis*, respectively. However, some were repeatedly observed at different retention times given the actual active principles to be relatively less than the appearance in retention time. In *M. poggei*, Cholest-2-ene-2-methanol, ethyl 9,12,15-octadecatrienoate, and 1,4-eicosadiene showed higher concentration of 12.80, 25.37, and 12.93%, respectively. However, in *T. occidentalis,* gamma-sitosterol,4,4,6a,6b,8a,11,12,14b-octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9, 10,11,12,12a,14, 14a,14b-octadecahydro-2H-picen-3-one and hexadecanoic acid were significantly present in higher concentrations with percentages given as 23.69, 5.24, and 6.50%, respectively.

Mineral Composition of Some Selected Minerals in Solvent Leaf Extracts of *M. poggei* and *T. occidentalis.*

The composition of some selected minerals in solvent leaf extracts of *M. poggei* and *T. occidentalis* indicated that the concentrations of the macro minerals K and P were significantly higher ($p < 0.05$) in *T. occidentalis* than in *M. poggei*, while the concentrations of Mg and Na were higher but not significantly ($p > 0.05$) in *T. occidentalis* than in *M. poggei.* Nevertheless, the concentration of the macro mineral Ca followed the reverse trend in that its concentration was significantly higher ($p < 0.05$) in *M. poggei* than in *T. occidentalis* (►**Table 3**). The concentrations of the micro minerals Fe and Zn were significantly higher ($p < 0.05$) in *M. poggei* than in *T. occidentalis*, while the concentrations of Mn and Cu were higher but not significantly ($p > 0.05$) in *M. poggei* than in *T. occidentalis.* The concentrations of the minerals in aqueous leaf extracts of *T. occidentalis* were of the trend Mg > Fe > Ca > Na > K > Mn > Zn > P> Cu. The range in *T. occidentalis* was 0.09 ± 0.004 mg/L of Cu to 19.61 ± 0.03 mg/L of Mg, while that of *M. poggei* was of the trend Mg > Ca > Fe > Na > Zn > Mn > K > P> Cu and the range in *M. poggei* was 0.10 ± 0.01 mg/L of Cu to 19.48 ± 0.03 mg/L of Mg. In both *T. occidentalis* and *M. poggei,* Mg had the highest concentration, while Cu had the lowest concentration.

In Vitro Antioxidant Activities of *M. poggei* and *T. occidentalis*

►**Fig. 1 A–D** is an indication of in vitro antioxidant assays for DPPH-free radicals, nitric oxide scavenging activity, phosphomolybdenum reduction assay, and FRAP percentage inhibition of the leaf extracts of *T. occidentalis* and *M. poggei* at different concentrations of 0.1, 0.5, 1.0, and 2.0 (mg/mL). *M. poggei* exhibited stronger inhibitory potentials against DPPH and phosphomolybdenum-free radicals (►**Fig. 1A** and **C**), while *T. occidentalis* showed a stronger inhibitory potential against NO and FRAP-free radicals. (►**Fig. 1B** and **D**)

Effect of Ethanol Leaf Extracts of *Mucuna poggei* and *Telfairia occidentalis* on Indices of Anemia

The result in ►**Table 4** showed the effect of ethanol leaf extracts of *Mucuna poggei* and *Telfairia occidentalis* on HCT

Table 2 Chemical profile of *Telfairia occidentalis* obtained from gas chromatography-mass spectrometry (GCMS) analysis

SN	RT	TC (%)	Library ID (NISTILL)	Molecular formula	Quality (%)
1	2.447	0.19	Butane	C_4H_{10}	80
2	6.493	0.18	2,6-Octadiene	C_8H_{14}	53
3	11.122	0.23	2,6,10-Dodecatrien-1-ol	$C_{12}H_{20}O$	80
4	11.694	0.15	Diethyl phthalate	$C_{12}H_{14}O_4$	96
5	12.547	2.25	1,4-Dimethyl-8-isopropylidenetricy clo[5.3.0.0(4,10)] decane	$C_{15}H_{24}$	50
6	12.638	1.28	1,4-Dimethyl-8-isopropylidenetricy clo[5.3.0.0(4,10)] decane	$C_{15}H_{24}$	58
7	12.844	2.43	2H-Cyclopentacyclooctene, 4,5,6,7, 8,9-hexahydro-1,2,2,3-tetramethyl-	$C_{15}H_{24}$	49
8	13.090	1.20	Nonanoic acid, 2,4,6-trimethyl-, ethyl ester, (2R,4S,6R)-(-)-	$C_9H_{18}O_2$	46
9	13.428	4.49	Phytol, acetate	$C_{22}H_{42}O_2$	72
10	13.599	0.82	1,4-Eicosadiene	$C_{20}H_{38}$	72
11	13.725	1.43	1,4-Eicosadiene	$C_{20}H_{38}$	70
12	14.017	0.92	2,3-Dimethoxy-5-methyl-6-dekaisoprenyl-chinon	$C_{59}H_{90}O_4$	53
13	14.246	0.74	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	94
14	14.303	0.56	Palmitoleic acid	$C_{16}H_{30}O_2$	78
15	14.458	6.50	Hexadecanoic acid	$C_{16}H_{32}O_2$	99
16	14.561	1.42	2,3-Dimethoxy-5-methyl-6-dekaisoprenyl-chinon	$C_{59}H_{90}O_4$	62
17	14.687	2.99	2,4,4-Trimethyl-3-hydroxymethyl-5a-(3-methyl-but-2-enyl)-cyclohexene	550281	41
18	14.807	3.12	2,2,6-Trimethyl-1-(2-methyl-cyclob ut-2-enyl)-hepta-4,6-dien-3-one	$C_{15}H_{22}O$	83
19	15.110	4.75	Olean-12-ene	$C_{30}H_{50}$	92
20	15.184	5.24	4,4,6a,6b,8a,11,12,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12 ,12a,14,14a,14b-octadecahydro-2H-p icen-3-one	$C_{30}H_{48}O$	95
21	15.522	2.15	Phytol	$C_{20}H_{40}O$	58
22	15.636	4.95	9,12-Octadecadienoic acid, l ester	$C_{18}H_{32}O_2$	99
23	15.842	1.68	Octadecanoic acid	$C_{18}H_{36}O_2$	98
24	17.216	23.69	Gamma-Sitosterol	$C_{29}H_{52}O_2$	99
25	17.433	13.11	Gamma-Sitosterol	$C_{29}H_{52}O_2$	98
26	17.748	7.01	Gamma-Sitosterol	$C_{29}H_{52}O_2$	99
27	18.023	4.87	Gamma-Sitosterol	$C_{29}H_{52}O_2$	92
28	18.818 0.42		2,4,4-Trimethyl-3-hydroxymethyl-5a -(3-methyl-but-2-enyl)-cyclohexene	$C_{15}H_{26}O$	35
29	19.384	1.16	β-Amyrin	$C_{30}H_{50}O$	99

Abbreviations: RT, retention time; TC, total concentration.

(PCV), RBC, and Hb count of PHZ-induced anemia in Wistar rats. The results indicated that HCT (PCV), RBC, and Hb counts significantly ($p < 0.05$) decreased in anemic control rats relative to the normal control rats, but were significantly ($p < 0.05$) increased on treatment with the standard multivitamin and ethanol leaf extracts of Mucuna *poggei* and *Telfairia occidentalis* at various concentrations of 100, 200, and 400 mg/kg body weight.

Effect of Ethanol Leaf Extracts of *Mucuna poggei* and *Telfairia occidentalis* on Lipid Peroxidation and Oxidative Stress Indices

The oxidative stress parameter, TBARS-MDA levels, significantly ($p < 0.05$) increased in anemic control rats relative to the normal control rats. This was significantly ($p < 0.05$) decreased on treatment with the leaf extracts and the standard multivitamin (►**Fig. 2A**). Similarly, catalase

Table 3 Comparative composition of some selected minerals in aqueous leaf extracts of *Mucuna poggei* and *Telfairia occidentalis*

Minerals	Concentration (mg/L) *T. occidentalis*	Concentration (mg/L) *M. poggei*
K	3.24 ± 0.03^{d}	1.42 ± 0.01^{b}
Na	3.88 ± 0.01^{c}	3.85 ± 0.05^{c}
Ca	4.27 ± 0.03^{c}	14.30 ± 0.01^{d}
Mg	19.61 ± 0.03^{e}	19.48 ± 0.03^{e}
Zn	1.68 ± 0.02^{c}	3.16 ± 0.05^{a}
Fe	6.28 ± 0.0^{f}	13.43 ± 0.05^{g}
Mn	1.93 ± 0.03^{e}	1.97 ± 0.05^{e}
Cu	0.09 ± 0.004^{g}	0.10 ± 0.01^{g}
P	0.99 ± 0.003^{a}	0.01 ± 0.01^{b}
Na/K	1.20: 1	2.71: 1
Ca/P	4.31: 1	1430: 1

Abbreviation: SD, standard deviation.
Values are means ± SD of three (3) replicate values. Values with different superscripts on the same column are significantly different at ($p < 0.05$).

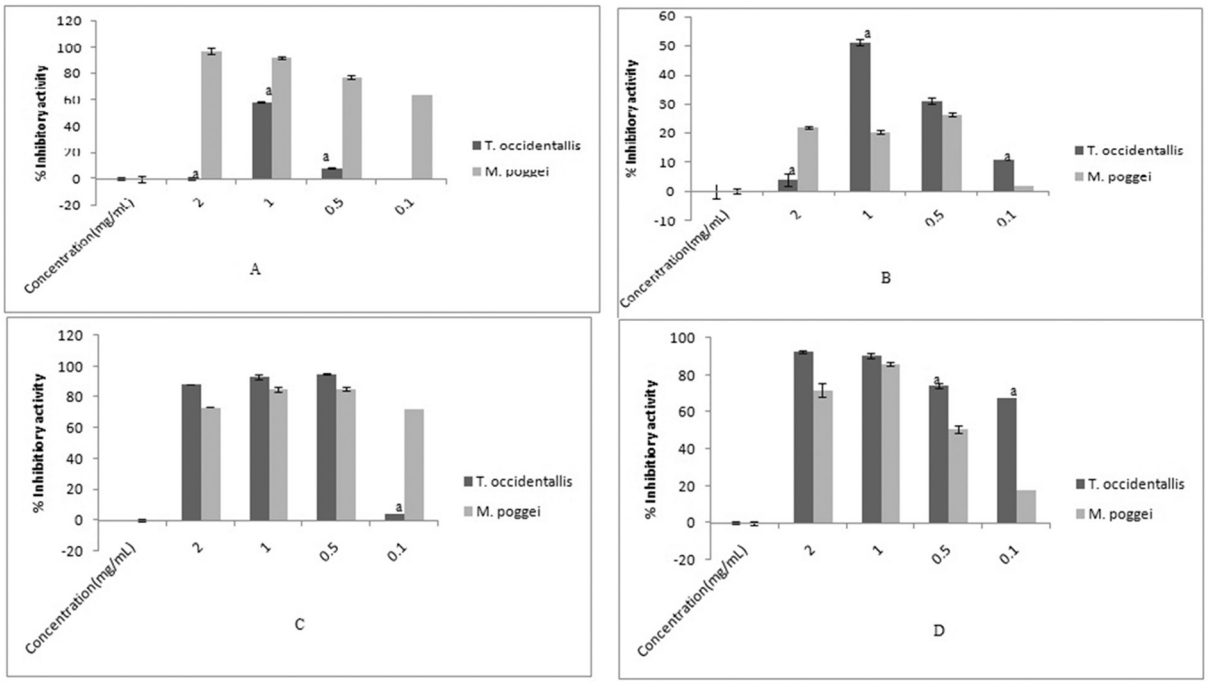

Fig. 1 (**A**) Percentage inhibition of aqueous leaf extracts of *Telfairia occidentalis* and *Mucuna poggei* on 2,2-diphenyl-1 picrylhydrazyl. Key a is significantly different versus *M. poggei* at $p < 0.05$. Mean ± SD. n =replica of three. (**B**) Percentage inhibition of aqueous leaf extracts of *T. occidentalis* and *M. poggei* on nitric oxide. Key a is significantly different versus *M. poggei* at $p < 0.05$. Mean ± SD. n = replica of three (3). (**C**): Percentage inhibition of aqueous leaf extracts of *T. occidentalis* and *M. poggei* on phosphomolybdenum. Key a is significantly different versus *M. poggei* at $p < 0.05$. Mean ± SD. n = replica of three (3). (**D**) Percentage inhibition of aqueous leaf extracts of *T. occidentalis* and *M. poggei* on reducing power. Key a is significantly different versus *M. poggei* at $p < 0.05$. Mean ± SD. n = replica of three (3). SD, standard deviation.

(►**Fig. 2B**), the activity of SOD (►**Fig. 2C**), and the activity of GPX (►**Fig. 2D**) significantly ($p < 0.05$) decreased in anemic control rats relative to the normal control rats. This, however, significantly ($p < 0.05$) increased on treatment with the two leaf extracts and the standard multivitamin in a dose-dependent manner. The level of reduced GSH significantly ($p < 0.05$) decreased in anemic control rats relative to the normal control rats. This, however, significantly ($p < 0.05$) increased on treatment with the two leaf extracts and the standard multivitamin in a dose-dependent manner (►**Fig. 2E**).

Discussion

The antioxidant and antianemic effects of ethanol leaf extracts of *M. poggei* and *T. occidentalis* in PHZ-induced anemia in Wister albino rats were investigated in this study. The phytochemicals composition of these two plants was

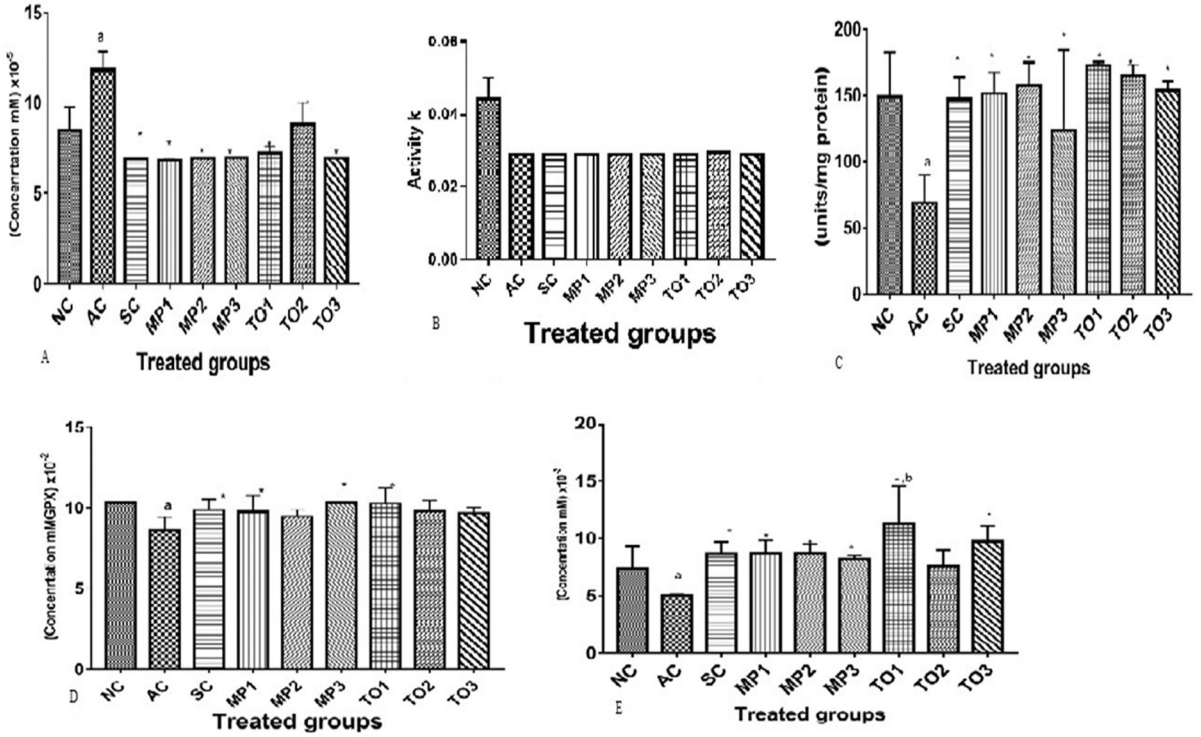

Fig. 2 (**A**) Effect of ethanol leaf extracts of *Mucuna poggei* and *Telfairia occidentalis* on oxidative stress parameter (malondialdehyde, MDA) of phenylhydrazine (PHZ)-induced anemia in Wistar rats. [a]Significantly different versus NC; * Significantly different versus AC. (**B**) Effect of ethanol leaf extracts of *M. poggei* and *T. occidentalis* on catalase activity of PHZ-induced anemia in Wistar rats. $n = 5$ mean ± SD. (**C**) Effect of ethanol leaf extracts of *M. poggei* and *T. occidentalis* on super oxide dismutase activity of PHZ-induced anemia in Wistar rats. [a]Significantly different versus NC; * Significantly different versus AC. $n = 5$ mean ± SD. (**D**) Effect of ethanol leaf extracts of *M. poggei* and *T. occidentalis* on glutathione peroxidase activity of PHZ-induced anemia in Wistar rats. [a]Significantly different versus NC; *Significantly different versus AC. $n = 5$ mean ± SD. (**E**) Effect of ethanol leaf extracts of *M. poggei* and *T. occidentalis* on reduced glutathione level of PHZ-induced anemia in Wistar rats. [a]Significantly different versus NC; * Significantly different versus AC, [b]Significantly different versus all treatment groups. $n = 5$ mean ± SD *($p < 0.05$).* AC, anemic control; NC, normal control; SC, standard control; SD, standard deviation.

earlier reported to be rich in alkaloids, flavonoids, saponins, terpenoids, steroid, and anthraquinones.[25] The rich presence of flavonoids in these plants suggests that the plants possess strong antioxidative activity and free radical scavenging capacity.

More so, **t**he GCMS analysis for the studied plants *M. poggei* and *T. occidentalis* was performed and the result indicated that Cholest-2-ene-2-methanol, ethyl 9,12,15-octadecatrienoate, and 1,4-Eicosadiene showed higher concentration of 12.80, 25.37, and 12.93%, respectively, in *M. poggei*, whereas in *T. occidentalis,* gamma-sitosterol,4,4,6a,6-b,8a,11,12,14b-octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadeca-hydro-2H-picen-3-one and hexadecanoic acid were significantly present in higher concentrations with percentages given as 23.69, 5.24, and 6.50% respectively. Some of these compounds have significance biological activity including antioxidant capacities that may be responsible for the observed antioxidant activities

Mineral content of the plants was also evaluated, and the concentrations of the minerals in the leaf extracts of *T. occidentalis* were of the trend Mg > Fe > Ca > Na > K > Mn > Zn > P> Cu. The range in *T. occidentalis* was 0.09 ± 0.004 mg/L of Cu to 19.61 ± 0.03 mg/L of Mg, while that of *M. poggei* was of the trend Mg > Ca > Fe > Na > Zn > Mn > K > P> Cu and the range in *M. poggei* was 0.10 ± 0.01 mg/L of Cu to 19.48 ± 0.03 mg/L of Mg. In both *M. poggei* and *T. occidentalis,* Mg had the highest concentration, while Cu had the lowest concentration (►**Table 3**). The concentrations of the micro minerals Fe and Zn were significantly higher ($p < 0.05$) in the leaves of *M. poggei* and *T. occidentalis*, while the concentrations of Mn and Cu were higher but not significantly ($p > 0.05$) in *M. poggei* than in *T. occidentalis*. Iron is a mineral that plays an essential role in many bodily functions, including oxygen transport, energy production, DNA synthesis, and body detoxification.[26] The significant presence of iron in these plants could play a major role in their antianemic properties, knowing the critical role of iron in RBC and Hb oxygen transport.

The in vitro antioxidant activities of the extract were also studied (►**Fig. 1A–D**), which is an indication of in vitro antioxidant activities of the plant extract using DPPH-free radicals, nitric oxide scavenging activity, phosphomolybdenum reduction assay, and FRAP indicating the percentage inhibition of the leaf extracts of *M. poggei and T. occidentalis* at different concentrations of 0.1, 0.5, 1.0, and 2.0 (mg/mL). *M. poggei* exhibited stronger inhibitory potentials against DPPH and phosphomolybdenum-free radicals (►**Fig. 1A**

Table 4 Antianemic effect of leaf extracts of *Mucuna poggei* and *Telfairia occidentalis* and HS12 on PCV, RBC, and Hb in the different experimental groups

Groups ($n = 5$)	HCT (PCV) (%)	RBC ($\times 10^{12} \mu L^{-1}$)	Hb(g/dL)
NC	38.5 ± 0.84	6.59 ± 0.49	12.55 ± 1.34
AC	25.8 ± 1.13 *a,c,d,e,f	3.6 ± 0.40 *a,c,d,e,f	10.30 ± 0.85*
PHZ+ HS-12 (SC) (0.1 mL)	45.6 ± 3.96	6.14 ± 0.01	13.95 ± 0.35
PHZ+ 400mg/kg (MP1)	45.85 ± 2.33	6.26 ± 0.26	14.15 ± 0.35
PHZ+ 200 mg/kg (MP2)	44.0 ± 8.91	5.04 ± 1.53	11.50 ± 2.69
PHZ+ 100 mg/kg (MP3)	48.10 ± 0.71	6.95 ± 0.46	14.85 ± 0.64
PHZ+ 400 mg/kg (TO1)	43.80 ± 0.71	6.55 ± 0.49	13.60 ± 0.71
PHZ+ 200 mg/kg (TO2)	43.15 ± 2.33	6.67 ± 0.50	13.00 ± 0.28
PHZ+ 100 mg/kg (TO3)	45.325 ± 1.80[f]	4.68 ± 0.70[f]	9.60 ± 2.69

Abbreviations: AC, anemic control; Hb, hemoglobin; HCT, hematocrit; MP, *M. poggei*; NC, normal control; PCV: packed cell volume; PHZ, phenylhydrazine; RBC, red blood cells; SC, standard control; SD, standard deviation; TO, *T. occidentalis.*
Values are expressed as mean ± SD ($p < 0.05$).
*Significantly different versus NC.
[a]Significantly different versus other groups.
[c]Significantly different versus MP1.
[d]Significantly different versus MP_2.
[e]Significantly different versus TO_2.
[f]Significantly different versus TO_1.

and **C**), while *T. occidentalis* showed a stronger inhibitory potential against NO and FRAP-free radicals (►**Fig. 1B** and **D**). This is also an indication of the antioxidative properties of the plant extract. This result is in line with the observed in vivo antioxidant activities.

One of the by-products of polyunsaturated fatty acid peroxidation in cells is malondialdehyde (MDA). MDA is one of the significant oxidation products and is regarded as the primary indicator of lipid peroxidation in oxidative stress, inflammation, and a variety of health conditions. Investigations were performed on the oxidative stress biomarker level. TBARS-MDA, a measure of oxidative stress was found to be considerably ($p<0.05$) higher in the anemic control group than in the normal control group. With the use of the leaf extracts and the regular multivitamin, this was significantly ($p < 0.05$) reduced (►**Fig. 2A**). The extracts' capacity to neutralize hydroxyl and peroxyl radicals through their antioxidant activity is thought to be the cause of the observed reduction[27]

The breakdown of cell membranes is caused by free radicals that are mediated by MDA. MDA levels were reported to be higher during hepatotoxicity and hepatocarcinogenesis caused by PHZ.[28] In a different study, evaluation of MDA levels in serum revealed a significant rise in the PHZ group compared with the control group. Recent research has shown that one of the key mechanisms of PHZ in the development of hemolytic anemia is an increase in MDA concentrations.[27,29] More so, in this present study, PHZ significantly ($p < 0.05$) reduced catalase activity as compared with the control group. *M. poggei* and *T. occidentalis* leaf extracts and the standard multivitamin, HS-12 treated groups significantly ($p > 0.05$) increased catalase activity in comparison with the PHZ-treated group (►**Fig. 2B**). Though the effect on catalase was not in a dose-dependent manner, the overall observation was that *M. poggei* and *T. occidentalis* leaf extracts exhibited a strong antioxidative potential. Due to constant exposure to high oxygen tension and erythrocyte membrane richness in polyunsaturated fatty acids, erythrocytes are more susceptible to lipid peroxidation and oxygen radicals formation. The reduction in the hematological parameters, PCV, HB, and RBC, counts due to PHZ-induced anemia (►**Table 4**) as observed in the present study are due to changes in the lipid composition of RBC membranes, which cause morphologically abnormal erythrocytes with decreased life span.[30] It has been proposed that membrane lipids are essential for maintaining erythrocyte structure,

function, and viability, as well as other aspects of cellular homeostasis. As previously stated, increased ROS causes more lipid peroxidation, which in turn creates chemicals that are toxic to cells and hemolyze to alter the structure of those cells.[31] Cell membrane degradation is caused by MDA, which is mediated by free radicals. MDA levels were reported to be higher during PHZ-induced hepatotoxicity and hepatocarcinogenesis.[32] These provide more support for how naturally occurring antioxidant molecules stabilize cell membrane based on their capacity to break down radical chain reactions and scavenge free radicals, thereby reducing oxidative stress-related damage.[33]

The normal hepatocytes are protected from free radicals and reactants by both enzymatic and nonenzymatic defense mechanisms. The enzymatic defense mechanism includes catalase (CT), SOD, and GPX, which quickly react with and remove ROS such as superoxide, hydrogen peroxide, and hydroxyl radicals.[34] The definition of oxidative stress is an imbalance between the generation of reactive species and the body's natural antioxidant defense. The definition of oxidative stress is an imbalance between the generation of reactive species and the body's natural antioxidant defense.[35] Also assessed were the antioxidant enzymes. Antioxidant indices, catalase, SOD, GPX activity, and GSH levels were all dropped considerably ($p < 0.05$) in anemic control compared with normal control. However, following administration of the two leaf extracts and the common multivitamin, these considerably ($p < 0.05$) rose in a dose-dependent manner (►**Fig. 2A–E**). These support the idea that natural antioxidant molecules stabilize cell membrane based on their level of free radical scavenging activity by disabling chain reactions and reducing oxidative stress-related damage.[33]

As compared with the control group, PHZ significantly ($p < 0.05$) decreased catalase activity in the current investigation. The regular multivitamin, HS-12, and the leaf extracts of *M. poggei* and *T. occidentalis* significantly ($p > 0.05$) improved catalase activity compared with the PHZ-treated group (►**Fig. 2B**). When anemic rats were given *M. poggei* and *T. occidentalis* leaf extracts along with the common multivitamin HS-12, there were significant ($p < 0.05$) increases in SOD levels in their serum as compared with anemic rats that were not given any treatment (►**Fig. 2C**). These encouraging outcomes are in line with a study that found antioxidant components included in traditional medicinal herbs could increase SOD function.[36] When compared with the normal control group in this investigation, PHZ induction significantly ($p < 0.05$) decreased serum SOD activity (►**Fig. 2C**), which may be because superoxide radicals are inhibiting SOD activity. It is possible that increased ROS formation, which outweighed the activities, caused the anemic control group's decreased SOD activity of these enzymes. However, treatments with *M. poggei* and *T. occidentalis* leaf extracts and the common multivitamin, HS-12, boosted SOD activity to levels equal to the normal control, with the dose of 400 mg/kg of *T. occidentalis* leaf extract demonstrating the largest increase ($p < 0.05$). These findings are consistent with an earlier study by Iweala et al in 2019, who reported increased SOD activity in N-nitrosodiethylamine exposed rats.[37] According to these findings, by Ben Salem et al, in 2017[38] administration of an ethanol extract of *C. scolymus* enhanced the SOD level in a condition associated with oxidative stress, such as diabetes, demonstrating the extract's antioxidant potential and in the same literature there was reported rich presence of flavonoids, a strong antioxidant phytochemical, a possible mechanism employed by *M. poggei* and *T. occidentalis*

In this investigation, PHZ induction considerably ($p < 0.05$) reduced serum GPX activity when compared with the healthy control group, supporting the finding that erythrocytes are susceptible to oxidative damage (►**Fig. 2D**). This finding echoes one from an earlier study.[39] The usual multivitamin HS-12, *M. poggei* and *T. occidentalis* leaf extracts all significantly ($p < 0.05$) boosted GPX activity in the treatment groups (►**Fig. 2D**). This rise may be the result of PHZ hepatotoxicity altering the tissue redox system by scavenging free radicals and boosting the antioxidant state in the liver.

PHZ significantly ($p < 0.05$) reduced GSH in the PHZ-treated group compared with the control group in the current study. A significant ($p < 0.05$) rise in GSH was observed in all groups following treatments with *M. poggei* and *T. occidentalis* leaf extracts and the common multivitamin, HS-12, with the highest dose of 400 mg/kg of *T. occidentalis* leaf extracts (►**Fig. 1E**). According to ►**Fig. 1E**, the increase in GSH in the groups that received standard multivitamins, HS-12, and leaf extracts from *M. poggei* and *T. occidentalis* indicated antioxidant protective properties and suggested that these plants' leaf extracts have a high capacity to increase GSH.

Overall considering the data obtained from this study, *M. poggei* and *T. occidentalis* leaf extracts showed strong potentials as potential antianemic and antioxidative agents. Worthy of note was the fact that the activity was not completely dose dependent but similar to the observed activity of standard multivitamins, HS-12. Causes of non-dose-dependent effects of drugs were reported earlier.[40] It was noted that although the majority of drugs work dose dependently, there are additional factors that influence a drug's bioavailability. The quantity of a drug that is not absorbed from the gastrointestinal tract as a result of hepatic, gastric, and/or intestinal first-pass effects and the absolute oral bioavailability may affect or result in a drug's dose-independent observed activity of a treatment.[40] More specifically, this study has offered these two plants as less expensive alternatives to HS-12 and other multivitamins that are available in conventional drug stores but are out of reach for the poor population in society, which makes up a larger portion of humanity and is in desperate need of blood-boosting alternatives to conventional drugs.

Conclusion

Data from this study suggest that *M. poggei* and *T. occidentalis* leaf extracts exert antioxidant and antianemic effects, making them viable alternatives in the management of oxidative stress-related complications.

Authors' Contributions

UAI and PAU conceived and designed the study. GUU, PAU UNO, JOM, ISI, and FOM performed literature research, COA, DEU, SKN, and PAU performed the laboratory analysis. PAU wrote the manuscript. UAI and DEU read, edited, and revised the manuscript for intellectual content. All authors have read and approved the final manuscript.

Compliance with Ethical Principles

This study was in compliance with the Convention on Trade in Endangered Species of Wild Fauna and Flora. Furthermore, the study was approved by the Department of Biochemistry Ethical Committee on Research, Innovation and Institutional Ethical Committee of Ebonyi State University Abakaliki, Nigeria.

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None.

Conflict of Interest

None declared.

References

1 Akindele AJ, Busayo FI. Effects of the hydroethanolic extract of Mucuna pruriens (L.) DC (Fabaceae) on haematological profile in normal and haloperidol treated rats. Nig Q J Hosp Med 2011;21 (02):93–98
2 Organization WH. Anaemia: Key Facts. WHO Monographs on Selected Medicinal Plants. Geneva: WHO; 2018,1:2
3 Obioma B, Eze D, Obeagu A, Emmanuel N, Okechukwu PC, Ifemeje JC. The effects of aqueous leaf extract of Mucuna pruriens (agbala) on some selected biochemical indices of wister albino rats. Int J Curr Microbiol Appl Sci 2014;3(01):6
4 Oladele JO, Oyewole OI, Bello OK, Oladele OT. Hepatoprotective effect of aqueous extract of Telfairia occidentalis on cadmium chloride-induced oxidative stress and hepatotoxicity in rats. J Drug Design Medicinal Chem 2017;3(03):5
5 Kayode AAA, Kayode OT. Some medicinal values of Telfairia occidentalis: a review. Am J Biochem Mol Biol 2011;1:9
6 World Health Organization. Micronutrient Deficiencies: Prevention and Control Guidelines. Geneva: WHO; 2015, 1:2
7 Gamit M, Talwelkar HS. Survey of different types of anemia. Int J Med Sci Public Health 2017;6:4
8 Ashish T, Sachin M, Rizwan S, Gadhpayle J, Bhongade SL, Vikas S. Antianaemic Potential of Swertia chirataon phenylhydrazine induced reticulocytosis in rats. Asian J Pharmaceutic Sci 2013;2(01):5
9 Okorie AU, Akuodor GC, Aja DOJ, et al. The effect of Justicia insularis ethanol leaf extract on haematological parameters in phenylhydrazine-induced anaemic Wistar rats. J Complement Med Res 2020;11(01):17
10 Ndem JI, Otitoju O, Akpanaiabiatu MI, Uboh FE, Uwah AF, Edet OA. Haematoprotective property of Eremomastax speciosa (Hochst.) on experimentally induced anaemic Wistar rats. Ann Biol Res 2013;4(06):5
11 Sung YM, Gayam SR, Wu SP. The oxidation of phenylhydrazine by tyrosinase. Appl Biochem Biotechnol 2013;169(08):2420–2429
12 Maurice MI. Pharmacognostical Profile of Selected Medicinal Plants. New York: *Handbook of African Medicinal Plants* CRC Press; 2014
13 Csuros CSaC. C.Z. Environment sampling and Analysis for metals. Boca Raton, CRC press, 2019
14 Kedare SB, Singh RP. Genesis and development of DPPH method of antioxidant assay. J Food Sci Technol 2011;48(04):412–422
15 Jagetia GC, Baliga MS. The evaluation of nitric oxide scavenging activity of certain Indian medicinal plants in vitro: a preliminary study. J Med Food 2004;7(03):343–348
16 Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem 1999;269(02):337–341
17 Güder A, Korkmaz H. Evaluation of in-vitro antioxidant properties of hydroalcoholic solution extracts urtica dioica L., Malva neglecta Wallr. and Their Mixture. Iran J Pharm Res 2012;11 (03):913–923
18 Shetlar MD, Hill HA. Reactions of hemoglobin with phenylhydrazine: a review of selected aspects. Environ Health Perspect 1985; 64:265–281
19 Ogunrinola OO, Olaitan SN, Fajana OO. Disruption of lipid profile and alteration of hepatic lipoprotein metabolism gene expression in anaemia-induced rat. J Appl Sci 2019;19:8
20 Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 1978;52:302–310
21 Aebi H. Catalase in vitro. Methods Enzymol 1984;105:121–126
22 Li X. Improved pyrogallol autoxidation method: a reliable and cheap superoxide-scavenging assay suitable for all antioxidants. J Agric Food Chem 2012;60(25):6418–6424
23 Hu ML. Measurement of protein thiol groups and glutathione in plasma. Methods Enzymol 1994;233:380–385
24 Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959; 82(01):70–77
25 Osukoya OA, Adegbenro D, Onikanni SA, Ojo OA, Onasanya A. Antinociceptive and antioxidant activities of the methanolic extract of *Telfairia occidentalis* Seeds. Anc Sci Life 2016;36(02): 98–103
26 Moll R, Davis B. Iron, vitamin B12 and folate. Medicine (Baltimore) 2017;45(04):198–203
27 Gheith I, El-Mahmoudy A. Laboratory evidence for the hematopoietic potential of beta vulgaris leaf and stalk extract in a phenylhydrazine model of anemia. Braz J Med Biol Res 2018;51 (11):e7722
28 Anbara H, Shahrooz R, Razi M, Malekinejad H, Najafi G. The effect of vitamin C on mice hemolytic anemia induced by phenylhydrazine: an animal model study using histological changes in testis, pre-implantation embryo development, and biochemical changes. Iran J Basic Med Sci 2018;21(07):668–677
29 Gaschler MM, Stockwell BR. Lipid peroxidation in cell death. Biochem Biophys Res Commun 2017;482(03):419–425
30 Stevens GA, Bennett JE, Hennocq Q, et al. Trends and mortality effects of vitamin A deficiency in children in 138 low-income and middle-income countries between 1991 and 2013: a pooled analysis of population-based surveys. Lancet Glob Health 2015; 3(09):e528–e536
31 Mladenov M, Gokik M, Hadzi-Petrushev N, Gjorgoski I, Jankulovski N. The relationship between antioxidant enzymes and lipid peroxidation in senescent rat erythrocytes. Physiol Res 2015;64 (06):891–896
32 Ezeigwe OC, Nzekwe FA, Nworji OF, Ezennaya CF, Iloanya EL, Asogwa KK. Effect of aqueous extract of *F. capensis* leaves and its combination with *C. aconitifolius* leaves on essential biochemical parameters of phenylhydrazine-induced anemic rats. J Exp Pharmacol 2020;12:191–201
33 Da Pozzo E, De Leo M, Faraone I, et al. Antioxidant and antisenescence effects of bergamot juice. Oxid Med Cell Longev 2018; 2018:9395804
34 Mansourian M, Sadeghi H, Doustimotlagh AH. Activation of the glutathione peroxidase by metformin in the bile-duct ligation induced liver injury: in vivo combined with molecular docking studies. Curr Pharm Des 2018;24(27):3256–3263
35 Elejalde E, Villarán MC, Alonso RM. Grape polyphenols supplementation for exercise-induced oxidative stress. J Int Soc Sports Nutr 2021;18(01):3

36 Ousaaid D, Ghouizi AE, Laaroussi H, et al. Anti-anemic effect of antioxidant-rich apple vinegar against phenylhydrazine-induced hemolytic anemia in rats. Life (Basel) 2022;12(02):x
37 Iweala EEJ, Evbakhavbokun WO, Maduagwu EN. Antioxidant and hepatoprotective effect of Cajanus cajan in N-nitrosodiethylamine-induced liver damage. Sci Pharm 2019;87(03):24
38 Ben Salem M, Ben Abdallah Kolsi R, Dhouibi R, et al. Protective effects of Cynara scolymus leaves extract on metabolic disorders and oxidative stress in alloxan-diabetic rats. BMC Complement Altern Med 2017;17(01):328
39 Mozafari AA, Shahrooz R, Ahmadi A, Malekinjad H, Mardani K. Protective effect of ethyl pyruvate on mice sperm parameters in phenylhydrazine induced hemolytic anemia. Vet Res Forum 2016; 7(01):63–68
40 Choi JH, Yoo JY, Kim SO, Yoo SE, Oh GT. KR-31543 reduces the production of proinflammatory molecules in human endothelial cells and monocytes and attenuates atherosclerosis in mouse model. Exp Mol Med 2012;44(12):733–739

