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Effects of Aqueous Extract of *Telfairia occidentalis* and *Mucuna pruriens* Leaves on the Biochemical Parameters of Phenylhydrazine Induced Anaemic Wistar Rats

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ABSTRACT

This work investigated the effect of aqueous extract of *Telfairia occidentalis* and *Mucuna pruriens* leaves on the biochemical parameters of phenylhydrazine induced anaemicWistar rats. The phytochemical compositions of *T. occidentalis* and *M. pruriens* were determined following standard procedures. The results obtained revealed the presence of flavonoids, alkaloids, saponins, tannins, phenols, terpenoids, steroids, proteins, reducing sugar and cardiac glycosides. The biochemical parameters were analyzed using Randox kits while the hematological parameters were determined following standard procedures. The result obtained shows that *T. occidentalis* and *M. pruriens* leaf extracts helped improve liver function and renal functions as seen from the liver marker enzymes (ALT, AST, ALP) and kidney markers (Urea and creatinine). The result indicates that the ALT level was higher in group 2 (anaemic group) (35.85±0.62 IU/L) whereas there was significant decrease ($P<0.05$) in Group 1 (23.73±0.55 IU/L), group 3 (25.73±0.91 IU/L), group 4 (32.73±0.56 IU/L), group 5 (30.51±0.55 IU/L) at $p<0.05$. This is a positive sign of improvement. Similar observation was found for AST and ALP levels. The effects of the aqueous extracts on the kidney function showed that the control group 1 (normal), had 22.33±1.25 mg/dl urea while the anaemic group had 29.40±1.07 mg/dl. The urea level then decreased in group 5 (27.73±0.60 mg/dl), group 3 (27.12±0.64 mg/dl), and group 4 (24.01±0.79 mg/dl), when compared to group 2. This is a positive indication for improvements for groups administered *T. occidentalis*, *M. pruriens* and blood tonic. The effects of the extracts on the creatinine levels show a decrease in serum creatinine levels. This suggests improved renal function. The results obtained from the heamatological study show that group 2 (anaemic rats) exhibited lower PCV (%), HB (g/dl) and RBC. The administration of the leaf extracts and HB12 blood tonic helped improve the RBC levels, justifying the use of the plants for treatment of anaemia Among the two plants, *T. occidentalis* possess better anti-anaemic properties. The plants were also found to have protective effect on the liver and kidney tissues against the deleterious effect of phenylhydrazine.

Keywords: *Telfairia occidentalis, Mucuna pruriens,* biochemical parameters, phenylhydrazine.

INTRODUCTION

Plants with medicinal properties have been utilized in the discovery of new drugs for the prevention, treatment and management of various diseases. Plants such as vegetables vary considerably in their nutrient contents and are good sources of vitamins, essential amino acids, proteins, as well as minerals and antioxidants [1]. They are included in meals mainly for their nutritional value although some are reserved for the sick due to their medicinal properties [2]. Generally, the active principles found in plants can be extracted and used in different forms which include infusions, syrups, concoctions, decoctions, infusion oils, essential oils, ointments and creams in the treatment/management and prevention of some diseases [3]. The use of traditional medicine in the treatment and management of diseases in the African continent cannot fade away and this could be attributed to the socio-cultural, socio-economic, lack of basic health care and qualified personnel. Plants contain active components such as anthraquinones, flavonoids, glycosides, saponins, tannins, etc., which possess medicinal properties that are harnessed for the treatment of different diseases. The active ingredients for a vast number of pharmaceutically derived medications contain components originating from phytochemicals. These active substances that contain the healing property are known as the active principles and are found to differ from plant to plant.

Anaemia is a condition in which the blood has a lower than normal number of red blood cells. This condition can also occur if the red blood cells do not contain enough haemoglobin (an iron-rich protein that gives blood its red colour). Haemoglobin helps red blood cells carry oxygen from the lungs to the rest of the body. If one have anaemia, the body does not get enough oxygen-rich blood and, as a result, one may feel tired, weak and short of breath. With severe or long-lasting anaemia, the lack of oxygen in the blood can damage the heart, brain and other organs in the body. Very severe anaemia may even cause death. Anaemia has three main causes: blood loss, lack of red blood cell production, or high rates of red blood cell destruction. These may be due to a number of diseases, conditions, or other factors.

The human body is known to produce billions of new red blood cells, and other blood components which replace blood cells that are lost due to normal cell turnover processes, illness or trauma. All the mature blood cells in the body are generated from a relatively small number of haematopoietic stem cells (HSCs) and progenitors. Each blood cell, red blood cells, white blood cells, and platelets play important roles in the body's normal physiological functions. However, certain diseases and conditions such as malaria, malnutrition, protozoan infections and pregnancy are among various conditions that could disrupt normal haematopoiesis thus predisposing one to anaemia. This is found to be more prevalent in both adults and children.

Fluted pumpkin (*Telfairia occidentalis* Hook F.) and *Mucuna pruriens* leaves used for various purposes in different countries of the world have been shown to have positive effects on some haematological parameters [3]; [4]. However, due to their ability to increase blood parameters, their extracts formulations are being used in several localities among housewives without any scientific investigation of their effect on haematological indices. It is therefore expedient to compare the blood boosting capacities of the individual extracts. This investigation will reveal their synergistic and antagonistic potential.

MATERIALS AND METHODS

Plant Materials

*M. prurient s*and *T. occidentalis* leaves were obtained from Uli Ihiala L.G.A, Anambra State, Nigeria and was identified by Dr. C.G Ukpaka, a botanist in the Department of Biological Sciences, Chukwuemeka Odumegwu Ojukwu University, Uli campus.

Animals

Thirty (30) weanling Wistar rats of approximately three (3) weeks old and weighing between 50-80g were used for the study. The rats were obtained from the Animal House of the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. The rats were fed with rat pellets and water *ad libitum.*

Equipment/Materials

The equipment/materials that were used are: Centrifuge, Glasswares, Haematocrit centrifuge, Micropipette, Microscope, Naubeur chamber and counter, Refrigerator, Spectrophotometer, Syringe, Thermometer, Water bath, and Weighing balance.

Chemicals and Reagents

The chemicals and reagents used are of analytical grade.

METHODOLOGY

Preparation of Plant Extracts

Known weights (200g) of dried leaves of *M. pruriens* and *T. occidentalis* were separated from the stem, washed with clean water to remove dirt and sand, drained, and chopped. They were macerated in 500 ml of water and then filtered to obtain homogenous aqueous extracts.

Phytochemical Analysis

The phytochemical screening and the quantitative analysis of *M. pruriens* and *T. occidentalis* leaves were carried out following standard procedures. The methods of [5]; [6]; [7] were adopted

Acute toxicity test of aqueous extracts of the leaves of *M. pruriens* and *T. occidentalis*

The method of [8] was used for the acute toxicity test of the aqueous extracts of leaves of *M. pruriens* and *T. occidentalis*. Thirteen (13) albino mice were utilized in this study. The test involved two stages. In stage one, the animals were grouped into three (3) groups of three rats each and were given 10, 100 and 1000 mg/kg body weight of the extracts respectively. The second stage involved the number of death that occurred in the different groups in stage one

Experimental Design

Thirty (30) weanling Wistar rats were used for the study. They were acclimatized for seven days with free access to feed and water. After acclimatization, they were randomly distributed into five (5) groups of 6 rats each. Anaemia was induced in the rat by injecting them with a single phenylhydrazine intraperioneal administration at a dose of 20 mg/kg b.w.[9].

Group 1 (Normal control group no Anaemia) was given rat feed and water *ad libitum*.
Group 2 (Positive control group Anaemic rats) was given rat feed and water *ad libitum*.
Group 3 (Anaemic rats) was given 200 mg/kg b.w. of the aqueous extract of *T. occidentalis* leaf.
Group 4 (Anaemic rats) was given 200 mg/kg b.w. of the aqueous extract of *M. pruriens* leaf.

Liver function test

Alanine amino transaminase (ALT) was determined by the [11] colorimetric method. Aspartate amino transferase (AST) determination by the Reitman-Frankel colorimetric method while, Alkaline phosphatase (ALP) was determined by phenolphthalein monophosphate method. Serum bilirubin was determined by the methods of Jendrassik-Grof for the *in vitro* determination of total bilirubin in serum or plasma [12],

Kidney function test

Creatinine level was determined by the modified method of Jaffe for the *in vitro* determination of creatinine in serum [13] while Urea level was determined by the modified method of [6] for the *in vitro* determination of urea in serum.

Group 5 (Anaemic rats) was given 200 mg/kg b.w. of blood tonic (HB12).

Animal Sacrifice and Sample Collection
The blood from the rats was collected through the ocular vein and allowed to clot, centrifuged and the serum separated from the cells. All rats were sacrificed on day 28.

Determination of Haematological Parameters

Haematological assay was performed with blood sample collected on an EDTA container from the animals. Packed Cell Volume (PCV) was determined by haematocrit method while Haemoglobin (Hb) was determined by cyanmethaemogloin method. The Total white blood cell count and platelet was done by the visual observation method whereas the red blood cell count was done by the Haemocytometer method [10].

Serum Protein Profile Test

Direct Biuret method for the *in vitro* determination of total protein in serum or plasma was adopted [14], while Bromocresol green method for the *in vitro* determination of albumin in serum/plasma was adopted [15]; [16]. Globulin levels was obtained by subtracting the quantity of albumins from that of total proteins.

Statistical Analysis

The data obtained were expressed as mean of 3 replicates ±SD. Statistical analysis was carried out using the Statistical Package for Social Sciences (SPSS). Two-way and one way analyses of variance were adopted for comparison, and the results were subjected to post hoc test using least square deviation (LSD). $p < 0.05$ was considered significant for all the results.

RESULTS

Phytochemical Screening Result

The results obtained for the phytochemical screening of the aqueous extracts of *T. occidentalis* and *M. pruriens* were presented in Table 1. The result revealed the presence of flavonoids, alkaloids, saponins, tannins, phenols, terpenoids, steroids, proteins and cardiac glycosides in *T. occidentalis*. Reducing sugar was found absent. Phytochemical screening of *M. pruriens* revealed the presence of flavonoids, alkaloids, saponins, tannins, phenols, terpenoids, steroids, proteins, reducing sugar and cardiac glycosides.

Table 1: Qualitative Phytochemical Analysis of the aqueous extracts of *T. occidentalis* and *M. pruriens* leaves

Phytochemicals	*T. occidentalis*	*M. pruriens*
Flavonoids	++	++
Alkaloids	+	++
Saponins	+	++
Tannins	+	+
Phenols	+	+
Terpenoids	+	+
Steroids	+	+
Proteins	+	+
Reducing sugar	-	+
Cardiac glycosides	+	++

Key: + = Present; ++ = Highly present; - = Absent.

Quantitative Phytochemical Analysis

Results obtained for the quantitative analysis of the aqueous extracts of *T. occidentalis* and *M. pruriens* leaves were presented in Table 2. The result revealed that *T. occidentalis* contained the higher level of flavonoids (6.14±0.70 %) compared to *M. pruriens*(4.58±0.59). Alkaloids was found higher in *M. pruriens*(2.04±0.14 %) compared to that of *T. occidentalis*(1.02±2.12 %). The level of saponins found was higher in *T. occidentalis*(5.67±0.10 %) than in *M. pruriens* (2.65±0.34 %). Tannins and phenols were found higher in *M. pruriens.*

Table 2: Quantitative Phytochemical Analysis of the aqueous extracts of *T. occidentalis*and *M. pruriens* leaves

Phytochemicals	*T. occidentalis*	*M. pruriens*
Flavonoids (%)	6.14±0.70	4.58±0.59
Alkaloids (%)	1.02±2.12	2.04±0.14
Saponins (%)	5.67±0.10	2.65±0.34
Tannins (%)	0.12±0.11	0.15±0.54
Phenols (%)	0.17±0.54	0.19±0.01

Results were presented as mean±SD of triplicate determination

Acute Toxicity Test

The acute toxicity tests (LD50) results were presented in Table 3 and 4. The results of the phase I and Phase II studies indicated no mortality. The result of the phase II studies indicated that there were signs of palpitations from the dose of 4000 mg/kg body weight. This is an indication that high concentration of the extracts will not cause mortality.

Table 3: Phase I of the acute toxicity (LD_{50}) of the aqueous leaf extracts of *T. occidentalis* and *M. pruriens* leaf extracts

Groups	Dosage (mg/kg body weight)	Mortality in group given *T. occidentalis extracts*	Mortality in group given *M. pruriens* extracts	Behavior
Group 1	10	0/3	0/3	Normal
Group 2	100	0/3	0/3	Normal
Group 3	1000	0/3	0/3	Normal

Table 4: Phase II of the acute toxicity (LD_{50}) of the aqueous leaf extracts of *T. occidentalis* and *M. pruriens* leaf extracts.

Groups	Dosage (mg/kg body weight)	Mortality in group given *T. occidentalis extracts*	Mortality in group given *M. pruriens* extracts	Behaviour
Group 1	2000	0/3	0/3	Normal
Group 2	4000	0/3	0/3	Signs of palpitation
Group 3	5000	0/3	0/3	Signs of palpitation

Heamatology Test Results

The results obtained for the heamatological study were presented in Table 5 and 6. The result indicates that group 2 (anaemic rats) exhibited lower PCV (%), HB (g/dl) and RBC. The group administered leaf extracts showed improved blood count. The PCV level was higher in group 5 (42.80±2.14 %) followed by group 3 (41.56±1.35 %). The HB levels was higher in group 1, with 14.90±0.14 g/dl followed by group 3 (13.85±0.45 g/dl) and group 5 (13.80±1.24 g/dl) with no significance difference at $p < 0.05$. The lowest HB was found in the anaemic group (6.93±0.38 g/dl). The total RBC count was highest in group 1 (7.99±0.12 10^6/ul) followed by group 3 (6.70±0.35 10^6/ul) and group 5 (6.52±0.68 10^6/ul). The administration of the leaf extracts and HB12 blood tonic helped improve the PCV, HB and RBC levels.

Table 5: Heamatology test results for the various rat groups.

Groups	PCV (%)	HB (g/dl)	RBC ($10^{\wedge 6}$/ul)
Group 1 (Control)	44.69±0.42	14.90±0.14	7.99±0.12
Group 2 (Positive control)	20.81±1.15	6.93±0.38	3.72±0.40
Group 3 (*T. occidentalis*)	41.56±1.35	13.85±0.45	6.70±0.35
Group 4 (*M. pruriens*)	38.14±1.87	12.72±0.63	5.07±0.51
Group 5 (HB12 blood tonic)	42.80±2.14	13.80±1.24	6.52±0.68

Results were presented as mean±SD of triplicate determination.

The results obtained for the total white blood cell (TWBC) count indicated that group 2 has the highest TWBC of (15.75±0.31 10^3/ul) while group 1 has the least TWBC (8.35±0.62 10^3/ul). The differential white blood cell count indicates that group generally showed increase levels of different white blood cells. The neutrophile level was highest in group 2 (26.57±1.67 %), while group 1 has the least neutrophile level (17.30±1.33 %). There was no significance difference in mean between group 3, 4,and 5 for neutrophile count. The result of the lymphocyte count shows that group 2 has a higher count (66.53±0.97 %) compared to the control group 1 (42.29±4.00 %). An increase in the lymphocyte count was also observed for group 3 (58.45±2.05 %), group 4 (57.10±1.84 %) and group 5 (60.20±1.92 %). The eosinophile levels for the group 1 is the lowest (0.33±0.58 %) compared to other groups. Groups 2, 3 and 5 had same level of eosinophile (1.33±0.58 %), while group 4 had 0.67±0.58 %. Similar trend was observed for the basophile levels of groups 2, 3 and 5 as each had 0.33±0.58 %. However, no basophil count was found for group 1 and group 4. The monocyte count was found higher in group 3 (1.33±0.58 %) while no significance difference existed between groups 2 and 5 with same level (1.33±0.58%).

Table 6: Total and differential white blood cell count

Groups	TWBC ($10^{\wedge 3}$/ul)	Neutrophile (%)	Lymphocyte (%)	Eosinophile (%)	Basophile (%)	Monocyte (%)
Group 1 (Control)	8.35±0.62	17.30±1.33	42.29±4.00	0.33±0.58	0.00±0.00	0.33±0.58
Group 2 (Positive control)	15.75±0.31	26.57±1.67	66.53±0.97	1.33±0.58	0.33±0.58	1.00±1.00
Group 3 (*T. occidentalis*)	12.31±0.46	23.70±0.94	58.45±2.05	1.33±0.58	0.33±0.58	1.33±0.58
Group 4 (*M. pruriens*)	10.23±0.56	24.1±1.35	57.10±1.84	0.67±0.58	0.00±0.00	0.33±0.58
Group 5 (HB12 blood tonic)	11.56±0.51	22.35±1.86	60.20±1.92	1.33±0.58	0.33±0.58	1.00±1.00

Results were presented as mean±SD of triplicate determination.

Liver Function

Effects of aqueous extracts of *T. occidentalis* and *M. pruriens* on the ALT levels

The effects of aqueous extracts of *T. occidentalis* and *M. pruriens* are presented in Figure 1. The result indicates that the ALT level was higher in group 2 (35.85±0.62 IU/L) whereas there was significant decrease in Group 1 (23.73±0.55 IU/L).

ALT levels in the groups 3, 4 and 5 are as follows: Group 3 (25.73±0.91 IU/L), Group 4 (32.73±0.56 IU/L), Group 5 (30.51±0.55 IU/L) at $p<0.05$.

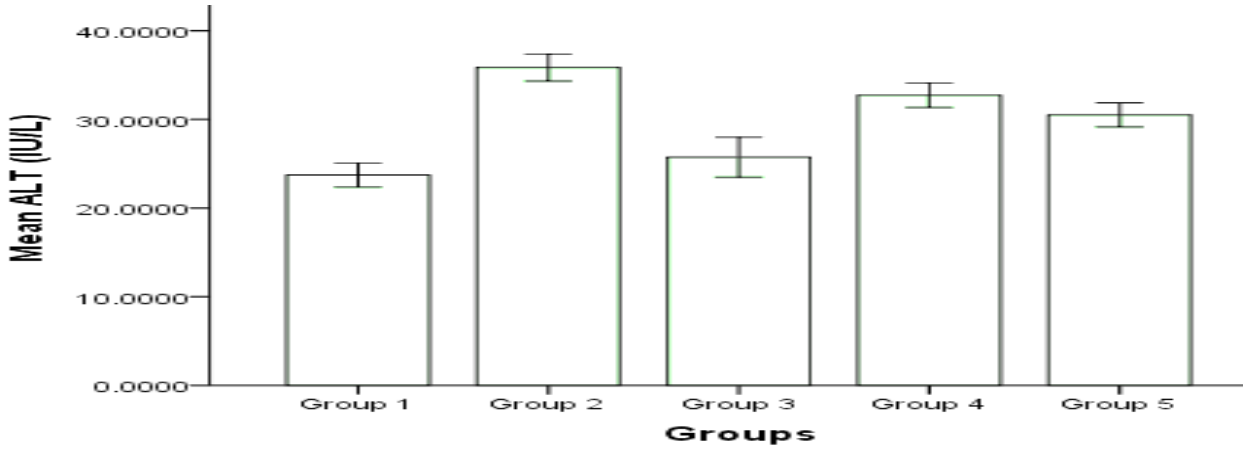

Figure 1: Effects of aqueous extracts of *T. occidentalis* and *M. pruriens* on the serum ALT level in Wistar albino rats.

Group 1 (Normal control group no Anaemia).
Group 2 (Positive control group Anaemic rats).
Group 3 (Anaemic rats) given 200 mg/kg b.w. *T. occidentalis* leaf aqueous extract.
Group 4 (Anaemic rats given 200 mg/kg b.w. *M. pruriens* leaf.
Group 5 (Anaemic rats) given 200 mg/kg b.w. of blood tonic (HB 12).

Effects of aqueous extracts of *T. occidentalis* and *M. pruriens* on the AST levels

The effects of aqueous extracts of *T. occidentalis* and *M. pruriens* on AST levels are presented in Figure 2. The result indicates that the AST level was higher in group 2 (43.42±1.35 IU/L) whereas there was significant decrease in Group 1 (36.09±0.35 IU/L). The level of AST in group 3 was 30.20±0.70 IU/L, while groups 4 and 5 had 38.14±0.35 IU/L and 27.85 IU/L respectively at $p<0.05$.

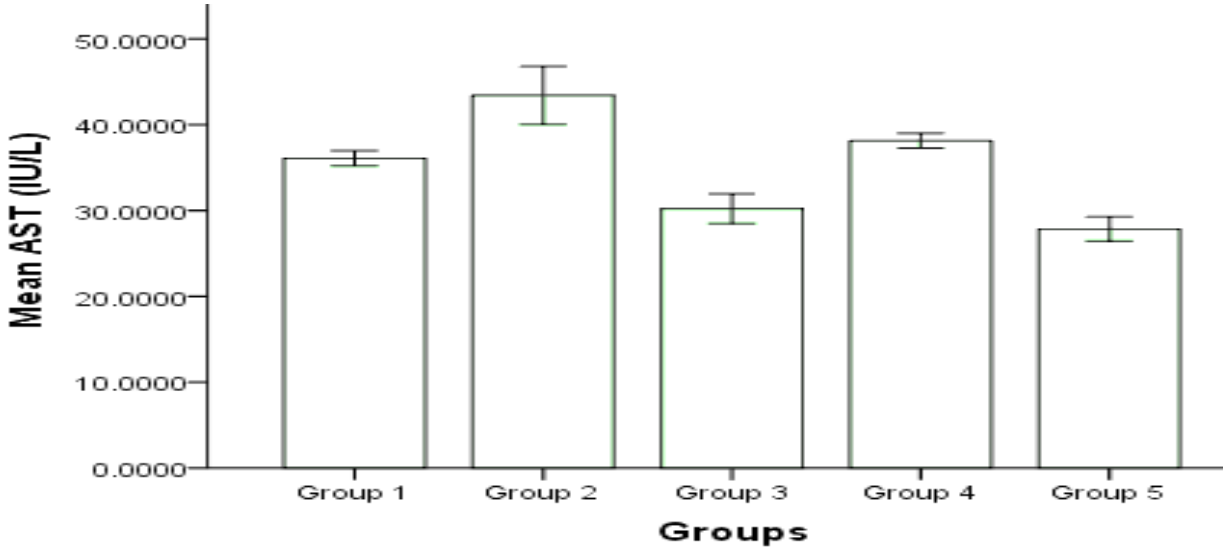

Figure 2: Effects of aqueous extracts of *T. occidentalis* and *M. pruriens* on the serum AST level in Wistar albino rats.

Effects of aqueous extracts of *T. occidentalis* and *M. pruriens* on the ALP levels

The effects of aqueous extracts of *T. occidentalis* and *M. pruriens* on the ALP levels are presented in Figure 3. The result indicates that the ALP level was higher in group 2 (74.00±0.56 IU/L) whereas there is significant decrease in Group 1 (61.17±0.86 IU/L), Group 3 (67.69±1.07 IU/L), Group 4 (69.44±0.76 IU/L), and Group 5 (67.17±1.00 IU/L) at *p<0.05.*

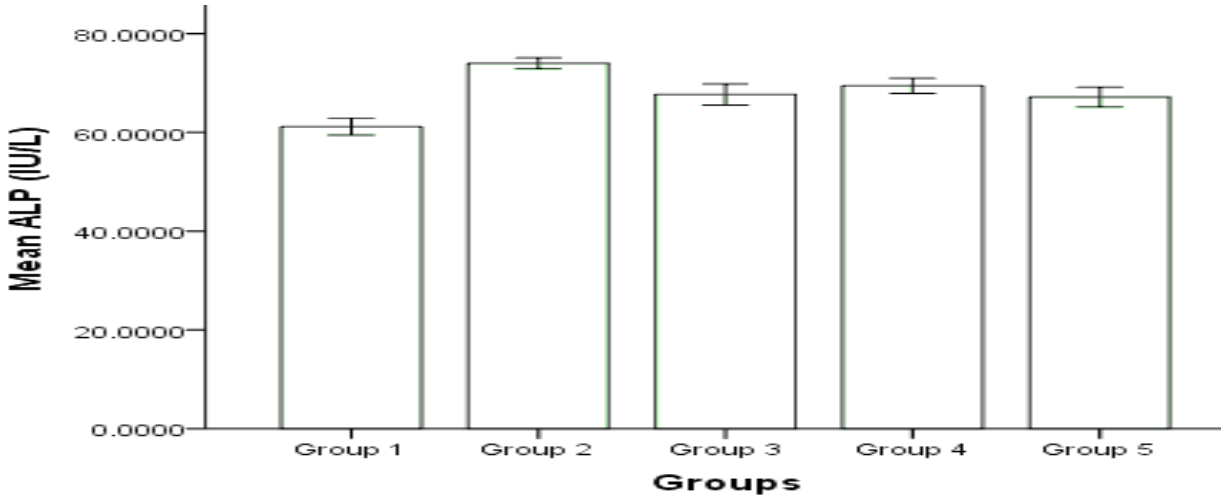

Figure 3: Effects of aqueous extracts of *T. occidentalis* and *M. pruriens* on the serum ALP level in Wistar albino rats.

Protein Profile

Effects of aqueous extracts of *T. occidentalis* and *M. pruriens* on the albumin levels

The effects of aqueous extracts of *T. occidentalis* and *M. pruriens* on the albumin levels are presented in Figure 4. The result indicates that the Albumin level was higher in group 1 (3.71±0.42 g/dl) whereas group 2 had the lowest albumin level (2.26±0.17 g/dl). There was a slight decrease in group 3 (3.37±0.20 g/dl) compared to the control with no significance difference between group 4 (2.70±0.03 g/dl) and group 5 (2.61±0.07 g/dl).

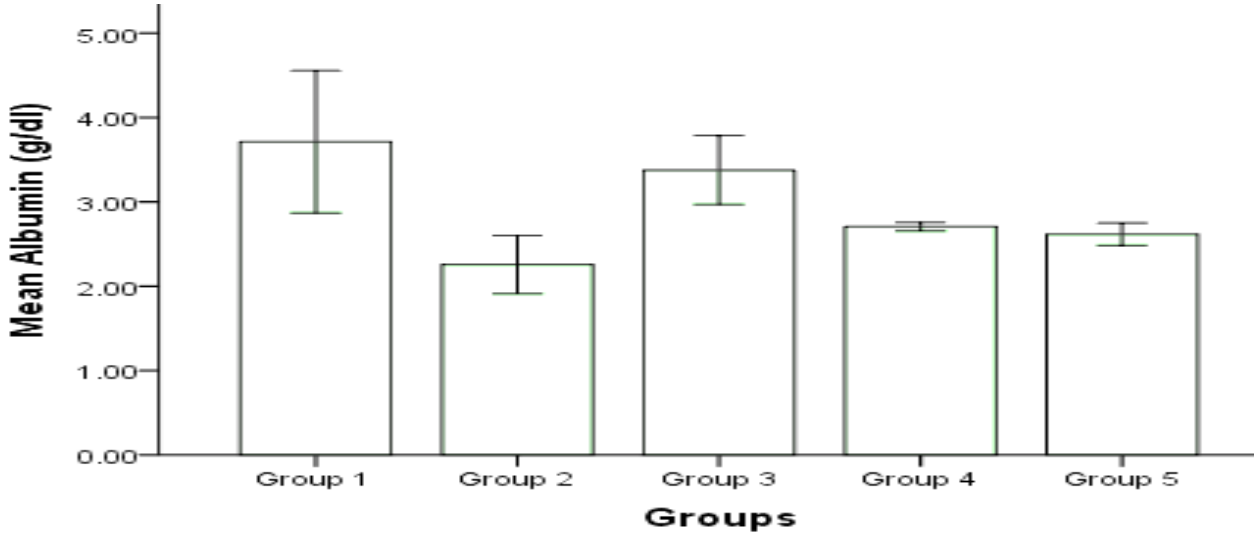

Figure 4: Effects of aqueous extracts of *T. occidentalis* and *M. pruriens* on the serum albumin level in Wistar albino rats.

Effects of aqueous extracts of *T. occidentalis* and *M. pruriens* on the globulin levels

The effects of aqueous extracts of *T. occidentalis* and *M. pruriens* on the globulin levels are presented in Figure 5. The result indicates that the globulin level was higher in group 3 (3.54±0.45 g/dl) than the control group 1 (3.35±0.22 g/dl). The levels of globulin in groups 2, 4 and 5 are 2.09±0.07 g/dl, 2.33±0.22 g/dl and 2.54±0.14 g/dl respectively.

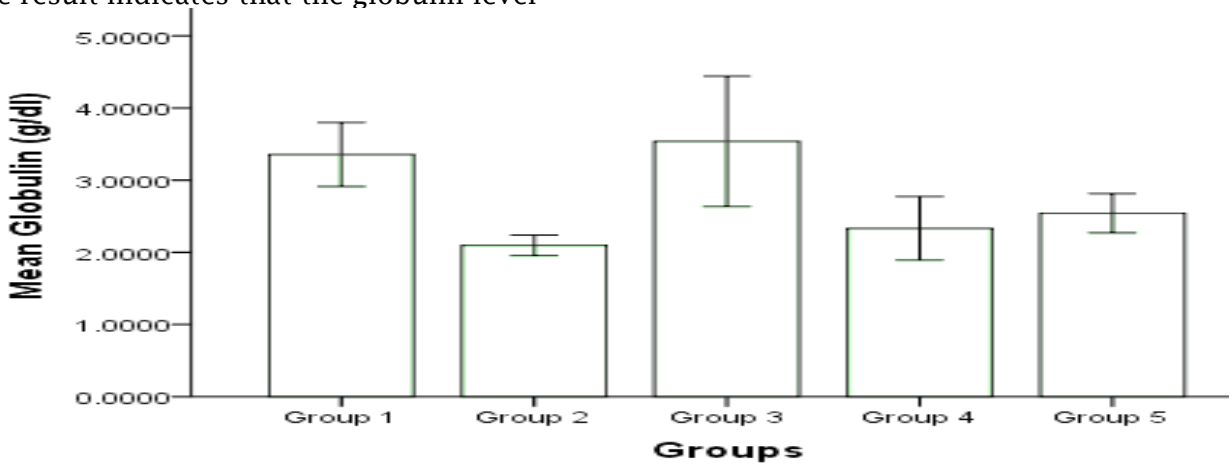

Figure 5: Effects of aqueous extracts of *T. occidentalis* and *M. pruriens* on the serum globulin level in Wistar albino rats.

3.4.3 Effects of aqueous extracts of *T. occidentalis* and *M. pruriens* on the total protein levels

The effects of aqueous extracts of *T. occidentalis* and *M. pruriens* on the total protein are presented in Figure 6. The result indicates no significance difference between the total protein level of group 1 (7.07±0.61 g/dl) and group 3 (6.91±0.37 g/dl). A decrease in the total protein levels of groups 4 and 5 showed no significant difference, 5.04±0.23 and 5.16±0.17 respectively. Group 1 has the lowest levels of total protein (4.35±0.22 g/dl).

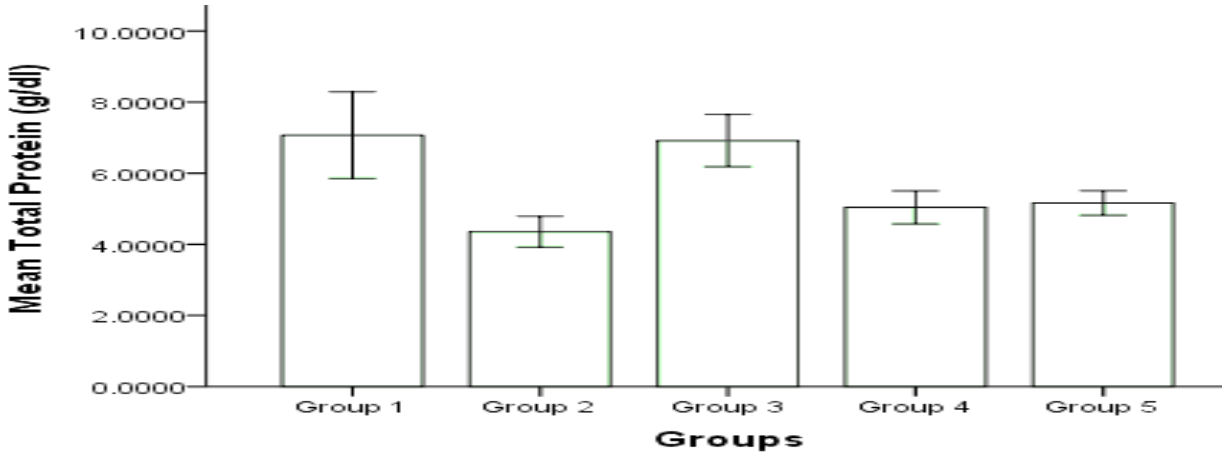

Figure 6: Effects of aqueous extracts of *T. occidentalis* and *M. pruriens* on the serum total protein level in Wistar albino rats.

Kidney Function

Effects of aqueous extracts of *T. occidentalis* and *M. pruriens* on the urea levels

The effects of aqueous extracts of *T. occidentalis* and *M. pruriens* on the urea levels are presented in Figure 7. The result indicates that the urea level increased in group 3 (27.12±0.64 mg/dl), group 4 (24.01±0.79 mg/dl, and group 5 (27.73±0.60 mg/dl) when compared to the control group (22.33±1.25 mg/dl). Group 4 had the urea level of 24.01±0.79 mg/dl while group 2 had 29.40±1.07 mg/dl.

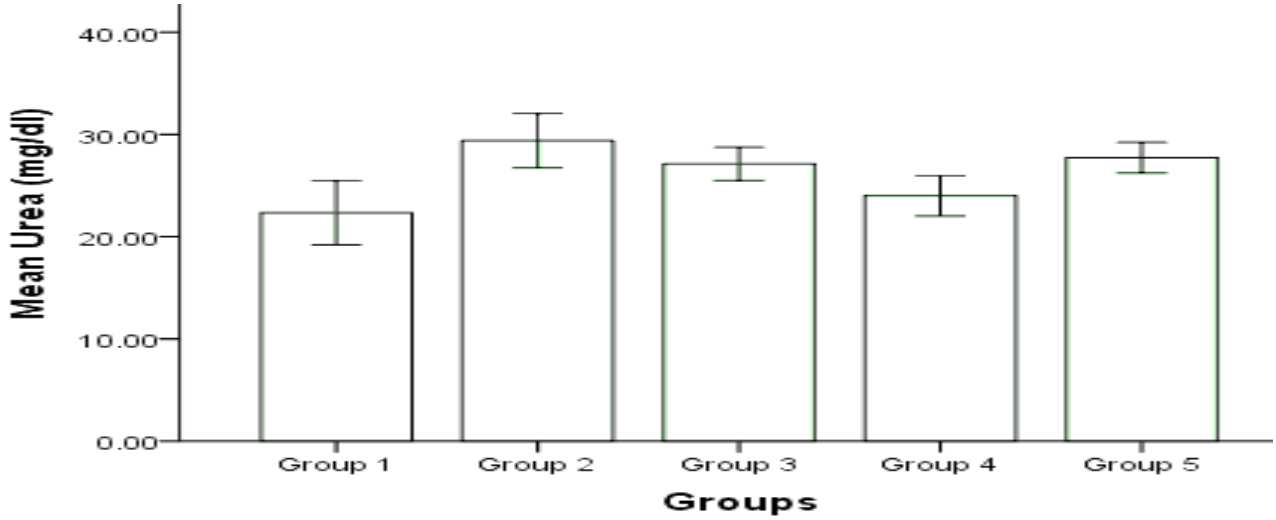

Figure 7: Effects of aqueous extracts of *T. occidentalis* and *M. pruriens* on the serum total protein level in Wistar albino rats.

Effects of aqueous extracts of *T. occidentalis* and *M. pruriens* on the creatinine levels

The effects of aqueous extracts of *T. occidentalis* and *M. pruriens* on the creatinine levels are presented in Figure 8. The result indicated that there was an increase in creatinine levels in group 2 (0.75±0.02 mg/dl), group 3 (0.66±0.01 mg/dl), and group 5 (0.71±0.01 mg/dl) while there was a decrease in group 4 (0.52±0.32 mg/dl) which suggests improved renal function compared to the control

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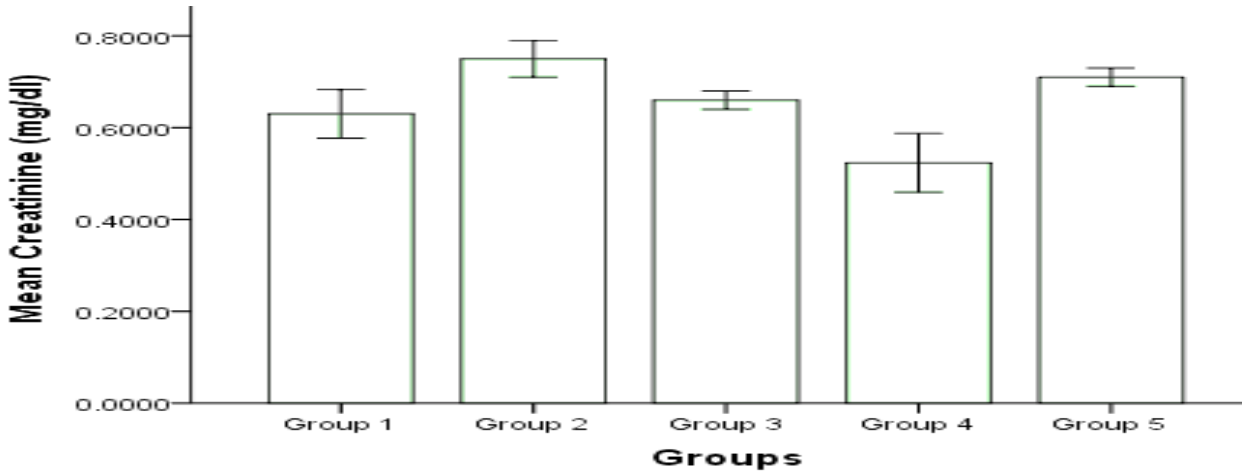

Figure 8: Effects of aqueous extracts of *T. occidentalis* and *M. pruriens* on the serum creatinine level in Wistar albino rats.

DISCUSSION

Medicinal plants are plants that have at least one of their parts (leaves, stem, barks or roots) used for therapeutic purposes. Recently, medicinal plants have become important for the treatment of different disease conditions, such as diabetes, malaria, and anaemia. The availability and relatively cheaper cost of medicinal plants in sub-Saharan Africa, makes them more attractive as therapeutic agents when compared to 'modern' medicines [17]. The importance of medicinal plants, and the contribution of phytomedicine to the well-being of a significant number of the world's population, has attracted interest from a variety of disciplines. Anaemia is the most common blood disorder, affecting about a third of the global population [18]. Research for screeing of medicinal herbs for anti-anemic effects form the basis of this work. The results obtained for the phytochemical screening of the aqueous extracts of *T. occidentalis* and *M. pruriens* were presented in Table 4.1. The result revealed the presence of flavonoids, alkaloids, saponins, tannins, phenols, terpenoids, steroids, proteins and cardiac glycosides in *T. occidentalis*. Reducing sugar was found absent. Phytochemical screening of *M. pruriens* revealed the presence of flavonoids, alkaloids, saponins, tannins, phenols, terpenoids, steroids, proteins, reducing sugar and cardiac glycosides. Results obtained for the quantitative analysis of the aqueous extracts of *T. occidentalis* and *M. pruriens* leaves revealed that *T. occidentalis* contained a higher level of flavonoids (6.14±0.70 %) compared to *M. pruriens*(4.58±0.59). Alkaloids was found

higher in *M. pruriens* (2.04±0.14 %) compared to that of *T. occidentalis*(1.02±2.12 %). The level of saponins found was higher in *T. occidentalis*(5.67±0.10 %) than in *M. pruriens* (2.65±0.34 %). Tannins and phenols were found higher in *M. pruriens.*The presence of these antioxidant phytochemicals is a supportive evidence in the ability of these plants to be used in the treatment of anaemia. Antioxidants are molecules that can delay or prevent an oxidative reaction [18] catalysed by free radicals. This antioxidant effect is mainly due to the presence of phenolic components such as flavonoids, phenolic acids and phenolic diterpenes [19]. Antioxidants protect plants against oxidative assault either by binding to metallic ions, eliminating free radicals or by decomposing peroxides [20]. Despite the availability of synthetic antioxidants, present research seeks at discovering new natural antioxidant compounds that may play a role in oxidative stress related disorders [21]. Epidemiological studies [22] have showed that decreases in the incidence of chronic diseases in some populations were related to the consumption of fruits and vegetables.

In this study, phenylhydrazine was used to induce anaemia. Phenylhydrazine is known to cause a reactive oxygen species formation, peroxidation of lipids and oxidative degradation of spectrin in the membrane skeleton. PHZ-induced haemolytic injury seems to be derived from oxidative alternations to red blood cell proteins [23]. It has been reported that phenyl hydrazine causes oxidative damage to red cells by increasing the formation of reactive oxygen species. However, alkaloids and flavonoids protect cells as powerful antioxidants which prevent or repair damage done to red cells by free radicals or highly reactive oxygen species [24]. Thus, it appears that the presence of these antioxidants in the plant sample reverse the damaging effect of phenyl hydrazine. Since there were improvements when the leaf extracts were administered the Wistar rats, this can be attributed to antioxidants bioactives such as flavonoids and other polyphenols.

The result obtained from the biochemical evaluation of the effects of the aqueous leaf extract on PHZ-induced anaemic rats revealed that *T. occidentalis* and *M. pruriens* leaf extracts helped improve liver function and renal functions as seen from the liver marker enzymes (ALT, AST, ALP) and kidney markers (Urea and creatinine). The result indicated that the ALT level was higher in group 2 (anaemic group) (35.85±0.62 IU/L) whereas there were significant decrease in Group 1 (23.73±0.55 IU/L), group 3 (25.73±0.91 IU/L), group 4 (32.73±0.56 IU/L), group 5 (30.51±0.55 IU/L) at $p<0.05$. This is a positive sign of improvement. Similar observation was found for AST Levels in which group 2 (43.42±1.35 IU/L) had the highest AST level compared to other groups, group 1 (36.09±0.35 IU/L), group 3 was 30.20±0.70 IU/L and group 4 and 5, had 38.14±0.35 IU/L and 27.85 IU/L respectively. The ALP level was higher also in the anaemic group i.e group 2 (74.00±0.56 IU/L) whereas there was significant decrease in group 1 (61.17±0.86 IU/L), group 3 (67.69±1.07 IU/L), group 4 (69.44±0.76 IU/L), and group 5 (67.17±1.00 IU/L) at $p<0.05$. This is an indication that the leaf extracts and the blood tonic improved liver function because an increase in the serum blood levels of these marker enzymes would have signaled negative attributes.

The effects of aqueous extracts of *T. occidentalis* and *M. pruriens* were presented in Figure 4. The result indicated that the Albumin level was higher in group 1 (3.71±0.42 g/dl) whereas group 2 had the lowest albumin level (2.26±0.17 g/dl). There was a slight decrease in group 3 (3.37±0.20 g/dl) compared to the control with no significance difference between group 4 (2.70±0.03 g/dl) and group 5 (2.61±0.07 g/dl).

The effects of the leaf extracts on the globulin level was higher in group 3 (3.54±0.45 g/dl) than the control group 1 (3.35±0.22 g/dl). The levels of globulin in group 2, 4 and 5 are 2.09±0.07 g/dl, 2.33±0.22 g/dl and 2.54±0.14 g/dl respectively. An increase in globulin levels for group administered *T. occidentalis* is a positive indication of renal improvements. Yet again, the result indicated no significance difference between the total protein level of group 1 (7.07±0.61 g/dl) and group 3 (6.91±0.37 g/dl). This is a positive indication for *T. occidentalis.* An increase in the total protein levels of group 4 and 5 with no significant difference, 5.04±0.23 and 5.16±0.17 respectively when compared to the normal control (Group 1) is also a positive attribute (6.52±0.6810^6/ul). The administration of the leaf extracts and HB12 blood tonic helped improve the PCV, HB and RBC level. The effects of the aqueous extracts of *T. occidentalis* and *M. pruriens* on the kidney function of Wistar albino rats were presented in Figure 7. The control group 1 (normal), had 22.33±1.25 mg/dl urea while the anaemic group had 29.40±1.07 mg/dl.The urea level then decreased in group 5 (27.73±0.60 mg/dl), group 3 (27.12±0.64 mg/dl), and group 4 (24.01±0.79 mg/dl), when compared to group 2. This a positive indication for improvements for groups administered *T. occidentalis, M. pruriens* and blood tonic. The effects of the extracts on the creatinine levels showed an increase in creatinine levels in group 2 (0.75±0.02 mg/dl) compared to group 1 (0.63±0.02 mg/dl). There was then a decrease in serum creatinine levels of group 3 (0.66±0.01 mg/dl), and group 5 (0.71±0.01 mg/dl) while there was a decrease in group 4 (0.52±0.32 mg/dl) compared to group 2. This suggests improved renal function.

The results obtained for the heamatological study were presented in Table 5 and 6. The result shows that group 2 (anaemic rats) exhibited lower PCV (%), HB (g/dl) and RBC. The group administered leaf extracts showed improved blood count. The PCV level was higher in group 5 (42.80±2.14 %) followed by group 3 (41.56±1.35 %). This is positive indicator for group administered *T. occidentalis.* The HB levels was higher in group 1, 14.90±0.14 g/dl followed by group 3 13.85±0.45 g/dl and group 5 (13.80±1.24 g/dl) with no significance difference at $p < 0.05$. The lowest HB was found in the anaemic group (6.93±0.38 g/dl). This indicates a positive result. The total RBC count was highest in group 1 (7.99±0.12 10^6/ul) followed by group 3 (6.70±0.35 10^6/ul) and group the results obtained for the total white blood cell (TWBC) count indicate that group 2 has the highest TWBC of (15.75±0.31 10^3/ul) while group 1 has the least TWBC (8.35±0.62 10^3/ul). The increased level of WBC suggests infection due to a compromised immune system. Infection may contribute to anaemia by reducing red blood cell survival, impairing iron bioavailability, impairing the response to erythropoietin and encouraging production of free radicals [25]. On the other hand, the reduced level of WBC and increased levels of other haematological indices may be due to the protective effect of the plant bioactive principles. In this regard, several studies have suggested that bioactive principles from plant sources may stimulate the release of the hormone erythropoietin in the kidney thereby increasing erythropoiesis in blood cells [25]; [26].

The differential white blood cell count indicated that groups generally showed increased levels of different white blood cells. The neutrophile level was highest in group 2 (26.57±1.67 %), while group 1 has the least neutrophile level (17.30±1.33 %). There was no significance difference in mean between groups 3, 4, and 5 for neutrophile count. The result of the lymphocyte count shows that group 2 has a higher count (66.53±0.97 %) compared to the control group 1 (42.29±4.00 %). An increase in the lymphocyte count was also observed for group 3 (58.45±2.05 %), group 4 (57.10±1.84 %) and group 5 (60.20±1.92 %). This is not a positive attribute. The eosinophile levels for the group 1 is the lowest (0.33±0.58 %)

compared to other groups. Groups 2, 3 and 5 had same level of eosinophile (1.33±0.58 %), while group 4 had 0.67±0.58 %. Similar trend was observed for the basophile levels of groups 2, 3 and 5 as each had 0.33±0.58 %. However, no basophil count was found for group 1 and group 4. The monocyte count was found higher in group 3 (1.33±0.58 %) while no significance difference existed between group 2 and 5 with same level (1.33±0.58 %). In general the differential white blood favors a positive result for group administered *T. occidentalis, M. pruriens* and blood tonic. It is thus recommended that the leaf extracts of *T. occidentalis* and *M. pruriens* could serve as prospect for drug formulation for the treatment of anaemia.

CONCLUSION

This study has therefore provided evidence of the anti-anaemic property of *T. occidentalis* and *M. pruriens* leaves in phenylhydrazine induced anaemic rats. The plant were also found to have protective effect on the liver and kidney tissues against the deleterious effect of phenylhydrazine. The bioactive principles in the plant possess antioxidative effect which may be useful for rapid haemopoiesis and erythropoiesis in the bone marrow. The results from this study support local claims on their efficacy in the treatment of anaemia. The result also revealed that *T. occidentalis* possess better anti-anaemic properties than *M. pruriens*.

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