

**Assessment of the haematopoetic potential of
Telfairia occidentalis leaf extract of on male wistar
rats**

ABSTRACT

Aims: This study assess the haematopoetic potentials of *Telfairia occidentalis* (TO) leaf extract on male wistar rat.

Methodology: A total of 20 wistar rats randomly distributed into four groups A to D were acclimatized for two weeks; group A served as control, while groups B C and D were gavaged 100 mg/kg b/w (UGU 1), 150 mg/kg b/w (UGU 2), 250 mg/kg b/w (UGU 3) of aqueous extract of TO respectively, once every 48 hours for 30 consecutive days. After the exposure period, the surviving rats were examined and sacrificed. Blood samples were collected for full blood count and blood film preparation.

Results: The result of the study showed that TO casued an increase in white blood cell count ($17.50 \pm 0.10 - 25.48 \pm 1.28 \times 10^9$ cells/L), lymphocyte ($2.45 \pm 1.35 - 15.86 \pm 2.66 \times 10^9$ cells/L), mid-sized cell ($3.38 \pm 0.42 - 4.61 \pm 0.60 \times 10^9$ cells/L) and granulocyte ($1.67 \pm 0.80 - 5.11 \pm 0.80 \times 10^9$ cells/L) when compared to the Control. There was equally an increase in the platelet count ($420.50 \pm 56.50 - 810.95 \pm 43.05 \times 10^9$ cells/L) and platetocrit ($0.33 \pm 0.05 - 0.58 \pm 0.03 \times 10^9$ cells/L) when compared to the Control. However, there was a reduction in the red blood count ($7.76 \pm 0.06 - 6.28 \pm 0.25 \times 10^9$ cells/L), haemoglobin ($16.67 \pm 0.37 - 12.18 \pm 0.58 \times 10^9$ cells/L) and hematocrit ($41.47 \pm 0.27 - 33.55 \pm 1.25 \times 10^9$ cells/L) of rats in treated group), ($6.92 \pm 0.34 \times 10^9$ cells/L) and when compared with the Control.

Conclusion: This present study have validated the haematopoetic potential of *Telfairia occidentalis* leaf extract as it improved the haematological parameters of male wistar rats. The antioxidants exerted a multitude of beneficial effect on cellular functions of both innate and adaptive immune system.

Keywords: blood film, haematology, full blood count, wistar rats, *Telfairia occidentalis*.

1. INTRODUCTION

Telfairia occidentalis is a type of climbing plant found in West Africa. It is cultivated for its leaves, which are used as a vegetable, and its seeds, which are edible. The plant is known by various names such as fluted gourd, fluted pumpkin and ugu. The plant is dioecious, perennial, and drought-tolerant. Typically, it is common to cultivate it in a trellised manner [1]. The plant being described is a crawling shrub that grows close to the ground. It has big leaves with divided sections and long, twisting tendrils. This plant prefers areas with high humidity and soil that drains well. People often grow it in gardens and small farms near their homes [2]. "*Telfairia occidentalis* is a member of the Cucurbitaceae family and possesses a single, dark green leaf with visible veins. The leaf can reach a width of 18 cm and a length of 35 cm. The Cucurbitaceae are reported to have been associated with man since 12,000 BC" [3]. Plants such as cucumber, watermelon, squash, and melon are commonly found within this plant family [4].

Telfairia occidentalis has been a traditional plant commonly utilized by a significant number of Nigerian indigenous communities, estimated to be around 30 to 35 million individuals. These communities include the Efik, Ibibio, and Urhobo [5]. "However, it is predominantly used by the Igbo ethnic group, who continue to cultivate the gourd for food sources and traditional medicines" [6]. "The shoots of *Telfairia occidentalis* have a lot of potassium and iron, while the seeds are made up of 27% crude proteins and 53% fats" [7]. "The leaves have a high amount of antioxidants and properties that are good for the liver and can fight against bacteria" [8]. In Nigeria, people use the young shoots and leaves of the female plant to make a soup called ofe egwusi. The seeds are large, about 5 cm in size, and dark red. They are full of fat and protein. People can eat them whole, grind them into a powder for soup, or turn them into a kind of fermented porridge.

Telfairia occidentalis is a well-known plant in herbal medicine. Its leaves are believed to have several health benefits. They are said to help maintain a balanced pH level in the blood, provide fiber that helps with digestion and prevents constipation, and support proper elimination of waste from the colon. By doing so, it may help prevent problems such as colitis, appendicitis, hemorrhoids, and anal fissures [9]. It is useful in the remedy of all cases of anaemia [10]. The leaves have been documented to stimulate bone marrow to produce blood cells and maintain body resistance to infection [11]. It has been employed in the treatment of anaemia, chronic fatigue and diabetes [12].

Throughout the history of medicine, natural products derived from plants have played a crucial role in maintaining human health. The active components found in plants have garnered significant scientific attention due to their important contributions to agriculture and medicine. However, our understanding of these substances and their potential benefits in nature is still limited because only a small number of plant species have been extensively studied. To gain a better understanding of natural products, it is important to conduct thorough investigations into the biological activities of various plants. One such plant is *Telfairia occidentalis*, although its leaves are commonly eaten by people in Southern Nigeria and other parts of West Africa, and they are also used in traditional medicine, we have limited knowledge about their effects. There are some claims made by herbal practitioners, but they have not been scientifically proven or verified. The few reported studies on this subject present a plethora of apparently conflicting results, supposedly due to their largely limited scope. It is therefore, paramount to scientifically investigate the haematopoietic potential of aqueous leaf extract of *Telfairia occidentalis* on male wistar rats.

2. MATERIAL AND METHODS

2.1 Collection, identification and laboratory analysis of edible plant

Fresh leaves of *Telfairia occidentalis* (fluted pumpkin) were purchased from Effurun Market in Effurun, Delta state in January, 2021; the taxonomic identity of the plant was confirmed at the Department of Plant Biology and Biotechnology, Faculty of Life Science, University of Benin, Benin City, Edo State, Nigeria. The plant was analyzed to determine its Phytochemical composition [13][14][15][16].

2.2 Preparation of plant extracts

The purchased leaves were air-dried to crispiness in the laboratory (prevailing room temperature of $30 \pm 2^\circ\text{C}$) for two weeks. The dried materials were reduced to coarse form using a pestle and mortar and further pulverized to very fine particles using Viking Exclusive Joncod pulverizing machine (Model: YLH2M2 - 4) China. The crude aqueous extract was prepared by decoction where 50g of the leaf powder extracted with 200mls of distilled water for 12hrs. The mixture was decanted and filtered using sterile whatman paper No 1. The filtrate was evaporated to dryness using a freeze dryer and reconstituted in distilled water to appropriate concentrations.

2.3 Experimental setup

Male wistar rats (6-7 weeks old) weighing within the range of 100g to 150g were obtained from the Anatomy Department, University of Benin, Nigeria. The rats were distributed randomly into four groups of six animals each for group A to D and allowed to acclimatized for 2 weeks. During acclimatization, the animals were housed in wooden cages with wire mesh covers and fed with standard rodent chow (Bendel Livestock Feeds Limited, Ewu, Edo state, Nigeria) and given distilled water ad libitum. After acclimatization, the rats were given different treatment protocol: Group A which was the Control (CTR) was given distilled water; Group B, C and D rats were gavaged 100 mg/kg b/w (UGU 1), 150 mg/kg b/w (UGU 2), 250 mg/kg b/w (UGU 3) of aqueous extract of TO for 60 consecutive days (once every 48 hour) respectively. The rats were maintained in laboratory conditions; and had access to drinking water and standard rodent chow (Bendel Livestock Feeds, Ewu, Edo state, Nigeria®) *ad libitum*. At the end of exposure period, survivors were fasted overnight and sacrificed under slight Anesthesia; then blood samples were collected. Blood was collected from the inferior vena cava of the rats with plain 5ml sterilized syringe into a vial containing 0.5 m EDTA for haematological analysis under a light anaesthesia. The blood sample was gently homogenised to ensure proper mixing of the blood with the anticoagulant, before taking it to the laboratory.

2.4 Laboratory analysis

In the laboratory, hematological analysis was carried out using Sysmx KX-21N automated machine (Sysmx corporation kobe, Japan) following the manufacturer's instructions. Briefly the sample was mixed and placed in contact with the sample probe for aspiration.

2.5 Data Analysis

All statistical analyses were conducted with Statistical Package for Social Scientists (SPSS) and Microsoft Excel computer software. Data are presented as mean \pm SEM (n=5). One-way ANOVA was used to determine the differences among various groups. When the corresponding F test for differences among the treated group means was significant pair wise, comparisons between treated groups and corresponding negative control were determined using multiple comparison procedure of the Dunnett

post-hoc test and differences were considered significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$ levels of significance.

3. RESULTS AND DISCUSSION

The result of the phytochemical composition of *Pterocarpus mildraedii* is shown in table 1. It reveals the presence of phytochemicals such as tannins, flavonoids, alkaloids, and saponin in the aqueous leaf extract of *Pterocarpus mildraedii*.

List 1: Phytochemical Composition of *Pterocarpus mildraedii* leaves extract

S/N	Parameters	Composition
1	Phenol	-
2	Tannins	+
3	Steroids	-
4	Flavonoids	+
5	Alkanoids	+
6	Saponin	+

NB: Indicates absent, + trace

Studies have shown that “haematological parameters represent a useful process in the diagnosis of many diseases as well as investigation of the length of damage to the blood” [17]. “This is relevant since blood constituents changes in relation to the physiological conditions of the animals. Haematological studies are vital because blood is a critical transport system of the body, and calculation of the haematological profile usually presents important information on the body's response to injury of all kinds, including hazardous injury” [18]. Haematological constituents depict the physiological functionality of the animal to its internal and external environments which include feed and feeding [19] as well as drugs [20].

The white blood cell and differential count is usually carried out in order to produce information on the amount of the different white cells present in circulating blood [21]. In this study the aqueous extract of *T. occidentalis* caused a significant increase in white blood cells. The result of the study showed that TO caused an increase in white blood cell count (17.50 ± 0.10 - $25.48 \pm 1.28 \times 10^9$ cells/L), lymphocyte (2.45 ± 1.35 - $15.86 \pm 2.66 \times 10^9$ cells/L), mid-sized cell (3.38 ± 0.42 - $4.61 \pm 0.60 \times 10^9$ cells/L) and granulocyte (1.67 ± 0.80 - $5.11 \pm 0.80 \times 10^9$ cells/L) in treated groups when compared to the Control (table 2) (plate 1b - 1d). The increases in the haematological indices observed in this study is consistent with the observation made when rats fed with the diet preparation of the air - dried leaves of *T. occidentalis* for 4 weeks [12]. The findings was also similar to the report of Eze et al. [22]; Obeagu et al. [23] In their result, the aqueous extract of *Telfairia occidentalis* also significantly increased ($p < 0.05$) the WBC count. The increases in the haematological indices observed following treatment with *T. occidentalis* leaves aqueous extract might not be unconnected with the chemical composition of its leaves. The chemical composition has shown to include proteins, fat, vitamin A, thiamine, riboflavin, nicotinamide, vitamin C [24] and minerals such as zinc, iron, calcium and magnesium [25]. “The amino acid profile of *T. occidentalis* had also been shown to be very rich and includes alanine, aspartate, glycine, glutamine, histidine, lysine, methionine, tryptophan, cysteine, leucine, arginine, serine, threonine, phenylalanine, valine, tyrosine and isoleucine” [26]. Some of these constituents are well established haematopoietic factors that have direct influence on the production of blood in the bone marrow.

Table 1: Hematological profile of male wistar rats given crude aqueous extract of TO leaf

	CTR	UGU 1	UGU 2	UGU 3	P-Value
White Blood Cell (x 10 ³ /μl)	17.50±0.10	18.40±5.80	12.85±2.65	25.48±1.28	P<0.05
Lymphocytes (x 10 ³ /μl)	12.45±1.35	16.00±5.40	9.15±0.95	15.86±2.66	P>0.05
Mid-Sized cell (x 10 ³ /μl)	3.38±0.42	1.70±0.20	2.20±1.00	4.61±0.60	P>0.05
Granulocyte (x 10 ³ /μl)	1.67±0.83	0.70±0.20	1.50±0.70	5.11±0.80	P<0.05
Lymphocyte (%)	73.03±9.13	86.20±2.20	72.85±7.55	62.65±7.95	P>0.05
Mid-Sized cell (%)	18.65±3.25	9.95±2.05	16.10±4.40	17.66±3.64	P>0.05
Granulocyte (%)	8.32±5.88	3.85±0.15	11.05±3.15	19.69±4.31	P<0.05
Red Blood Cell (x 10 ⁶ /μl)	7.76±0.06	6.92±0.34	6.75±0.42	6.28±0.24	P>0.05
Hemoglobin (g/dl)	16.67±0.37	14.10±0.80	13.70±0.70	12.18±0.58	P>0.05
Hematocrit (%)	41.47±0.27	36.65±1.75	35.30±1.40	33.55±1.25	P>0.05
Mean Corpuscular Volume (μm ³)	53.48±0.78	52.95±0.05	52.40±1.20	53.40±0.11	P>0.05
Mean Corpuscular Hb (pg)	21.43±0.63	20.35±0.15	20.30±0.20	19.32±0.12	P>0.05
Mean Corpuscular Hb.conc. (g/dl)	40.25±0.65	38.45±0.35	38.75±0.45	36.20±0.30	P>0.05
Coefficient of variation RDW (%)	16.20±0.30	17.40±0.70	17.55±0.95	18.65±0.46	P>0.05
Standard deviation of RDW (μm ³)	30.88±0.68	32.10±1.90	31.15±2.95	37.01±1.09	P>0.05
Platelet (x 10 ³ /μl)	420.50±56.50	493.00±75.00	558.00±13.00	810.95±43.05	P<0.05
Mean Platelet Volume (μm ³)	8.03±0.13	7.05±0.25	7.25±0.25	7.14±0.06	P>0.05
Plateletocrit (%)	0.33±0.05	0.35±0.04	0.40±0.00	0.58±0.03	P>0.05
Platelet Distribution Width (%)	17.88±0.58	16.70±2.20	15.95±0.35	15.97±0.14	P>0.05
P-LCR (%)	11.27±0.73	6.90±0.70	8.20±0.60	7.01±0.39	P<0.05

NB: RDW = Red cell distribution width; All values are expressed as Mean±SE; N = 5; P<0.05 and P>0.05 indicates significant and non-significant difference respectively

On the contrary, there was a non-significant reduction in red blood blood count (7.76±0.06 - 6.28±0.25 ×10⁹cells/L), haemoglobin (16.67±0.37 – 12.18±0.58 ×10⁹cells/L) and hematocrit (41.47±0.27 – 33.55±1.25 ×10⁹cells/L) of rats in treated group), (6.92±0.34 ×10⁹cells/L) in treated groups when compared with the Control. This however does not agree with the findings of Udosen and Osu [27] who reported that *T. occidentalis* extracted with n-butanol increased red blood cell, and haemoglobin in wistar. The increase according to them may be associated with amino acids and the iron present in *T. occidentalis* [22][23]. However, Adias et al. [28] had earlier recorded “a significant increase in Hb concentration with no significant difference in the mean PCV level following their study on the effect of pumpkin extract (*Telfairia occidentalis*) on routine haematological parameters in acetone-Induced oxidative stress albino rats”. Ossamulu et al. [29] reported the abundance of saponins, an abundant bioactive ingredient in *T. occiendentalis* may trigger hemolysis in red blood cells [30]. This may be responsible for the non-significant increase RBC, heamoglobin and heamatocrit in treated rats. The non-significant changes observed indicates that *T. occidentalis* may also have blood detoxifying potentials, as has been seen in various vegetables [31][32]. Ths may have played a physiological role in maintaining the levels of RBC, HCT, and HCB of treated group of wistar rats.

Increased platelet count (420.50±56.50 - 810.95±43.05× 10⁹ cells/L) and platetocrit (0.33±0.05 – 0.58±0.03 ×10⁹cells/L) in rats administered aqueous extract of *Telfairia occidentalis* may not also be unconnected with abundant amino acids present in the leaves as earlier stated. Similar trends have been reported by Eze et al. [22], Obeagu et al. [23] and Udosen and Osu [27] in rats as a result of *Telfairia occidentalis* leaf extracts in Wistar albino rats. “Platelets are cytoplasmatic fragments of bone marrow megakaryocytes” [33]. “They are dynamic blood particles whose major function, along with the coagulation factors, is haemostasis, or the stoppage of bleeding. Platelets interact with each other, as

well as with leukocyte and endothelial cells, searching the vascular bed for sites of injury, where they become activated” [34]. “In addition to their vital role in haemostasis and thrombosis, accumulating evidence demonstrates that platelets contribute to the inflammatory process, microbial host defense, wound healing, angiogenesis, and remodeling” [35].

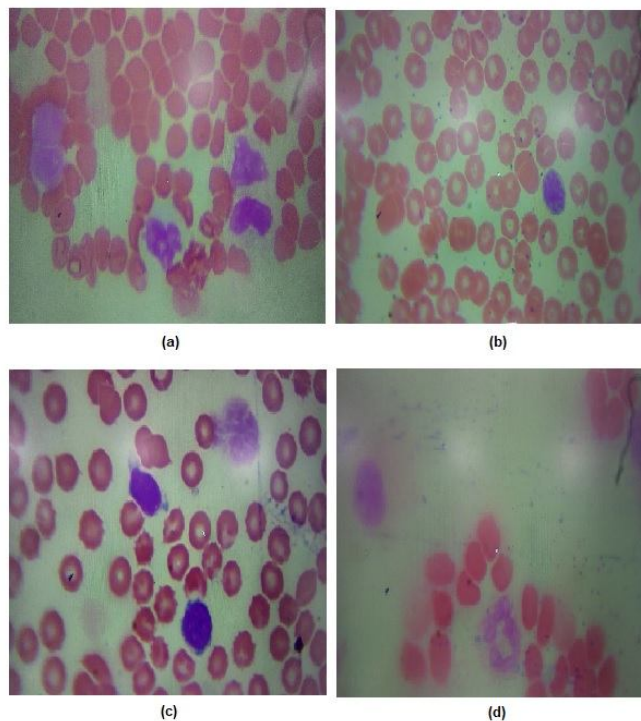


Plate 1: Blood film of (a) control rats (CTR) (b) rats exposed to 100 mg/kg b/w crude aqueous extract of TO (c) rats exposed to 150 mg/kg b/w crude aqueous extract of TO (d) rats exposed to 250 mg/kg b/w crude aqueous extract of TO (Eosin stain X 100)

4. CONCLUSION

This present study have validated the haematopeotic potential of *Telfairia occidentalis* leaf extract as it improved the haematological parameters of male wistar rats. The antioxidants exerted a multitude of beneficial effect on cellular functions of both innate and adaptive immune system.

ETHICAL APPROVAL

This research design was reviewed and approved by the College of Science Ethical board, Federal University of Petroleum Resources, Effurun (CS/EMT/2021/006).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

Author A' (Albert C. Ibezute) designed the study, performed the statistical analysis and wrote the protocol, 'Author B' (Reuben O. Eshogba) managed the literature searches and wrote the first draft of the manuscript. All authors read and approved the final manuscript.

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