

Comparative Study On The Phytochemical Composition Of *Telfairia Occidentalis* And *Ipomea Batata* Leaves Extract And Their Haematological Effects On Wistar Rats

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Abstract

The current study focuses on the comparative phytochemical analysis between *Telfairia occidentalis* (Ugu) and *Ipomea batata* (sweet potato) leave extracts and also haematological analysis of blood samples obtained from Wistar rats administered with leaves extracts of the two plants. Parameters considered in this study include Alkaloids, Saponins, Tannins, Red Blood Cell (RBC), White Blood Cell (WBC), Haemoglobin Concentration (Hgb), Packed Cell Volume (PCV)/ Haematocrit (HCT) and Platelet Counts (PLT). The two leave extracts show the presence of the following phytochemicals: Alkaloid, Flavonoid, Phenols, Terpenoids, Glycosides and Saponins. Automated Hematological analysis of the blood samples revealed: WBC $9.0 \times 10^3/\mu\text{L} \pm 0.1$, RBC $6.7 \times 10^6/\mu\text{L} \pm 1.0$, Hgb $12.4 \text{ g/dL} \pm 1.5$, PLT $988 \times 10^3/\mu\text{L} \pm 41.3$ and PCV/HCT $37.5\% \pm 0.1$ for *I. batata* while *T. occidentalis* gave WBC $8.5 \times 10^3/\mu\text{L} \pm 1.2$, RBC $7.2 \times 10^6/\mu\text{L} \pm 0.3$, Hgb $12.7 \text{ g/L} \pm 0.3$, PLT $750 \times 10^3/\mu\text{L} \pm 68.4$ and PCV/HCT $40.1\% \pm 1.1$. From the result, it can be concluded that *Ipomea batata* contain phytochemical and haematological parameters that are close related to *Telfairia occidentalis*, especially in WBC Hb and HCT. However, the latter register significantly higher RBC, Hgb and PCV values. This increase is more pronounced in groups fed with mixtures of two extracts, suggesting synergistic effect in samples. Similarly, significant increase in weight of the Albino rat fed with extracts was observed during the period of the study compared to control.

Keywords: Phytochemicals, Haematological Parameters, *Telfairia Occidentalis* (Ugu Leaf), *Ipomea Batata*

INTRODUCTION

Vegetables are the fresh and edible portions of herbaceous plants, which can be eaten raw, or cooked [1]. Vegetables are valuable in maintaining alkaline reserve of the body. They are valued mainly for their high carbohydrate, vitamin and mineral contents. Vegetable may be edible roots, stems, leaves, fruits or seed. Each part contributes diet in its own way [2]. However, there are some used and inexpensive leafy vegetables whose nutritive and anti-nutritive potentials are yet to be adequately studied and utilized. Among these leafy vegetables, *Ipomea batata* (sweet potato leaf) and *Telfairia occidentalis* (Fluted pumpkin) [3]. Several medicinal plants including leafy vegetables have been documented to have essential macro and micro nutrients which can also augment the pharmacological functionality of the phytochemicals found in plants [4].

Telfairia occidentalis Hooker, it is called 'Ugwu' in Igbo language, a tropical vine grown in West Africa as a leafy vegetable and for its edible seeds. Belonging to the family Cucurbitaceae, it has two species in Africa namely: *Telfairia Occidentalis* Hooker and *Telfairia Pedata* Hooker from West and East Africa respectively [5]. It is a large, perennial, climbing plant which plays important role in human and livestock nutrition. It contain nutrients such as protein, carbohydrates, vitamin,

minerals and fibre [6] [7]. The traditional herbal preparation of the plant *Telfairia Occidentalis* leave has been employed in the treatment of Anemia, sudden attack of convulsion and Malaria [8]. The major phytochemicals present in the leaves are Alkaloids, flavonoids, saponin, phenol and glycoside [5]. The proportions of various minerals varies with age of the plant. Anti-nutrient of the young stems and leaves of the plants part was found to be rich than that found in the older leaves and stem. [9]. The high content of Iron (Fe) in the young tender fluted pumpkin leaves was given as the basis for which the leaf extract is administered traditionally as blood tonic in treatment of anemia and as an anti-convulsion to patients. The leaves are also good source of Iron (Fe) and Manganese (Mn), moderate sources of Magnesium (Mg) and (Zn) which are essential in human and animal Nutrition [6]

Ipomea batata (L.) Lam. (sweet potato) belongs to the Ipomoea genus, the largest species within the Convolvulaceae family [10]. It is called 'sweet potato leaf' in English and 'ganyen dankali' in Hausa. There are several varieties of *Ipomea batata*, which differs based on the color of the peel and flesh of the tuber, including purple- purple, purple- orange, yellow - yellow purple and yellow - yellow orange (YYO) [11]. It is tuberous rooted perennial plant that

is usually grown as an annual crop. *Ipomea batata* (sweet potato leaf) have traditional uses in Malaysia, Pakistan, Philippines, Indonesia and Tanzania including a source of dietary fiber, for treating allergies and anemia and providing energy for people with diabetes mellitus[12],[13],[14]. Sweet potatoes contain various phytochemical compounds such as phenolics compound, flavonoids, anthocyanins, carotenoids and tannins in their roots, tubers, leaves and stem [15][16].

AIM AND OBJECTIVES

The study aimed at comparing the phytochemical and hematological composition of *Telfairia Occidentalis* (ugu leaf) and *Ipomea batata* (sweet potato leaf) using experimental animals, fed with *Ipomea batata* extract, *Telfairia Occidentalis* and mixture of both extract.

METHODS

Sample Collection and Preparation

The fresh leaf of *Telfairia Occidentalis* (fluted pumpkin) was purchased at Tudun Wada market of Gusau local government area. While the *Ipomea batata* leaves (sweet potato) was obtained local farmers Dam site of same Local Government in Zamfara State, Nigeria. The samples were authenticated at Biological Science Department of the University. The leaves were washed thoroughly to remove contaminants, shade dried for one week and reduced to a powdery form by pounding in a clean pestle and mortar.

Maceration of the Powdered Sample

Obeagu [8] method was adopted with slight modifications. Exactly 100g of the powdered leaves of *T. occidentalis* was soaked in 80% ethanol, stirred for 72 hrs after every 1hr interval. After 72hrs, it was filtered with filter paper. The extract was evaporated to concentration by using rotary Evaporator and was dried further using the hot air oven. One (1g) of extract was then dissolved in 50ml of normal saline. The same method was applied in extracting *I. batata* extract while in extracting mixture of *T.occidentalis* and *I. batata extract* 100g of each powdered sample was measured and soaked in 80% ethanol stirred for 24 hrs after every 1hr interval. After 24 hrs., it was filtered with filter paper. The extract was evaporated to concentration by using the steam method and was dried further using the hot air oven. One gram (1g) was then dissolved in 50ml of normal Saline. Also, the same method was applied in extracting mixture of *I. Batata* and *T. Occidentalis*.

PHYTOCHEMICAL SCREENINGS

Test for Alkaloids (Wagner's and Hager's Test)

Exactly 2ml of the ethanol extract (filtrate) was measured and transferred into a test tube and 5 drop of Wagner's reagent was added. A reddish-brown

precipitate indicates the presence of alkaloids. And similarly, method was performed by drop 5 drops of Hager's reagent. A yellow precipitate indicates the presence of alkaloids [17]

Test for Flavonoids (Alkaline Test)

Exactly 2ml of the ethanol extract was measured and then transferred into a test tube, 2ml of 2% NaOH was added, and the mixture turn to yellow and 2 drop of dilute acid was added the colour then disappeared. This indicates the present of flavonoids [18].

Test for Phenols (Ferric Chloride Test)

Exactly 2ml of the ethanol extract was measured and then transferred into a test tube, 2ml of distilled water was added, followed by 10% FeCl₃ solution. A bluish black colour indicates the presence of phenol [19].

Test for Terpenoids (Sulphuric Acid Test)

Exactly 2ml of the ethanol extract was measured and then transferred into a test tube, 2ml of chloroform was added into it, 2ml of H₂SO₄ was also added then it formed a layer in the test tube. The presence of a reddish-brown coloration at the interface indicates presence of terpenoids [20].

Test for Steroids

Two ml (2ml) of the ethanol extract was measured and then transferred into a test tube, 2ml of chloroform was added into it, and 2ml of conc H₂SO₄ was also added. In the lower chloroform layer, a red colour indicates the presences of steroids [21]

Test for Glycoside (Salkowski's and Keller-kilani Test)

Salkowski's Test

Two ml 2ml of the ethanol extract was measured and then transferred into a test tube, Then 2ml of chloroform was added and the few drops of conc H₂SO₄ was added and the mixture was shake. Formation of reddish-brown color indicates the presence of glycosides [19].

Keller-kilani Test

Exactly 2ml of the ethanol extract was measured and then transferred into a test tube, 2ml of acetic acid was added and few drops of 2% FeCl₃ solution. The mixture was poured into a test tube containing 2ml conc H₂SO₄. A brown ring at the interface/junction indicates the presence of glycosides [19]

Test for Tannins (Ferric chloride test)

Two ml (2ml) of the ethanol extract was measured and then transferred into a test tube, few drops of 2% FeCl₃ solution was added. Formation of blue colour indicates the presence of hydrolysable tannins. [18].

Test for Saponins (Emulsion test)

Exactly 2ml of the ethanol extract was measured and then transferred into a test tube then 2ml of distilled

water was also added and shake vigorously for a stable persistent froth. Formation of emulsion indicates the presence of saponins when few drops of olive oil was added [20].

The experimental design by Obeagu [8] was adopted, 16 Albino rats were used as animal models. They were housed in four wire mesh cages separately containing four rats in each cage. The weight of rats was taken before the experiment. The rats were made to acclimatize for 1 week before experiment; they were feed on commercial packaged feed and water. . Plants extract were orally administer for 14 days.

Wistar Rat grouping Order

Sixteen (16) Albino rats were divided in to four groups, each containing four albino rats. The groups were labeled as group1,2,3 and 4. The groups received the following treatments:

Group 1: The four (4) rats in this group were administered with normal packaged feed, distilled water and 100mg/Kg of *T.Occientalis* extract.

Group 2: This group was administered with normal packaged feed, distilled water and 100mg/Kg of *I. batata* extract.

Group 3: This group was also administered with normal packaged feed, distilled water and 100mg/Kg of mixture of *T.Occidentalis* and *I.batata*

Group 4: This group served as control, administered normal packaged feed and distilled water only.

This feeding process continues for fourteen (14) uninterrupted days.

Collection of Blood Samples

$$\text{Hgb} = \frac{\text{OD of test} \times \text{Concentration of standard} \times \text{Dilution factor}}{\text{OD of standard}}$$

Where: OD = Optical density, Dilution factor = $1/200 \div 1/200 = 1/200 \times 200/1 = 1$

Platelet Count (PLT)

The platelet count was obtained using haemocytometer. Exactly 0.38ml of ammonium oxalate was dispensed into each of the tube; 200ml of mixed EDTA and blood was added to each tube and mixed properly. A Pasteur pipette, one of the grids of the chamber was filled with the samples; the chamber was left undisturbed for 20 minutes to allow the cells

$$\text{Platelet count/Liter} = \frac{\text{Number of cells count} \times \text{Dilution factor}}{\text{Volume counted}}$$

Where: Dilution factor (D_f) = 20, Volume count = Total area of square count $\times$ Depth.

Total White Blood Cell (TWBC)

The total white blood cell (TWBC) is estimated using the haemocytometer. 0.38ml of the diluting fluid was measured into each of the tubes, 20 μ L of EDTA and blood was added to it and well mixed. The counting chambers were assembled making sure that the central grid areas of the chamber and the special haemocytometer cover glass are completely cleaned

At the end of the treatment, blood samples of the rats according to the group were collected by direct cardiac puncture into sterile containers with anticoagulants: Ethylenediaminetetraacetic acid (EDTA).

Analysis of Blood Sample

Packed Cell Volume (PCV) estimation

Sterilized capillary tubes (vacuum blood sample tube) were filled up to its three quarter 3/4 mixed with EDTA and sealed at one end using a sealant. The tubes were placed on the slots of micro haematocrit rotors and balanced with the sealed ends against the rim gasket to avoid breakage. The inner lid was positioned carefully to avoid dislodging of the tubes. It was a centrifuged for 5mins at 12000round/min, immediately after centrifuging, the PCV was read using a Haematocrit reader and recorded [8].

Haemoglobin (Hgb) Concentration

The Haemoglobin (Hgb) Concentration was measured using spectrophotometer by cyanometha haemoglobin method. Exactly 0.02ml of mixed EDTA and blood was carefully dispensed into 4ml Drabkin's neutral diluting fluid with the aid of a micropipette, the solutions in the tube were gently swirled with blood for homogeneous mixture and left at room temperature for a while away from sunlight for 4-5 minutes. The spectrophotometer was switch on allowed to set and the wavelength was set and calibrated at 540nm. The Drabskin's fluid was used to zero the spectrophotometer and the absorbance of the mixture samples was read.

to settle. Using the 10x objective lens magnification, the rulings were focused and the central square of the chamber was brought into view. The 40x objective lens magnification was then used to focus on the small platelets which appeared as small bright fragments. The platelets in the small squares were counted and the number of platelet was reported per liter of blood.

and dried. The diluted blood sample was remixed, using a Pasteur pipette; the grids of the chamber were filled with the sample. The chamber was left undisturbed for 2 min to allow time for the white blood cells to settle, using the 10x objective with the condenser iris closed sufficiently to give full contrast, the rulings of the chamber and white blood cells were focused until they appeared as small black dots. The

cells in the four corners square of the chamber were counted. The number of the white cells per little of blood was reported by dividing the total number of cells counted by 2 then divides the number [8].

$$\text{TWBC} = \frac{\text{Cell count} \times 2 \times 10^6}{4 \times 0.1}$$

RESULT

The results obtained in the laboratory for the experiment Table 4.1-4.2 are for the extract of *T. Occidentalis* and *I. Batata*, clinical analysis of blood sample result obtained are in table 4.3 – 4.7 the values are entered accordingly.

TABLE 4.1: RESULT OF PHYOCHEMICAL SCREENING

S/No.	Phytochemicals experimental methods	<i>T. Occidentalis</i>	<i>I. batata</i>
1	Alkaloids (1)Wagners test <i>Hagers test</i> (2)	- - ++	++ ++
2	Flavonoids Alkaline test	++	++
3	Phenols Ferric chloride test(FeCl_3)	++	++
4	Glycosides Keller - Kilani Test Sakowski's Test	++ - -	++ ++
5	Terpenoid Test Sulphuric Acid Test	++	++
6	Saponin Test	++	++

Table 4.2: Stock solutions from two selected dose of *T. Occidentalis* and *I. Batata* extract.

Standard Dose	Stock Solution	Animal body weight(g)	Calculated dose (mg)	Equivalent dose (ml)
Low dose, 200mg/Kg	320mg/16ml	100	20.0	1.0
	361.6mg/17.6ml	113	22.6	1.1
	384mg/19.2ml	120	24.0	1.2
	432mg/22.4ml	135	28.0	1.3
	448mg/22.4ml	140	30.4	1.4
	486mg/24ml	152	45.2	1.5
High dose, 400mg/Kg	640mg/19.2ml	100	40	2.0
	723mg/36.8ml	113	45.2	2.3
	768mg/38.4ml	120	48.0	2.4
	864mg/43.2ml	135	54.0	2.7
	896mg/24ml	140	56.0	2.8
	972.8mg/48ml	152	60.8	3.0

Table 4.3: Control free extract administered

S/No.	Parameters	Control (1)	Control (2)	Control(3)	Control (4)	Average
1	WBC	$14.9 \times 10^3/\mu\text{L}$	$9.1 \times 10^3/\mu\text{L}$	$10.1 \times 10^3/\mu\text{L}$	$7.7 \times 10^3/\mu\text{L}$	$10.5 \times 10^3/\mu\text{L} \pm 3.1$
2	RBC	$6.7 \times 10^6/\mu\text{L}$	$8.1 \times 10^6/\mu\text{L}$	$6.29 \times 10^6/\mu\text{L}$	$6.79 \times 10^6/\mu\text{L}$	$6.97 \times 10^6/\mu\text{L} \pm 0.8$
3	HGB	12.5	13.6	12.3	12.5	$12.7 \text{ g/dL} \pm 0.6$
5	HCT	36.3%	45%	34.5%	39.0%	$38.7\% \pm 0.1$
6	MCHC	34.4	30.2	35.7	32.1	$33.1 \text{ dL} \pm 2.4$
7	PLT	916	974	998	904L	$947 \times 10^3/\mu\text{L} \pm 45.2$

Table 4.4: *Ipomea Batata* Extract administered

S/No.	Parameters	<i>I. Batata</i> (1)	<i>I. Batata</i> (2)	<i>I. Batata</i> (3)	Average
1	WBC	7.2	13.0	6.8	$9.0 \times 10^3/\mu\text{L} \pm 0.1$
2	RBC	7.87	5.85	6.5	$6.7 \times 10^6/\mu\text{L} \pm 1.0$
3	HGB	14.1	11.1	12.1	$12.4 \text{ g/dL} \pm 1.5$
4	HCT	44.1%	32.1%	36.2%	$37.5\% \pm 0.1$
5	MCHC	32.0	34.6	33.4	$33.3 \text{ g/dL} \pm 1.3$
6	PLT	1034	976	954	$988 \times 10^3/\mu\text{L} \pm 41.3$

Table 4.5: *T. Occidentalis* Extract administered

S/No.	PARAMETER	T.O 1	T.O 2	T.O 3	T.O 4	AVERAGE
1	WBC	8.5	9.3	10.8	5.4	$5 \times 10^3/\mu\text{L} \pm 1.2$
2	RBC	6.82	6.87	7.28	8.06	$7.2 \times 10^6/\mu\text{L} \pm 0.3$
3	HGB	12.8	12.2	12.3	13.4	12.7 g/L ± 0.3
4	HCT	40.0 %	37.9%	39.0%	42.8 %	40.1% ± 1.1
5	MCHC	31.6	32.2	31.5	31.3	31.7 g/dL ± 0.4
6	PLT	732	645	780	841	$750 \times 10^3/\mu\text{L} \pm 68.4$

Table 4.6: Mixed of *I. Batata* and *T. Occidentalis* Extract administered

S/No.	PARAMETERS	M1	M2	M3	AVERAGE
1	WBC	7.4	11.5	11.5	$10.1 \times 10^3/\mu\text{L} \pm 2.4$
2	RBC	7.34	6.85	7.85	$7.34 \times 10^3/\mu\text{L} \pm 0.5$
3	HGB	14.34	13.0	12.7	13.3 g/dL ± 0.9
4	HCT	45.5%	41.6%	44.1%	43.7% ± 0.1
5	MCHC	31.4	31.3	28.8	30.5 g/dL ± 1.5
6	PLT	805	869	934	$869 \times 10^3/\mu\text{L} \pm 64.6$

Table 4.7: Comparison between Control, *I. Batata* and *T. Occidentalis* EXTRACT Administered to the animal.

S/No.	PARAMETERS	Control	<i>T. Occidentalis</i>	<i>I. Batata</i>	Mixed (T.O and I.B)
1	WBC	$10.5 \times 10^3/\mu\text{L} \pm 3.1$	$8.5 \times 10^3/\mu\text{L} \pm 1.2$	$9.0 \times 10^3/\mu\text{L} \pm 0.1$	$10.1 \times 10^3/\mu\text{L} \pm 2.4$
2	RBC	$6.97 \times 10^6/\mu\text{L} \pm 0.8$	$7.2 \times 10^6/\mu\text{L} \pm 0.3$	$6.7 \times 10^6/\mu\text{L} \pm 1.0$	$7.34 \times 10^3/\mu\text{L} \pm 0.5$
3	HGB	12.7 g/dL ± 0.6	12.7 g/dL ± 1.1	12.4 g/dL ± 1.5	13.3 g/dL ± 0.9
4	HCT	38.7% ± 0.1	40.1% ± 1.1	37.5% ± 0.1	43.7% ± 0.1
5	MCHC	33.1 g/dL ± 2.4	31.7 g/dL ± 0.4	33.3 g/dL ± 1.3	30.5 g/dL ± 1.5
6	PLT	$947 \times 10^3/\mu\text{L} \pm 45.2$	$750 \times 10^3/\mu\text{L} \pm 68.4$	$988 \times 10^3/\mu\text{L} \pm 41.3$	$869 \times 10^3/\mu\text{L} \pm 64.6$

T.occidentalis =*Telfairia occidentalis*, *I.Batata* =*Ipomea batata* ,*T.O* and *I.B.*= Mixture of *Telfairia occidentalis* and *Ipomea batata*

DISCUSSION

From the result of the study on qualitative phytochemical screening of the two leaves extract. Both leaves contain as much variety of phytochemicals except of Alkaloids and Glycosides which was absent in *T. Occidentalis* extract when Wagner's test and Sakowski's tests were carried out. However, Hagers test indicate the presence of Alkaloid in both *T.Occidentalis* and *I.Batata* extracts. The presence of phytochemicals in both the two leaves samples (Table 4.7) are indication of their richness in the biochemicals which are very essential to the plants and human ethnomedicinal uses as reported by [5] & [6]. The presence of the many tested phytochemicals exhibit a range of pharmacological effect, these activities: Anti-inflammatory, anti-parasite, and anti-virus with the presence of saponin, that is to say the leaves extract are a good anti-cholesterolemic due to their ability to prevent absorption [21]. The Anti-viral and inflammatory activity of some of these phytochemicals have a connection with their improve reading of the white blood cells count (WBC) in the Haematological study of this research.

This study also reveal that the Haematological parameters from WBC - PLT have appreciably registered significance effect on the albino rats white

blood cells (WBC) in *T.Occidentalis* and *I.Batata* are having a close reading with a difference margin of ± 1.2 and ± 0.1 microliter of the blood, this may not be far away from the fact that both leaves contain Alkaloids.

Red blood cells count (RBC) both the two leaves extract reading was higher than that of the control, and mixture has the highest, this improvement have confirmed the report of [22], [23] & [24] that animal with low white blood cells are more expose to high risk of disease infection while those with higher WBC count are capable of generating anti-bodies to resist disease attack. The increase in packed cell volume (PCV) or Haematocrit (HCT) shows a better transportation and this may result in an increase of primary and Secondary polycythemia according to [23]. However in Chineke [25] postulated that high PCV or HCT indicate either an increase in number of red blood cells or reduction in circulating plasma volume it can be said that the result is with the specification as the reading of HCT in the study indicate relatively close with that of the control $38.7\% \pm 0.1$ and *T.Occidentalis* $40.1\% \pm 1.1$ and $43.7\% \pm 0.1$ of the mixed, but the mixture of the extract come close to that of the control. The mixture is having higher reading compare to *T. Occidentalis* and *I. Batata*. This indicate that the group of animals

that have taken the mixture are having appreciable blood level condition. Comparatively I.Batata have shown that it contain similar composition of the *Telfairia Occidentalis* especially in terms of increase blood level in human beings.

CONCLUSION

From the result of this study, it can be concluded that *Ipomea batata* contains phytochemical constituents that are close related to those of *Telfairia Occidentalis*. They equally share similarities in the hematological parameters especially in White Blood Cells (WBC), Haemoglobin (Hb) and Haematocrit (HCT). Furthermore, combination of the two leaves extracts yielded relatively high (RBC) and (WBC), suggesting the likelihood of synergistic effect of the extracts. The leave extract have really proven to be rich in the nutrient looking at the high increase in weight of the Albino rat during the period of the study. *Ipomea batata* leave is comparatively rich in nutrient as the *Telfairia Occidentalis* leave.

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