

## Original Research Article

# Pharmacognostic, phytochemical, antioxidant and toxicological properties of aqueous extract of *Telfairia occidentalis* Hook. F. leaves

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## Abstract

**Purpose:** To determine the phytochemical composition, toxicological profile, pharmacognostic and therapeutic potential of aqueous extract of *Telfairia occidentalis* (*T. occidentalis*) in phenylhydrazine-induced oxidative stress in Wistar rats.

**Methods:** The pharmacognostic and phytochemical parameters were assessed using established analytical standards. Acute toxicity study (LD<sub>50</sub>) was evaluated on Wistar rats using Lorke's method, while the subacute toxicity was conducted using liver markers (aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP)) and kidney function tests (creatinine, uric acid, and urea). Furthermore, antioxidant activity was investigated by spectrophotometrically monitoring stress markers (malondialdehyde, superoxide dismutase, glutathione peroxidase and catalase activity in vivo).

**Results:** Aqueous extract of *T. occidentalis* at 100 - 400 mg/kg significantly reduced stress markers compared to non-treated group, surpassing standard Orheptal at 50 mg/kg ( $p < 0.05$ ). The extract showed very high LD<sub>50</sub> ( $> 5000$  mg/kg) with no marked adverse effect on hepatic and renal functions. Phenolic content was highest in the extract (30.673 mg/g).

**Conclusion:** Aqueous extract of *T. occidentalis* reduces oxidative stress, thus highlighting its promising role as a natural blood tonic and antioxidant. The high number of phenolic compounds in *T. occidentalis* is largely responsible for its activities.

**Keywords:** *Telfairia occidentalis*, Pharmacognostic, Phytochemistry, Stress markers, Toxicology

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## INTRODUCTION

*Telfairia occidentalis* (Cucurbitaceae), commonly known as Fluted Pumpkin or Fluted Gourd, is a staple vegetable in Nigeria, known for its rich

nutritional composition and health benefits. It is one of the most important natural blood tonics used extensively by the Igbo people of southeastern Nigeria; the other two being *Mucuna pruriens* (L.) DC. (Fabaceae) and *Justicia carnea* Lindl. (Acanthaceae) [1]. Blood

boosting property of the leaves of the plant has been reported in experimental animals [2]. The leaves extract has also been reported to exhibit hepato-protective and anti-oxidant activities [5] and stabilize human red blood cells exposed to stress [3]. The leaves are also known to be rich in iron, calcium, phosphorus and vitamins. It is locally known in Nigeria as *ugu* (Igbo) or *oworoko* (Yoruba) [4]. Despite its widespread use, complete investigations into the pharmacognostic parameters and antioxidant properties of *T. occidentalis* leaf aqueous extract are mainly narrow in existing literature. The increasing occurrence of oxidative stress-related disorders necessitates a thorough understanding of plant samples with potential anti-oxidative capabilities. Owing to growing interest in natural sources for therapeutic effects, the need to discover and espouse the pharmacognostic and antioxidant aspects of *T. occidentalis* leaves extract becomes important. Detailed information on the pharmacognostic properties and antioxidant efficacy of *T. occidentalis* aqueous extract, particularly under oxidative stress conditions induced by phenylhydrazine, remains inadequate.

Furthermore, lack of comprehensive studies exploring dose-dependent effects highlights the urgency for a systematic investigation of the antioxidant properties of this plant. In order to fill this knowledge gap, the current study evaluated the pharmacognostic characteristics and antioxidant effect of aqueous extract of *T. occidentalis*, creating an avenue for potential therapeutic applications and offering valuable insights into the pharmacological properties of *T. occidentalis*.

## EXPERIMENTAL

### Plant material

The leaves of *T. occidentalis* (10 kg) were harvested from Nsukka in Enugu State of Nigeria, in May 2023 and taxonomically authenticated by Mr. C. J. Onyeukwu of the Department of Botany, University of Nigeria, and a voucher no. (UNN/11772) was assigned. The leaves were air-dried at 28 °C away from direct sunlight, pulverized into tiny particles and stored.

### Solvent extraction

The powdered plant materials (500 g) were cold macerated in 5 L of deionized water at room temperature in a closed vessel with periodic stirring for 12 h. The mixture was filtered and the filtrate lyophilized to obtain a dry extract.

## Pharmacognostic studies

### Microscopic evaluation

Powdered plant sample (10 mg) was placed on a glass slide. Two drops of chloral hydrate were added and covered with a cover slip. The sample was passed 3 times through a flame. Glycerol (1 drop) was put on the side of the cover slip. The slide was allowed to cool before mounting on the microscope for observation. The image was focused with the lowest objective first before adapting to a higher one [8].

### Ash content

#### Total ash

*T. occidentalis* powdered sample (8 g) was burnt in a furnace at 450 °C and dried in a desiccator to constant weight. All the methods used under analytical standard were as previously described [8]. Total ash was calculated using Eq 1.

$$\text{Total ash (\%)} = (W_1/W_2)100 \dots\dots\dots (1)$$

Where  $W_1$  is the weight of remnant ash, and  $W_2$  is the weight of initial powdered medication

#### Acid insoluble ash

The ash (0.28 and 0.39 g) derived from the total ash was heated with 25 mL of 2 N hydrochloric acid for few minutes and filtered. The filter paper was then burned at 450 °C and residual ash calculated using Eq 2.

$$\% \text{ acid insoluble ash value} = (W_1/W_2)100 \dots (2)$$

Where  $W_1$  is weight of acid-insoluble ash, and  $W_2$  is weight of powdered medication

#### Percentage water soluble ash (PWSA)

The ash (0.28 and 0.39 g) derived from the total ash was heated with water (25 mL) for 20 min and the mixture was filtered. The filter paper was burned to constant weight at 450 °C and weighed. Percentage water-soluble ash value (PWSA) was calculated using Eq 3.

$$\text{PWSA} = (W_1/W_2)100 \dots\dots\dots (4)$$

Where  $W_1$  is the weight of water soluble,  $W_2$  is the weight of powdered sample.

#### Sulphated ash value (SAV)

The ash (0.28 g and 0.39 g) derived from the total ash was respectively soaked in conc.  $\text{H}_2\text{SO}_4$

(2 mL) and burned to constant weight at 80 °C. The SAV was then calculated using Eq 5.

$$\text{SAV} = (W_1/W_2)100 \dots\dots\dots (5)$$

Where  $W_1$  is the weight of sulphated ash,  $W_2$  is weight of powdered sample

### **Qualitative phytochemical analysis**

The screening of the plant extract for the presence of different phytochemicals was done according to standard protocols [9].

### **Quantitative phytochemical analysis**

The quantitative analysis was carried out using spectrometric methods. The readings were taken three times and the findings presented as mean  $\pm$  standard deviation (SD). The amount present of the test substance was calculated by computation based on absorbance at the same wavelength as the standard. This was done using an equation for regression. The results were given as the equivalent of the standard compound per gram [9].

#### *Determination of alkaloid content*

The extract (1 mg) was soaked in 20 % sulphuric acid ( $\text{H}_2\text{SO}_4$ ) for 3 h and the mixture was filtered. Thereafter, 5 mL of 60 %  $\text{H}_2\text{SO}_4$  was added to 1 mL of the filtrate and incubated for 3 h. The absorbance was measured at 490 nm. Total alkaloid content was computed from the calibration curve of atropine standard [9].

#### *Total saponin content (TSC)*

The Vanillin–Sulfuric acid model was followed to determine total saponin content [9]. Absorbance was measured at 527 nm and the TSC was estimated from the standard diosgenin curve.

#### *Determination of tannin content (TC)*

The TC was measured spectrophotometrically by incubating 1 mg/mL solution of the extract with ferric chloride and potassium hexacyanoferrate (III) appropriately as previously reported [9]. The absorbance was recorded at 530 nm and the TC was estimated from the Tannic acid calibration curve.

#### *Determination of total steroids*

The extract (1 mg), 2 mL of 4 N  $\text{H}_2\text{SO}_4$ , 2 mL of 0.5 % ferric chloride and 0.5 mL of 0.5 % potassium hexacyanoferrate (III) solution were maintained at 70 °C for 30 min and made up to

10 mL. The absorbance of the mixture was measured at 780 nm against the reagent blank. Total steroidal content was read from the calibration curve for the cholesterol standard.

#### *Total terpenoid content (TPC)*

The TPC was also determined spectrophotometrically. The extract (1 mg) was incubated with 1 mL of phosphomolybdic acid and 1 mL of conc.  $\text{H}_2\text{SO}_4$  for 30 min and absorbance was measured at 700 nm. The terpenoid calibration curve was constructed and the total content was computed from the regression equation of the cholesterol standard, represented as mg/g of the standard [9].

#### *Determination of total flavonoid content*

Aluminium chloride ( $\text{AlCl}_3$ ) colorimetric technique [9] was used to estimate flavonoid content. The extract (1 mg), 3 mL of distilled water, 0.3 mL 1 %  $\text{NaNO}_2$  and 0.3 mL 10 %  $\text{AlCl}_3$  were mixed. Thereafter, 2 mL 1 M NaOH was added after 5 mins, and the solution was made up to 10 mL with distilled water. Absorbance of the mixture was recorded at 510 nm. The standard reference used was rutin.

#### *Total phenolic content (TPC)*

The Folin-Ciocalteu technique [9] was employed. The extract (1 mg) was combined with 0.5 mL Folin's reagent and 3.9 mL distilled water and the mixture was allowed to stand for 10 min at 28 °C. Thereafter, 2 mL of 25 %  $\text{Na}_2\text{CO}_3$  was added and the mixture heated in boiling water for 1 min. The absorbance was recorded at 650 nm, and TPC was determined by utilizing a reference curve based on gallic acid.

#### *Estimation of cyanogenic glycosides*

The extract (1 mg) was mixed with 4 mL of alkali picrate solution and boiled for 5 min at 90 °C. Absorbance was measured at 490 nm. The amount of cyanide in the extract was determined using an equation of regression equation of the standard [9].

### **Animals**

Adult male albino Wistar rats (121 – 220 g) reared in the animal house of the Faculty of Pharmacy, University of Nigeria, were used. The animals were made to acclimatize for 2 weeks under standard conditions (12 h light / dark cycle, room temperature with unrestricted access to feed and water). The experimental protocol was in accordance with the guidelines of the Ethics

Committee of the University of Nigeria, registered by the National Health Research Ethics Committee of Nigeria (ref no. NHREC/05/01/2008B). Ethical approval was obtained from the Ethics Committee, Faculty of Pharmacy (approval no. FPSRA/UNN/25/00124). The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care as found in European Community Guidelines (EEC Directive of 1986; 86/609/EEC) and complied with the internationally accepted guide for the Care and Use of Laboratory Animals [10].

### Acute toxicity studies (LD<sub>50</sub>)

Acute toxicity of the plant extract was determined in rats according to the modified Lorke's protocol [11]. The investigation involved two phases. Twenty-one rats (150 - 200 g) were divided into 7 groups (n = 3). Rats were administered 10, 100 and 1000 mg/kg, respectively. In the second phase, doses of 1600, 2600, 3900 and 5000 mg/kg were administered to four animal groups (n = 3). A control group (n = 3) received 0.5 mL of distilled water. The LD<sub>50</sub> was derived as a geometrical average of the highest non-lethal dose and the least hazardous dose.

### Evaluation of anti-oxidant activity

A total of 30 Wistar rats were employed for the investigation. This assay involved 30 animals randomly assigned to 6 groups (n = 5 in each group). Each group was labelled suitably from group A to group F for simple identification. Treatments and animal care were done for 2 weeks, after which the animals were humanely sacrificed by cervical dislocation and a blood sample was taken to assess the level of selected antioxidant markers. Group 5 (E, untreated control) received 0.2 mL phenylhydrazine alone, water and feed during the entire test. Group F received only water and feed (normal control). Group D received 5 mL of the standard drug (Orheptal) with phenylhydrazine. Groups A, B and C received 100, 200 and 400 mg/kg doses of *T. occidentalis* extract following phenylhydrazine (PHZ; 0.2 mL) administration. The doses were given once daily orally for 2 weeks.

### Sample preparation

Plasma/serum from the experimental animals was centrifuged at 3500 rpm for 10 min, and the supernatant was collected. Clarified plasma/serum was diluted into different concentrations with normal saline to do a pre-experiment.

### Oxidative stress markers

#### Malondialdehyde (MDA) assay

Plasma MDA was carried out using the improved thiobarbituric acid technique [12]. A solution of 10 nmol/mL of MDA was used as a standard. A mixture of the test sample, 50 µL of the clarificant, 1.5 mL of dilute acid reagent and 500 µL of dilute chromogenic agent was incubated at 95 °C for 40 min. Serum MDA levels were measured against 50 µL of absolute ethanol serving as the blank.

#### Superoxide dismutase (SOD) assay

The hydroxylamine method [13] was employed in the determination of SOD concentration. The test samples and control containing all the appropriate reagents were incubated at 37 °C for 40 min. The data were recorded against distilled water on the SOD-68 Elabscience program of the Diatek Semi-automated Blood Biochemistry Analyzer. The SOD levels were further calculated using Eq 6.

$$\text{SOD activity conc (IU/mL)} = \{(AC-AS)/AC\}68 \dots\dots\dots (6)$$

Where AC is the absorbance of control, and AS is absorbance of sample

#### Determination of catalase (CAT) activity

Absorbance of the test samples and that of the Contrast Blank in the Diatek CATALASE-ELS x325 program mode was read against distilled water as the blank at 405 nm. Plasma catalase activity was calculated using Eq 7 [13].

$$\text{Catalase activity (IU/mL)} = (AC-AS)325 \dots\dots (7)$$

Where AC is absorbance of control, and AS is absorbance of sample.

#### Glutathione peroxidase (GPx) assay

Determination of GPx activity level in the plasma was done using the Elab Science Glutathione Peroxidase (GSH-Px) assay kit (Elabscience Biotechnology Co. Ltd., Texas, USA) set at GPx-ELS Program Mode. The method used was the dithio-bis(2)-nitrobenzoic acid (DNTB) colorimetric method [13].

Absorbance (A) of the samples, standard (200 µL of 20 µmol/L GSH), control and blank (diluent for the standard) were recorded. The result was printed out and the GPx activity (IU) calculated using Eq 8.

GPx activity (IU) =  $(A_c - A_s) / (A_{st} - A_b) \times \text{Conc of standard} \times \text{DF}$  ..... (8)

Where  $A_c$  is absorbance of control,  $A_s$  is absorbance of sample,  $A_{st}$  is absorbance of standard, DF is dilution factor.

### Liver marker enzymes assay

#### Serum aspartate aminotransferase (AST) activity

Serum AST activity was determined using the Reitman-Frankel colorimetric method [14].

#### Alanine aminotransferase (ALT)

Serum ALT activity was determined using the Reitman-Frankel colorimetric method [14].

#### Serum total bilirubin

Serum total bilirubin was measured based on the Jendrassik and Grof protocol [14], using Randox Total Bilirubin test kit (Randox Lab. Ltd., Crumlin, UK).

#### Serum alkaline phosphatase (ALP) activity

Serum ALP was determined using the thymol pthalein monophosphate method [14].

#### Serum total proteins

Serum total protein was assayed using the Biuret method [15].

#### Serum albumin

Serum albumin was assayed using the Bromocresol green technique [15].

### Evaluation of kidney function

#### Serum creatinine level

Serum creatinine was determined spectrophotometrically following previous method [16]. The serum (100  $\mu$ L) and the standards (2 mg/dL of Creatinine) were mixed with 1 mL of the working reagent and the absorbance was recorded after 20 and 80 secs against distilled water (blank) at 510 nm.

#### Determination of the uric acid levels

The enzymatic colorimetric method [16] was used to assay uric acid levels.

### Assay of serum urea

Serum urea was quantified following the method of Lamb and Price [16].

### Statistical analysis

Data was analysed using GraphPad Prism version 7.0 (GraphPad software, San Diego, USA). Data were presented as mean  $\pm$  standard deviation (SD) and compared using one-way analysis of variance (ANOVA), followed by Dunnett's test.  $P < 0.05$  was considered statistically significant.

## RESULTS

### Phytochemical composition

Results showed that the plant extract was rich in phenolic compounds (30.673 mg/g) in addition to other phytochemicals such as carbohydrates, alkaloids, saponins, tannins, proteins, fats and oils, terpenoids, and steroids (Table 1).

**Table 1:** Phytochemical composition of the aqueous leaf extract of *T. occidentalis*

Phytochemical	Qualitative composition	Quantitative (mg/g equivalent) of the standard
Carbohydrates	+++	2.46 $\pm$ 0.006
Alkaloids	++	6.25 $\pm$ 0.126
Reducing sugars	-	-
Saponins	+++	0.12 $\pm$ 0.021
Tannins	+++	3.82 $\pm$ 0.416
Flavonoids	++	-
Proteins	++	3.04 $\pm$ 2.024
Fats and oils	+	1.06 $\pm$ 2.024
Terpenoids	++	7.51 $\pm$ 3.024
Steroids	++	2.46 $\pm$ 0.006
Total phenolics	+++	30.67 $\pm$ 5.476

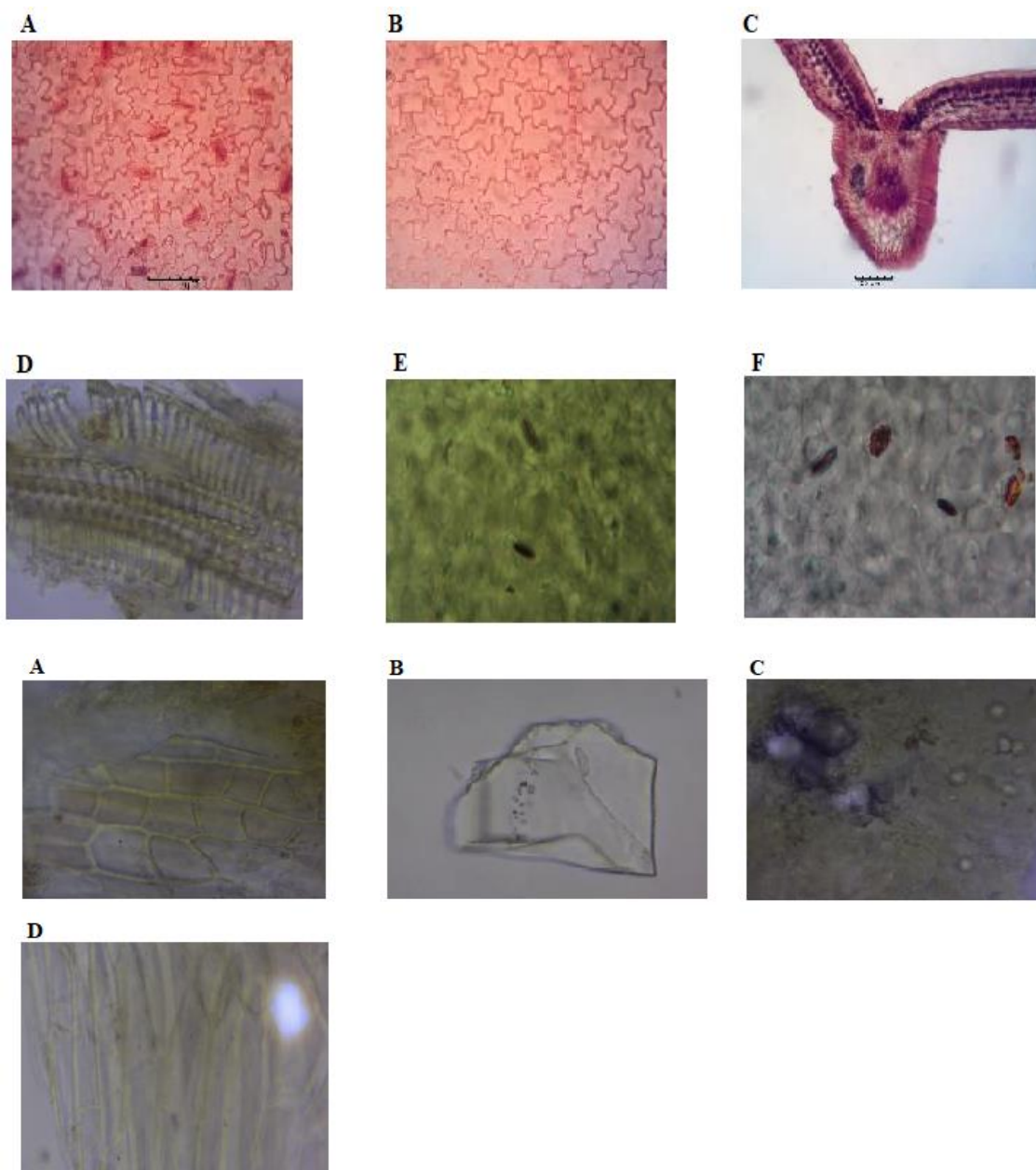
**Note:** + marginally present; ++ moderately present; +++highly present; -not detected

### Pharmacognostic microscopy

The pharmacognostic microscopy of the leaf of *T. occidentalis* is given in Figure 1.

### Acute toxicity profile of *T. occidentalis* extract

There were no deaths or adverse reactions recorded in the animals, even at the highest dose of 5000 mg/kg. The LD<sub>50</sub> is therefore much greater than 5000 mg/kg (Table 2).



**Figure 1:** Pharmacognostic microscopy of the leaf of *T. occidentalis*. A: Lower epidermis of the leaf, B: upper epidermis of the leaf, transverse section of the leaf, C: transverse section showing bundles of annular xylem tissues, D: transverse section showing the anomocytic stomata cells, E: anomocytic stomata cells in the lower epidermis, F: epidermal cells from the mid rib, G: group of calcium oxalates, H: irregular shape of calcium oxalate, I: phloem parenchyma cells

**Table 2:** Acute toxicity profile of the *T. occidentalis* extract

Treatment	Dose (mg/kg)	No of deaths
Phase 1	10	0/3
	100	0/3
	1000	0/3
Phase 2	1600	0/3
	2600	0/3
	3900	0/3
	5000	3

#### Antioxidant activity

The effect of *T. occidentalis* extract on the plasma levels of the various oxidative stress markers, after 8-day treatment at different doses, is given in Table 3.

#### Effect of the test extract on hepatic functions

The effect of *T. occidentalis* extract on plasma levels of various liver function markers, after 14-day treatment at different doses, is presented in Table 4.

**Table 3:** Levels of the oxidative stress markers

Animal (group)	Initial weight (g) At day 1	Final Weight (g) At day 8	Mean value of SOD (IU/mL)	Mean value of MDA (μmol/mL)	Mean value of CAT (IU/mL)	Mean value of GPx (IU/mL)
A	169.71	154.68	51.08±2.19	22.36±2.23*	65±14.81	385.58±17.88
B	173.34	150.65	43.18±1.83*	13.06±1.60	125.02±10.18	403.94±0.67
C	167.76	157.48	53.15±1.73	11.95±1.14	134.34±4.91	381.64±7.3
D	170.44	157.66	52.67±2.97	18.61±6.41	55.03±6.16	388.85±7.30
E	173.30	155.11	49.81±2.26	9.16±0.64	120.68±15.72	340.98±5.54
F	172.38	170.19	55.07±2.78	7.36±0.50	73.77±20.22	377.70±6.91

A: Group received PHZ + 100 mg/kg dose of extract, B: group received PHZ + 200 mg/kg dose of extract, C: group received PHZ + 400 mg/kg dose, D: group received PHZ + standard haematinic (Orheptal®), E: group received PHZ + no treatment (Untreated Control), F = no PHZ and no treatment (normal control). \* $P < 0.05$  compared to normal control

**Table 4:** Effect of the extract on liver function markers

Animal group	ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dl)	Total Proteins (g/L)	Albumin (g/L)
A	19.35	74.45	35.12	0.43	67.03	37.57
B	20.04	68.63	35.09	0.39	68.20	38.19
C	14.08	59.69	34.82	0.29	62.40	35.48
D	18.58	53.10	33.59	0.25	62.55	37.85
E	12.01	55.44	32.51	0.32	65.72	37.53
F	17.33	43.82	32.21	0.29	60.11	34.40

**Key:** A = Animal group given dose of 100 mg/kg and PHZ, B= group given dose of 200 mg/kg and PHZ, C= Group given dose of 400 mg/kg and PHZ, D = Group given dose of Standard 2.5 ml of haematinic (Orheptal®) syrup and PHZ, E= Group given only PHZ with rat feed as control, F= Group given only feed and water

### Effect of the extract on renal function

Effects of *T. occidentalis* extract on plasma levels of kidney function markers, after 14-day treatment with different doses, are presented in Table 5.

**Table 5:** Effect of extract on serum level of kidney function markers

Animal group	Urea (mg/dl)	Uric acid (μmol/L)	Creatinine (mg/dL)
A	42.52	374.96	0.97
B	40.50	382.44	1.04
C	42.49	270.93	0.82
D	40.63	178.12	0.90
E	39.74	212.55	0.82
F	34.71	134.71	0.71

A: Group administered 100 mg/kg dose + PHZ, B: group received 200 mg/kg + PHZ, C: group received 400 mg/kg + PHZ, D: group received standard haematinic (Orheptal®) + PHZ, E: group received only phenylhydrazine (PHZ) with rat feed as control, F: group received only feed and water

## DISCUSSION

Phytochemical screening revealed several secondary metabolites. Alkaloids present in the extract have been reported to exhibit analgesic and antimicrobial properties, while tannins exhibit antioxidant and anti-inflammatory effects. Saponins are known for their cholesterol-lowering and immune-boosting effects. The

presence of carbohydrates indicates the nutritive value of *T. occidentalis*, while the presence of proteins and fats/oils further contributes to its nutritional profile. Terpenoids and steroids are known for their various pharmacological properties, such as antineoplastic, anti-inflammatory, and antioxidant [17]. Phenolics, on the other hand, contribute to the antioxidant potential of *T. occidentalis* [18].

Acute toxicity test revealed no death in either phase 1 or phase 2 at doses ranging from 10 to 5000 mg/kg. This indicates that the extract of *T. occidentalis* is safe at tested doses, with no observed mortality. The extract, therefore, has a high margin of safety and is unlikely to cause acute toxicity in man. Results of the anti-oxidant assay showed varying levels of oxidative markers among the rats treated with different doses of the extract, standard drug, and control group. There was an increase in SOD, CAT, GPx and an increase in MDA which indicated increased oxidative stress. Rats which received only feed and water, exhibited highest mean values of SOD, CAT, and GPx, indicating better antioxidant activity and lower oxidative stress compared to other groups, and this was because the system was not disturbed with phenylhydrazine (PHZ) which causes oxidative stress in red blood cells by generating reactive oxygen species [12,13].

Liver function markers such as ALT, AST, and ALP are used to assess liver health, with higher levels indicating potential liver damage or dysfunction. Group F showed the lowest levels of ALT (IU/L), AST (IU/L), and ALP (IU/L), suggesting healthy liver function. This is in contrast to rats treated with the extract or the standard drug, which showed higher levels of these markers, particularly at higher doses (groups C and D). However, total bilirubin concentration (mg/dL), total protein concentration (g/L), and albumin concentration levels (g/L) were within normal ranges in all groups, indicating overall normal liver function.

The results revealed that *T. occidentalis* had a mild effect on liver function, as evidenced by the minimal elevation in liver function markers in treated groups compared to normal control group. However, further studies are needed to determine the long-term effects of the extract on liver health and function. Kidney function markers such as urea, uric acid, and creatinine are used to assess kidney health, with higher levels indicating potential kidney damage or dysfunction [19]. Group F (received only feed and water) exhibited the lowest levels of urea, uric acid, and creatinine, suggesting healthy kidney function. This is in contrast to rats treated with the extract or standard drug, which showed higher levels of these markers.

Group C (received the highest dose of extract) exhibited the highest levels of urea and uric acid, indicating potential kidney stress. However, creatinine levels were within normal ranges in all groups, indicating overall normal kidney function. The results suggest that the extract of *T. occidentalis* may have a mild impact on kidney function, as evidenced by the minimal elevation in urea and uric acid levels in some treated groups compared to the normal control group. Further studies are needed to establish the long-term effects of the extract on kidney health and function.

## CONCLUSION

The aqueous extract of *Telfairia occidentalis* shows high phenolic content, a wide safety profile up to 5000 mg/kg dose, effective antioxidant activity, dose-dependent increase in AST, ALT, ALP, urea and uric acid levels, suggesting potential kidney stress and mild impact on liver function. Further investigation is recommended to ascertain the underlying mechanisms, potential clinical applications in oxidative stress-related disorders, and long-term impact on liver and kidney function.

## DECLARATIONS

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### Availability of data

The data that support the results of this study are available from the Departments of Pharmacognosy and Environmental Medicine, and Pharmaceutical & Medicinal Chemistry, University of Nigeria, Nsukka, and can be obtained from the authors upon request and with the permission of the Tropical Journal of Pharmaceutical Research.

### Conflict of interest

No conflict of interest is associated with this work.

### Ethical approval

Ethical approval was obtained from the Ethics Committee, Faculty of Pharmaceutical Sciences, University of Nigeria Nsukka (approval no. FPSRA/UNN/25/00124).

### Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Conceptualization: Patience N Ugwu and Estella U Odo; Methodology/bench work: Patience N Ugwu, Fabian I Eze, Vincent O Chukwube, Chinonso E Mgbeokwere, David O Ezenwanne, Charity C Ezea and Eleje O Okonta; Writing—original draft: Patience N Ugwu; Writing – Review & Editing: Fabian I Eze; Supervision: Estella U Odo.



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