Phytochemical screening and acute toxicity evaluation of *Telfairia occidentalis* aqueous extracts on rats

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Abstract: The phytochemical composition and acute toxicity of *Telfairia occidentalis* aqueous extracts were investigated in this study. Phytochemical screening was carried out on the pulverized leaf, root, pod and stem samples. Proximate analysis was also conducted for the root to ascertain the effect of drying procedures on its composition. Fifty-six (56) Wister albino rats, male and female were divided into two broad groups of 28 animals per group. The first group was randomly separated into seven (7) groups of four (4) animals per group. The control group received distilled water alone while the other groups received varied doses (1500mg/kg, 2250mg/kg and 3000mg/kg) of the Soluble and Insoluble *Tefairia occidentalis* root fraction. The second group of 28 animals was also distributed into 7 groups of 4 animals per group. Six test groups received varied doses (1500mg/kg, 2250mg/kg and 3000mg/kg) of *Telfairia occidentalis* fruit and stem extracts. The animals were observed for the first 12hr for any toxic symptoms and for 48 hr for mortality rate. Surviving animals were sacrificed after 48 hours. Phytochemical screening results reveal the presence of tannins, flavonoid, steroid, terpenoids, saponin, alkaloid, glycosides, proteins and carbohydrates. Flavonoid and saponin was not detected in stem sample; alkaloid is present in all samples except pod; and cyanogenic glycoside was found in both root and pod samples. Except for the fibre content, the method of preparation of the root had no significant effect on the proximate composition of the sample. The root extracts cause insignificant reduction in Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) activities, except for the significant reduction in ALT activity at highest dose. The pod extract significantly increased the ALT and AST activities, which is dose dependent, while the stem extract only caused increased activity of ALT, but not AST. None of the extracts administered had any significant effect on the levels of serum creatinine and urea. Thus, while the root extract may exhibit some hepatoprotective effect (or nephrotoxic due to cyanogenic glycoside) and its proximate composition, not affected by heat treatment, the pod and stem extracts of *Telfairia occidentalis* may have some effects on rat hepatocytes.

Keywords: *Telfairia occidentalis*, phytochemical screening, acute toxicity, aqueous extract.

INTRODUCTION

Plant toxicity may be as old as plant itself or evolved with the inception of herbivorism. Organisms often synthesize or use toxins in their survival strategy. The biologically active chemicals inherent in toxic plants are products of secondary metabolism; phytochemicals or antinutritional factors, whose roles in normal plant physiology are unknown, until recently. In many cases, the presence of these chemicals in plants may confer some degree of protection from plant predators; microbes, insects, ruminants and humans, as well as other plants that may compete with it for nutrients and light (Wink and Schimmer, 1999). Their functional roles are seen in ecology and defense. They also serve as attractants or repellents, and also as colors and scents of reproductive organs (Molyneux *et al*., 2007). Toxins within a plant can be confined to one or more plant parts: seed or nuts, rooting structures, cotyledons, stems, leaves or bark; or all parts (Acamovic and Brooker, 2005). This confinement may be dependent on the part of the plant that is most at risk of predation; the location of the storage organs and/or where they can be easily and more effectively discharged into the environment. Toxic plants have been known for centuries, as they were used by primitive cultures for medicinal purposes as well as arrow poisons (Habermehl, 1989).

Telfairia occidentalis (Family: *Curcubitaceae*) is a tropical vine grown in Nigeria and other West African region for its leaf and edible seeds. The young shoots and leaves of the female plant are vegetable ingredients of a Nigerian delicacy. The seeds of *T. occidentalis* are high in carbohydrate, fat and phosphorus. They also contain Vitamin A (Christian, 2007). The Iodine value of the Fluted pumpkin oil indicates high degree of unsaturation compared to palm oil, thus, can make good cooking oils and suitable for margarine production (Agatemor, 2006). Using polarized light and scanning electron microscopy, Okoli and McEven (1986) showed that calcium crystals are abundant in the leaf laminae of *T. occidentalis*. Oboh (2005) demonstrated a dose- dependent hepatoprotective effect of the leaf extract of *T. occidentalis*. He suggested that the water extractable component of the leaf could be more potent than the ethanolic extract because of its higher antioxidative activity as compared to the ethanolic

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extract. The ethanolic fruit extract has also been shown to have a dose dependent hypercholesterolemic, hyper-proteinemic, hypertriglyceridemic and hyper- conjugated bilirubinemic effect on rats, suggesting that the fruit may not be safe for consumption (Olorunfemi *et al.,* 2006). The root has been reported by various workers to be either toxic (Akubue, 1980; Togun *et al*., 1994; Ajibesin et al., 2002) or non toxic (Eseyin *et a*l., 2006).

The stem which has no known nutritional importance is usually disposed as a waste or used as organic manure in composting. The fruit pod and the mesocarp are usually discarded after extracting the seeds. The root remains underground throughout the harvest period and is usually uprooted during seasonal land clearing activities. Thus, this study is aimed at investigating the phytochemical composition of these inedible parts; root, stem and pod of *T. occidentalis* and to assess possible toxicity on Wistar albino rats

MATERIALS AND METHODS

All the chemicals and reagents used in this investigation are products of Sigma-Aldrich Laborchemikalien and BDH Laboratory, Poole England. Randox (UK) reagent kits were used for estimation of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST).

Plant materials

Telfairia occidentalis roots, stem, and pods were collected from a farmland in Obigbo, Port Harcourt, South- South Nigeria. Samples were identified and deposited at the Hebarium of Plant Science and Biotechnology Dept. of University of Port Harcourt. The samples were washed, cut into smaller bits and dried under shade. Plant samples were given mild heat treatment prior to blending in mechanical grater mill. The plant samples were ground into fine powder, and part of the powdered samples kept for phytochemical analysis. A portion of the root sample was oven-dried, pulverized and kept for comparison of proximate composition with air-dried root sample. Each sample (200g) was macerated in adequate distilled for 24 hours to obtain the crude extracts. The extract solutions were filtered and filtrates concentrated and reduced to constant weight at 50^{o}C. The extracts were preserved in a refrigerator until used.

Phytochemical analysis

Determination of phytochemical composition was carried out on the pulverized specimen using standard procedures, as described by Harborne (1973), Trease and Evans (1989) and Sofowara (1993).

Test for alkaloids was carried out by Mayer's and Wagner's reagent method as described by Iweala (2009); flavonoid, saponins, tannins, cardiac glycosides, steroids as described by Edeoga *et al.* (2005); anthraquinone, steroidal nucleus as described by Ajaiyeoba (2000); terpenoids by Libermann-Burchard test; cyanogenic glycoside by picrate paper test; protein by millon's test and carbohydrate by molish test.

Proximate composition of root sample

The determination of moisture, ash, crude protein, crude fat, total carbohydrate and fiber composition of the samples was carried out in accordance with the recommended methods of AOAC (1984).

Animal treatment

Fifty-six (56) male and female Wister albino rats, male and female, were obtained from animal house in Uzuakoli Bende L.G.A., Abia State, Nigeria. Animals were kept in animal house of the Department of Biochemistry, University of Port Harcourt and allowed to acclimatize for two weeks with unlimited access to food and water. Animals were separated into two broad groups of 28 animals per group. The first group was randomly divided into seven (7) groups of four (4) animals per group. The control group received distilled water alone while the other groups received varied doses (1500mg/kg, 2250mg/kg and 3000mg/kg) of the Soluble and Insoluble *Tefairia occidentalis* root fraction.. The second group of 28 animals was also distributed into 7 groups of 4 animals per group. Six test groups received varied doses (1500mg/kg, 2250mg/kg and 3000mg/kg) of *Telfairia occidentalis* fruit and stem extracts. The animals were observed for the first 12hr for any toxic symptoms and for 48 hr for mortality rate. Surviving animals were sacrificed after 48 hours.

Collection of blood

Animals were placed under mild chloroform anesthesia, and blood collected by jugular laceration. Blood was collected into appropriate sample bottles for analysis.

Biochemical analysis

The analyses for Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were done colorimetrically based on the methods of Reitman and Frankel (1957). Estimation of urea was carried out by diacetyl monoxime method (Rosenthal, 1955) and creatinine by alkaline picrate method.

STATISTICAL ANALYSIS

Data obtained were analyzed statistically using Analysis of Variance (ANOVA). The Bonferroni's test was used for post-hoc comparisons. $P<0.05$ was considered statistically significant.

RESULTS

The qualitative phytochemical analysis result (table 1) shows the presence of tannins, terpenoids, steroids, steroid nucleus, cardiac glycosides, proteins and

carbohydrates in all the plant samples investigated. Alkaloid was found in leaf, root and stem samples; flavonoid in leaf, root and pod; saponin in leaf and root; and cyanogenic glycosides in root and pod. There was high presence of flavonoids, alkaloids, saponin, tannins and carbohydrates in the root of *Telfairia occidentalis*. The pod also showed high levels of tannins and steroids.

The result also indicated the absence of anthraquinone in all the samples. While the stem showed absence of flavonoid, saponin and cyanogenic glycosides, the pod contains little or no alkaloid and saponin.

Table 1: Preliminary Qualitative Phytochemical Analysis Result

Phytochemcals		Availability/ Composition		
	Leaf	Root	Stem	Pod
Alkaloid	+	++	+	-
Flavonoid	++	++	-	+
Saponin	+	++	-	-
Tannins	+	++	+	++
Terpenoids	+	+	+	+
Cardiac glycosides	+	+	+	+
Cyanogenic glycosides	-	+	-	+
Anthraquinones	-	-	-	-
Steroids	+	+	+	++
Steroid nucleus	+	+	+	+
Proteins	+	+	+	+
Carbohydrates (%)	+	++	+	+

+ = Present; ++ = highly present; - = absent or undetected

Table 2 shows the result of proximate composition of *T. occidentalis* roots processed by different drying procedures. Except for the fibre content, the air-dried sample showed no significant difference in composition compared with the oven-dried sample.

Table 3 shows the effect of 48-hour exposure of experimental animals to *T. occidentalis* root extract on serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine and urea. There was an insignificant ($P>0.05$) decrease in the activities of ALT and AST with increased dose of the soluble aqueous root extract (SLE). The levels of creatinine and urea were not affected. Similar trend was observed for the groups administered increasing doses of insoluble aqueous root extract (ISL).

The result of 48-hour exposure to *Telfairia occidentalis* pod and stem extracts on serum ALT, AST, creatinine and urea is shown in Table 4. The pod extract caused a dose-dependent significant ($P<0.05$) increase in the activities of ALT and AST compared with control, while the levels of creatinine and urea did not change significantly ($P>0.05$). However, the stem extract caused a significant ($P<0.05$) increase in the activity of ALT, while the other parameters (AST, creatinine and urea) were not affected.

Table 2: Proximate Composition of *Telfairia Occidentalis* Root

Constituent	Composition (%)	
	Air-dried	Oven-dried
Moisture	14.35±4.78[a]	9.7±2.00[a]
Ash	8.15±1.82 [a]	6.45±1.23 [a]
Crude Protein	5.2 ±1.93[a]	6.12±2.09 [a]
Crude fat	2.95±0.96[a]	4.05±0.96[a]
Carbohydrate	42.3±10.4[a]	30.77±5.37[a]
Fibre	65.07±8.03[a]	42.90±4.65[b]

Values are means ±standard deviations of triplicate determinations. Values in the same row bearing the same superscript are not significantly different ($P<0.05$)

DISCUSSION

The result of phytochemical screening of *T. occidentalis* leaf, *root*, pod and stem (table 1.0) showed the presence of flavonoid, alkaloids, saponins, terpenoids, tannins, steroids and glycosides. Protein and carbohydrates were also observed. The root had more of these phytochemicals than the other samples. Anthraquinone was absent in all the samples. With the exception of cyanogenic glycosides, the root sample contains all the phytochemicals that are found in the leaf of *T. occidentalis*. Ethnobotanically, the leaves of *Telfairia occidentalis* are useful in the treatment of convulsion, anaemia, cardiovascular disease, artherosclerosis, hypertension, impotence and malaria (Iwu, 1983; Sofowora, 1996**;** Odoemene and Onyeneke, 1998; Obute and Adubor, 2007). The leaf extract was reported to be important in the management of hyper- cholesterolemia**,** liver ailments and immune defence system impairment (Eseyin *et al*., 2005). Its antioxidant, antimicrobial, hypoglycaemic and antidiabetic activities have also been reported (Oboh *et al*., 2006; Abo *et al*., 2008). Thus, depending on the concentration of the cyanogenic glycosides and any other inherent anti-nutritional factor, the root may compare favorably with the leaf in medicinal importance.

The application of heat in the preparation of the root did not affect the proximate composition of the sample. While air-drying could achieve moisture level similar to oven-drying, the significant reduction in the fiber content of the oven-dried sample could result from heat-enhanced pulverization that destroyed most of the sample fibre. Oven-drying may also reduce or eliminate the cyanide content of the sample.

Reduction in the serum activities of ALT and AST of normal rat has been observed as a means through which

Table 3: Result of forty-eight (48) hour exposure to *Telfairia occidentalis* root extract

Group	Treatment	ALT(U/L) Mean± SEM	AST (U/L) Mean± SEM	Creatinine (µmol/L) Mean± SEM	Urea (mmol/L) Mean± SEM
1	Distilled Water	45.0±4.0	129±22.2	60.0±10.0	5.7±0.3
2	Soluble Root Extract (1500mg/kg)	44.0±5.29	127.3±26.8	63.3±6.7	5.6±0.1
3	Soluble Root Extract 2250mg/kg)	45.0±3.5	110.0±16.0	50.0±0.0	5.5±0.1
4	Soluble Root Extract (3000mg/kg)	34.3±5.9	109.3±14.4	63.3±6.7	5.8±0.4
5	Insoluble Root Extract (1500mg/kg)	36.0±1.7	108.0±11.2	50.0±0.0	5.6±0.1
6	Insoluble Root Extract (2250mg/kg)	45.0±2.6	114.7±11.3	50.0±0.0	5.4±0.2
7	Insoluble Root Extract (3000mg/kg)	24.±2.9[a]	90.3±20.3	56.7±6.7	5.4±0.2

n=4; [a]Significant difference (P< 0.05) compared to Group 1.

Table 4: Result of forty-eight (48) hour exposure to *Telfairia occidentalis* pod and stem extracts

Group	Treatment	ALT (U/L) Mean± SEM	AST (U/L) Mean± SEM	Creatinine (µmol/L) Mean± SEM	Urea (mmol/L) Mean± SEM
1	Distilled Water	40.75±3.1	51.50±1.3	81.25±13.1	6.68±0.6
2	Pod Extract (1500mg/kg)	55.00±1.4[a]	93.5±1.3[a]	82.50±12.0	6.23±1.1
3	Pod Extract 2250mg/kg)	62.75±3.0[a]	105.00±2.9[a]	82.50±6.93	6.93±1.0
4	Pod Extract (3000mg/kg)	63.00±1.3[a]	129.25±2.2[a]	91.25±5.0	5.23±0.8
5	Stem Extract (1500mg/kg)	42.75±2.6	55.00±3.0	85.00±11.0	5.75±1.7
6	Stem Extract (2250mg/kg)	56.00±1.4[a]	59.00±8.1	87.50±12.0	5.23±0.7
7	Stem Extract (3000mg/kg)	53.00±2.0[a]	51.25±1.0	93.75±3.0	5.28±1.0

n=4; [a]Significant difference (P<0.05) compared to Group 1.

plant extract offer some protection to the liver (Olaleye *et al*., 2006). Also low aminotransferase levels have been reported to occur secondary to either dialysate or pyridoxine deficiency (Thapa and Walia, 2007). Saoudi *et al*. (2011) had reported a decrease in plasma transaminase (AST and ALT), ALP, LDH and γ-GT activities as indicative of kidney damage. Nobre *et al*. (2003), reporting a decrease in ALT and AST suggested that cyanotoxins promoted renal alterations and affected renal physiology. Although insignificant, the reductions in serum ALT and AST resulting from 48-hour exposure to *Telfairia occidentalis* root extract may be due to the presence of glycosides. The significant increase in both parameters compared with control on administration of pod extract may result from the effect of some biologically active principle that interferes with the liver cell integrity. The increase in ALT activity as a result of administration of stem extract may also result from effect on liver cell membrane integrity. ALT has been noted to be basically located in the liver, while AST was present in tissues such as the skeletal muscle, heart, brain, kidney and liver (Thapa and Walia, 2007). The "no effect" observation on urea and creatinine for all the extracts may be an indication that the extracts within the duration of administration did not affect the renal function.

Thus, while the root extract may exhibit some hepatoprotective effect (or nephrotoxic due to cyanogenic glycoside) and its proximate composition, not affected by heat treatment, the pod and stem extracts of *Telfairia occidentalis* may have some effects on rat hepatocytes.

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