

Comparative phytochemical analysis and antioxidant activity of the stem and seed of *telfairia occidentalis* hook. F. (cucurbitaceae)

N.S. NJINGA^{*1}, M.T. BAKARE-ODUNOLA¹, S.T ABDULLAHI¹, J.A OKONKWO², O.H OLADIMEJI³

1. Department of Pharmaceutical and Medicinal Chemistry, University of Ilorin, Ilorin, Nigeria.

2. Faculty of Pharmaceutical Sciences, University of Ilorin, Ilorin, Nigeria.

3. Department of Pharmaceutical and Medicinal Chemistry, University of Uyo, Uyo, Nigeria.

*Corresponding Author's E-mail: ngastanjin@yahoo.com. GSM: +2347064933604

ABSTRACT

This research analyzed and compares the phytochemicals and antioxidant activity of the different fractions (petroleum ether, chloroform, ethyl acetate, acetone and methanol) of the seed and stem extracts of *T. occidentalis*. Phytochemical studies were done using standard methods and spectrophotometric techniques. The antioxidant activities were evaluated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging effects. The phytochemistry of the seed fractions showed the presence of cardiac glycosides, alkaloids, steroids and terpenoids while that of the stem extract showed the presence of reducing sugars, alkaloids, cardiac glycosides, flavonoids, steroids and terpenoids. Only the chloroform and ethyl acetate fractions of the stem contain flavonoid and phenols while the other seed did not contain any flavonoid and phenols. The petroleum ether and chloroform fractions of the seed contain more alkaloid than the stem. The difference was statistically significant at $p < 0.05$. Also, only the ethyl acetate and acetone fractions of the stem contains alkaloid while those of the seed did not. The antioxidant activity of the seed fractions (0.5 mg) was found to range from 40.5 to 76.75 % scavenging activity for all the fractions while only the chloroform and ethyl acetate of the stem showed scavenging activity of 69.27 and 76.75 % respectively. The phytochemicals have been reported to be responsible for the pharmacological effect of most medicinal plants. Compounds from the chloroform and ethyl acetate fractions of the stem may be potent leads for potential antioxidant.

Key words: *Telfairia occidentalis*, phytochemical, antioxidant, cucurbitaceae

Introduction

Poverty and limited access to modern medicine has caused about 80% of the world's population, especially in the developing countries to use herbal medicine as their source of primary healthcare^{1,2}.

Medicinal plants are widely distributed throughout the world but most abundantly in tropical countries. It is estimated that about 25% of all modern medicines are directly or

indirectly derived from higher plants³. The use of herbs as medicine is the oldest form of healthcare known to humanity and has been used in all cultures throughout history⁴.

In many communities, traditional medical practice is often viewed as an integral part of their culture. Herbal medicines are often viewed as a balanced and moderate approach to healing. In the West, like our ancestors, it is believed they will help us live

healthier lives. Individuals who use them as home remedies and over-the-counter drugs spend billions of dollars on herbal products. As such, they represent a substantial proportion of the global drug market ^{3,5}.

Although it is generally believed that most herbal preparations are safe for consumption, some herbs like most biologically active substances could be toxic with undesirable side effects ⁶. A well-defined and constant composition of the drug is therefore, one of the most important prerequisites for the production of a quality drug. Given the nature of products of plant origin, which are not usually constant and are dependent on or influenced by many factors, ensuring consistent quality of products is vital for the survival and success of the pharmaceutical industry ⁷.

Telfairia occidentalis commonly called fluted pumpkin occurs in the forest zone of West and Central Africa, most frequently in Benin, Nigeria and Cameroon ⁸. The plant is dioecious, perennial, and drought-tolerant. It is a creeping vegetable shrub that spreads low across the ground with large lobed leaves, and long twisting tendrils. Herbal preparations of the plant has been employed in the treatment of anaemia, GIT disorders, malaria, convulsion, impaired defense immune systems and diabetes ^{9,10,11}. The plant has been reported to contain flavonoids, tannins, reducing sugars, glycosides, saponins, steroids and triterperoids ¹². This plant has also been reported to have antioxidant, antidiabetic, anti-inflammatory, hepatoprotective, haematinic, properties ^{13,14,15}.

GC-MS analysis of hexane and dichloromethane fractions of the seed showed that the seed contained compounds such as pentadecanoic acid, hexadecanoic acid; 16- octadecenoic acid methyl ester; 9, 12- octadecadienoyl chloride (Z,Z); 9- Octadecadienoic acid (Z)-, 2, 3-dihydroxypropyl ester; Octadecanoic

acid ; hexadecanoic acid, 2,3 bis [(trimethylsilyl) oxy] propyl ester in the hexane fraction and 2,4-heptadien-6-ynal, (E,E); benzoic acid ; dodecanoic acid ; linoleic acid ethyl ester ; hexadecanoic acid, methyl ester ; α -phellandrene ; α -campholene aldehyde;terpinen- 4-ol ; trans- β -ocimene ; borneol ; stigmastan-3-ol, in the dichloromethane fraction ¹¹.

The research aimed to analyse the phytochemicals and compare the antioxidant activities of the petroleum ether, chloroform, ethyl acetate, acetone and methanol fractions/extract of the seed and stem of *Telfairia occidentalis*.

Materials and Methods

Materials and Equipments

The materials and chemicals used in this study were all BDH products and they include: chloroform, ethylacetate, acetone, methanol, aluminium chloride, sulphuric acid, hydrochloric acid, ferric chloride, glacial acetic acid, acetic anhydride. Other reagents used include: Fehling's solutions A and B, Wagners' reagent which were all of analytical grade obtained from the Department of Pharmaceutical and Medicinal Chemistry.

The equipment used include: OHAUS analytical balance, electric oven, water bath, rotary evaporator (SHZ-III, Brinkmann Instruments, Inc., Westbury, New York), UV-Vis spectrophotometer (752N), Milling machine (Viking Type YL8024).

Methods

Collection and identification of plant

Fresh shrub and pumpkin of *Telfairia occidentalis* were obtained from Olunlade and Pipeline areas, Ilorin, Kwara state, Nigeria. A Taxonomist in the Department of

Plant Biology, University of Ilorin, Nigeria, identified the plant as *Telfairia occidentalis* with voucher number: UILH/001/1063.

Preparation and Extraction

The stem and seeds were cut differently into smaller pieces and air dried at room temperature for a week. It was further dried in the oven at 40 °C. The dried plant part was then pounded using a mortar and pestle and then milled in a milling machine.

The milled stem (232g) and seed (300g) of *T. occidentalis* each were weighed, placed in different extraction bottles and 600 ml each of petroleum ether was poured into it followed by stirring at two hour interval for up to 12 hours after which it was decanted. This process was repeated for 3 days for exhaustive extraction. The decanted supernatant was filtered and the filtrate concentrated using a rotary evaporator. The marc was dried and sequentially extracted with chloroform, ethyl acetate, acetone and methanol respectively, to obtain different fractions/extract of the stem and seed of *T. occidentalis*.

The percentage yield for each fraction of the extracts was calculated using the expression

$$\% \text{ yield} = \left(\frac{\text{Weight of extract}}{\text{Weight of powdered plant part}} \right) \times \frac{100}{1}$$

Qualitative phytochemistry

The different fractions of the stem and seed of *T. occidentalis* were tested for the presence of phytochemicals using the following standard methods^{16,17}.

Test for carbohydrates

Fehling's test

Equal volumes of Fehling's solution A and B reagents were mixed together and 2ml of the resulting solution was added to each of the extract and gently boiled. A brick red precipitate appearing at the bottom of the test tube indicated the presence of reducing sugars.

Molisch's test

Each extract was mixed with 2ml of Molisch's reagent and the mixture was shaken properly. After that, 2ml of concentrated sulphuric acid (H₂SO₄) was poured carefully along the side of the test tube. Appearance of a violet ring at the interphase indicated the presence of carbohydrate.

Test for phenols and tannins

Each extract was mixed with 2ml of a 2% solution of FeCl₃. A blue-green or black coloration indicated the presence of phenols and tannins.

Test for flavonoids

Each extract was mixed with 2ml of a 2% solution of NaOH. The formation of an intense yellow colour which turned colourless on addition of few drops of diluted HCl indicated the presence of flavonoids.

Test for saponins

Each extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously. The formation of a stable foam was taken as an indication for the presence of saponins.

Keller-kilani test for glycosides

Each extract was mixed with 2ml of glacial acetic acid containing 1-2 drops of a 2% solution of FeCl₃. The mixture was then poured into another test tube containing 2ml of concentrated H₂SO₄. A brown ring at the interphase indicated the presence of cardiac glycosides.

Test for steroids

Each extract was mixed with 2ml of chloroform and concentrated H₂SO₄ was added sidewise. A red colour produced in the lower chloroform layer indicated the

presence of steroids. Another test was performed by mixing the crude extract with 2ml of chloroform, and then 2ml of each of concentrated H₂SO₄ and acetic anhydride were poured into the mixture. The development of a greenish colour indicated the presence of steroids.

Test for terpenoids

Each extract was dissolved in 2ml of chloroform and 2ml of concentrated H₂SO₄ was carefully added. A layer of reddish-brown colour at the interphase indicated the presence of terpenoids.

Test for alkaloids

Each extract was mixed with 2ml of 1% HCl and heated gently. Wagner's reagent was then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

Quantitative phytochemistry

Total Phenolic content

The amount of phenol in the extract was determined by the Folin-Ciocalteu reagent method¹⁸ with some modifications. 3.6 ml of 10 % Folin-Ciocalteu reagent and 2.9 ml of 7 % solution of Na₂CO₃ were added to 3.6 ml of the plant extract. The resulting mixture was incubated for 30 minutes at room temperature and the absorbance of the solution was measured at 765 nm. All extracts were evaluated at a final concentration of 0.1 mg/ml. Total phenolic content was expressed as mg/g tannic acid equivalent using the following equation based on the calibration curve: $y = 0.1216x$, $R^2 = 0.9365$, where y was the absorbance x was the concentration.

Total Flavonoid content

The aluminium chloride colorimetric method¹⁹ was used with some modifications to determine the flavonoid content. 0.5 ml of sample plant extract was mixed with 2 ml of

methanol, 2.5 ml of 10 % aluminium chloride and kept at room temperature for 30 minutes. The absorbance was then measured at 420 nm. Extracts were evaluated at a final concentration of 0.1 mg/ml. Total flavonoid content were calculated as quercetin equivalents (mg/g) using the following equation based on the calibration curve: $y = 0.0255x$, $R^2 = 0.9812$, where y was the absorbance x was the concentration.

Total Alkaloid determination

0.1 g of the each extract was weighed into a sample bottle and 4 ml of 20 % acetic acid in ethanol was added. The sample bottle was covered and allowed to stand for 4 h. It was then filtered and the extract was concentrated on a water bath at 55°C to one quarter of its original volume. Concentrated ammonium hydroxide was added dropwise to the extract until precipitation was complete. The precipitate was collected from the solution and washed with dilute ammonium hydroxide and filtered. The residue, which contained the alkaloid was weighed after dryness to completion and the percentage was calculated²⁰ using the formular:

$$\% \text{ alkaloid} = \left(\frac{\text{Weight of alkaloid precipitate}}{\text{Weight of powdered sample}} \right) \times \frac{100}{1}$$

Antioxidant Assay

This was done using DPPH²¹. 0.5 mg/ml of each extract was added, at an equal volume, to methanolic solution of DPPH (100 µM). The mixture was kept in the dark at room temperature for 30 minutes. The absorbance (A) was then measured at 518 nm and converted into the percentage antioxidant activity using the following equation:

$$\% \text{ DPPH} = \left(\frac{\text{Absorbance (DPPH)} - \text{Absorbance (Extract)}}{\text{Absorbance (DPPH)}} \right) \times \frac{100}{1}$$

Statistical Analysis

All experiments were done in triplicates and data obtained were represented in tables as

Mean $\pm$ Standard error of mean (SEM). Analysis of Variance (ANOVA) was used to statistically analyze the data where applicable. T-test was used to compare the mean data obtained for each extract of the seed and stem of *T. occidentalis*. Differences between samples were taken as being significant at $P < 0.05$.

Results and discussion

After exhaustive extraction of the different fractions of the seed and stem extracts of *T. occidentalis*, chloroform gave the highest yield for the seed and stem (7.61 ± 0.05 and 1.07 ± 0.03 % respectively) while ethyl acetate afforded the lowest yield ($0.09 \pm 0.01\%$) as shown in table 1. Extractive value

in the order: $\text{CHCl}_3 > \text{petroleum ether} > \text{ethyl acetate} > \text{methanol} > \text{acetone}$ and $\text{CHCl}_3 > \text{petroleum ether} > \text{acetone} > \text{methanol} > \text{ethyl acetate}$ for the seed and stem fractions respectively (Table 1). This indicated that the phytochemicals extracted are mostly less polar as can be observed from the Table 3 in which the chloroform fraction of the seed contains mostly alkaloids, cardiac glycoside, steroids and terpenoids.

The methanol fraction of the stem is soluble in water because of the presence of flavonoid (Table 2) while that of the seed is soluble in water because of the presence of mostly steroids and cardiac glycoside.

Table 1. Percentage yield of the seed and stem extract of *T. occidentalis*

Plant part	fractions	Weight of powdered drug (g)	Weight of extract (g)	% yield $\pm$ SEM
Seed	Petroleum ether	300	14.28	4.76 ± 0.10
	Chloroform	300	22.82	7.61 ± 0.05
	Ethyl acetate	300	6.17	2.06 ± 0.06
	Acetone	300	2.05	0.68 ± 0.03
	Methanol	300	3.50	1.17 ± 0.01
Stem	Petroleum ether	232	1.79	0.77 ± 0.03
	Chloroform	232	2.49	1.07 ± 0.03
	Ethyl acetate	232	0.21	0.09 ± 0.01
	Acetone	232	0.60	0.26 ± 0.01
	Methanol	232	0.42	0.18 ± 0.01

The qualitative phytochemistry of *Telfairia occidentalis* stem (Table 2) showed the presence of reducing sugars and alkaloids in all the fractions except the methanol extract; cardiac glycosides in only the methanol extract; flavonoids in the chloroform and acetone extracts. Steroids were present only in the chloroform extract and terpenoids in only the acetone extract. Saponins and

tannins were absent in the extracts of the stem of *Telfairia occidentalis*. This correlates with previous work where carbohydrates was present²², and this may imply that the stem of *T. occidentalis* can be a good source of energy.

Telfairia occidentalis seed showed the presence of cardiac glycosides, steroids and

terpenoids in all the extracts and alkaloids in only the petroleum ether and chloroform extracts. Cardiac glycosides were present in high quantity in *Telfairia occidentalis* seeds. Carbohydrates, reducing sugars, flavonoids, saponins and tannins were absent in all the fractions of *Telfairia occidentalis* seed extract. This contradicts the work done by Eseyin *et al.*²² in which they indicated the presence of carbohydrates and reducing sugars. This could be due to the

environmental and climatic factors associated with its plantation, maturity and harvesting.

Secondary metabolites (phenols, flavonoids, alkaloids) are known to have antimicrobial activity²³, Cardiac glycosides use for the treatment of heart failure and atrial arrhythmia²⁴, are potent inhibitors of DNA double-strand break repair²⁵, thus suggesting the possible uses for the this plant for medicinal purposes

Table 2. Qualitative Phytochemistry of the seed and stem of *Telfairia occidentalis*

Phytochemical constituent	Test	Petroleum ether		Chloroform		Ethyl acetate		Acetone		Methanol	
		seed	stem	seed	stem	seed	stem	seed	stem	Seed	stem
Carbohydrates	Molish's Test	-	-	-	-	-	-	-	-	-	-
Reducing sugars	Fehling's Test	-	+++	-	+++	-	+++	-	+	+	+
Alkaloids	Wagners' Test	+	+	+	+	-	+++	-	+	-	-
Cardiac glycosides	Keller-kilaniTest	+++	-	++	-	++	-	+++	-	+	++
Flavonoids	Alkaline reagent Test	-	-	-	+	-	-	-	+	-	-
Saponins	Frothing Test	-	-	-	-	-	-	-	-	-	-
Steroids	Acetic anhydride Conc. H ₂ SO ₄	++	-	++	++	++	-	+	-	+	-
Tannins	FeCl ₃ Test	-	-	-	-	-	-	-	-	-	-
Terpenoids	CHCl ₃ + H ₂ SO ₄	+	-	+	-	+	-	+	+	+	-

KEY: + = presence; - = absence

Quantitative Phytochemistry of *Telfairia occidentalis*

The result of the total percentage phenolic content showed that the acetone extract contained more phenolic content (4.02%) than the chloroform extract (3.59%) as

shown in Figure 1. Phenols have been associated with the antimicrobial activity of the plant²³ thus the presence of phenol in this plant justifies its antimicrobial activity as reported in literature²⁶

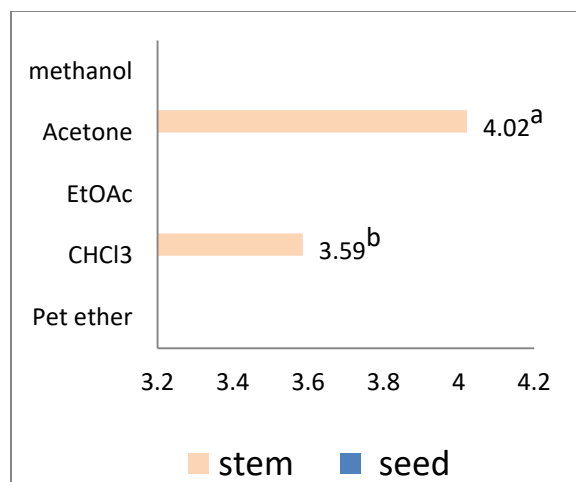
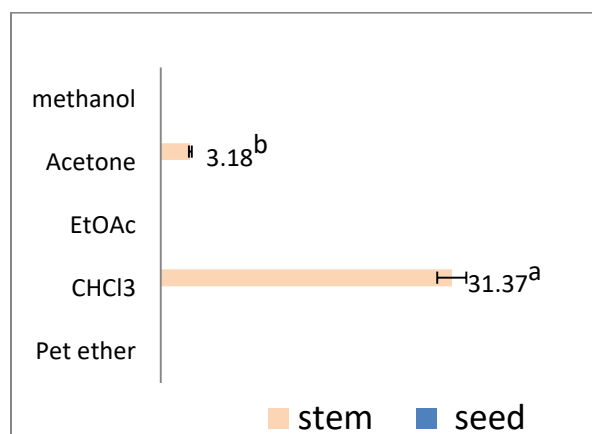


Figure 1. Total phenolic content in the seed and stem of *Telfairia occidentalis*. (Values with a superscript are significantly different [$P < 0.05$]).

Only the chloroform and acetone extracts of the stem significantly contained flavonoid (31.37 and 3.18%) as shown in Figure 2. Flavonoids are polyphenolic compounds found in a wide variety of plants and they impart a yellow or white pigment to flowers and fruit and have a wide range of activity. Flavonoids are particularly useful for maintaining healthy circulation and some are antioxidant, while others are anti-inflammatory, anti-viral or capillary strengthening. This shows the high medicinal potency of the chloroform and acetone fractions as antioxidants (Figure 4).



The antioxidant activity of the acetone extract of the stem was greater than that of

Figure 2. Total flavonoid content in the seed and stem of *Telfairia occidentalis*. (Values with a superscript are significantly different [$P < 0.05$]).

The seed of *Telfairia occidentalis* showed a significantly high ($P < 0.05$) alkaloid content for both petroleum ether and chloroform extracts with total percentage alkaloid content of 40.64% and 29.22% respectively, whereas, the acetone and ethyl acetate extracts of the stem only contained alkaloid (Figure 3).

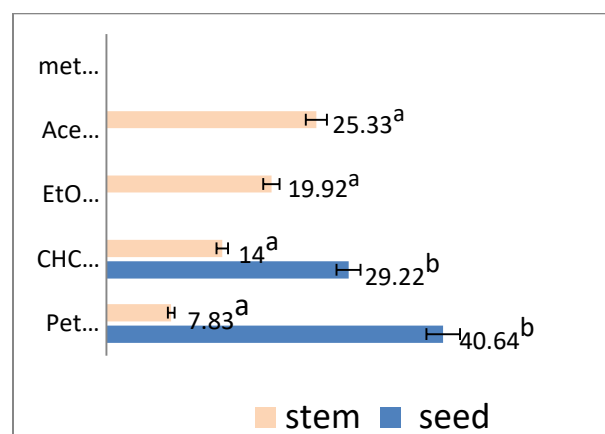


Figure 3. Total alkaloid in the seed and stem of *Telfairia occidentalis*. Values with the same letter superscript within the same constituent are not significantly ($P < 0.05$).

Antioxidant activity

The percentage antioxidant activity of the chloroform extract of both the seeds and stem extracts of *T. occidentalis* showed that the seeds had a higher antioxidant activity though not statistically significant. The antioxidant activity of the seed CHCl₃ is mostly due to the presence of alkaloids present as shown in Figure 2. While that of the acetone might be due to the presence of cardiac glycoside, steroids and terpenoids as shown in Table 2.

the seed though not statistically significant (Figure 4). Antioxidant activity of plant

extracts have been attributed to the flavonoids present in such plant extracts²⁷.

Thus presence of flavonoid in the stem is responsible for its high antioxidant activity.

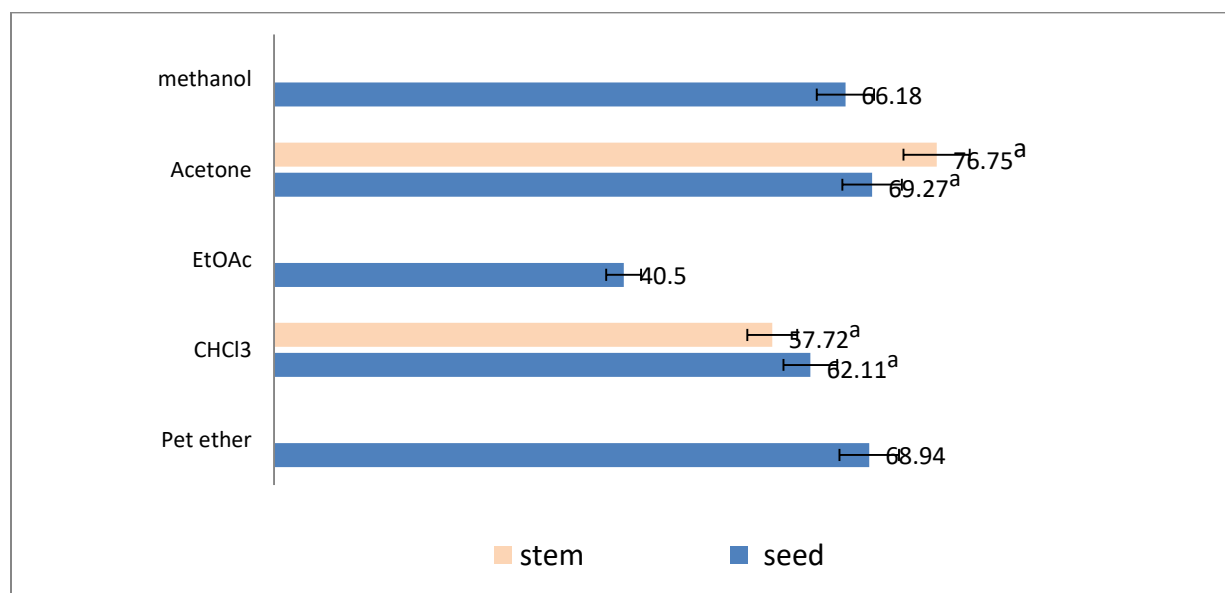


Figure 4. % DPPH scavenging activity at 0.5 mg/ml. Values with the same letter superscript within the same constituent are not significantly ($P < 0.05$).

CONCLUSION

The phytochemical analysis carried out on the seeds and stem extracts of *Telfairia occidentalis* showed the presence of phenolics, reducing sugars, cardiac glycosides, alkaloids, flavonoids, steroids and terpenoids. The DPPH antioxidant assay showed a relatively good antioxidant activity with the acetone fraction of both the

seed and stem extracts. These results confirm the folkloric claim of this plant.

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