The Pharmaceutical and Chemical Journal, 2017, 4(6):131-140

Available online www.tpcj.org

Research Article

ISSN: 2349-7092
CODEN(USA): PCJHBA

Anti-nutrients Composition of Fluted Pumpkin Leaf Grown in Different Geoponic Media

***Kalu Okonwu, Love A Akonye, Stephen I Mensah**

Department of Plant Science and Biotechnology, University of Port Harcourt, P.M.B. 5323, Port Harcourt, Nigeria

Abstract The anti-nutrient compositions of fluted pumpkin leaf grown in three different geoponic media were quantified. These media were white-sand (WS), humus soil (HS) and sawdust (SD). The anti-nutrients composition of matured pumpkin leaf assessed were alkaloids, flavonoids, saponins, hydrogen cyanide, trypsin-inhibitor, oxalate, tannins and phytate. Standard analytical methods were followed in the determination of the anti-nutrient compositions. Phytate, tannin, oxalate, trypsin-inhibitor, hydrogen cyanide, and saponins contents (ppm) in fluted pumpkin leaf grown in WS, HS and SD were 7.131, 8.857 and 8.866; 1.436, 2.127 and 4.348; 3.814, 5.927 and 4.882; 0.959, 2.584 and 1.556; 0.007, 0.001 and 0.002; 4.175, 7.253 and 5.108, respectively while total flavonoids and total alkaloids contents (g/100g) were 7.679, 8.064 and 13.387, and 17.848, 9.077 and 12.445 in that same order. Among the groups of alkaloids, indole/benzypyrole (5.959, 1.059 and 3.885 g/100g) and quinoline (4.493, 4.507 and 5.041 g/100g) were the most abundant while acridine (0.008, 0.009 and 0.011 g/100g) and imidazole (0.001, 0.003 and 0.001 g/100g) were the least in the three treatments. Also, isoflavones (2.321, 3.731 and 5.551 g/100g) and flavan-3-ols (4.648, 3.918 and 7.226 g/100g) were the most concentrated subgroups of flavonoids while anthocyanin was the least (0.083, 0.083 and 0.082 g/100g) for WS, HS and SD, respectively. The study revealed that the bioactive content of *T. occidentalis* changes depending on the growth medium.

Keywords anti-nutrient, geoponic media, pumpkin, grown

Introduction

Secondary metabolism helps plants to understand changes in the environment [1]. Also, some by product of primary metabolism are involved in stress response thereby serving as agent of protection for the plant. Cuin and Shabala [2] reported that amino acid level rises when plants are grown in saline area. Amino acids such as glycine, lysine, threonine and glutamate assist in preserving the stomach from harm [3, 4]. According to Croteau *et al*. [1], alkaloids confer some level of resistance to plants against invaders and have high health benefits.

Studies have shown that anti-oxidants can inhibit chemical impairment caused by free radicals produced in many ways such as pesticide, organic solvents and environmental pollution [5]. Synthetic antioxidant like butylated hydroxyanisole and butylated hydroxytoluenehave the capacity of increasing the longevity of plant products and are utilized in food industries [6]. However, synthetic antioxidant possess health challenge in the area of tumour formation and liver destruction in human [7, 8]. Ascorbic acid, tocopherol and vitamin A are frequently used artificial antioxidants, generally sold in the market and studies have revealed its potential to cause death in adults who consumed them. The precise mode of oppression is yet to be established but it has been suggested that it could be due to the severe poisons that they possess compared to natural antioxidants [9]. In recent times, studies on natural phenolics have been on the increased with majority of them occurring in plants and intake of plant material containing high level of phenolic compounds may minimized the danger in the formation of so many illnesses due to their antioxidant properties, among other factors. The antioxidant capacity in plants was found to be influenced by

cultivars, maturity and other environmental factors such as sunlight exposure [10]. Studies have shown that irradiation can affect the antioxidant level in cabbage and beetroot leaves while pre-treatment before the extraction process and stage of leaf maturity affect the antioxidant activity in the guava's leaves [11, 12].

Literatures have shown that plants contain various bioactive compounds which are involved in its metabolism. These substances play different roles in plants based on their biosynthesis and constituents. Hence, the consumption of these substances in plants by humans have a health implications. This health concern, necessitated the evaluation of the bioactive compounds in *Telfairia occidentali s*grown in three different solid media.

Materials and methods

Source of materials

The seeds of *Telfairia occidentalis* and sawdust (SD) used were sourced from Choba Market and Rumuosi Sawmill, respectively while the white sand (WS) and humus soil (HS) were obtained from Choba River and a garden in University of Port Harcourt in that order. The seeds were planted in three different medium namely; white sand, sawdust and humus soil. After germination, the seedlings were allowed to stand for one month when it is matured and can be harvested to prepare food. The leafy vegetables were harvested and the following analyses were carried out: phytochemicals, flavonoids, alkaloids and organic acids.

Cyanogenic glycosides determination

Sample (5 g) was ground into a paste. The paste was dissolved in 50 ml distilled in a corked conical flask and allowed to stay overnight for cyanide extraction. The extract was filtered and the filtrate used for cyanide determination. To 1 ml of the filtrate, 4ml alkaline picrate (1 g of picrate and 5 g sodium carbonate dissolved in a volume of minimally warm water and the volume made up to 200 ml with distilled water) was added and incubated in a water bath for 5 minutes. After colour development (reddish brown colour), the absorbance of the corked test tube read in spectrophotometer at 490 nm. Also, the absorbance of the blank containing only 1ml distilled water and 4ml alkaline picrate solution. Then, the cyanide content was extrapolated from a cyanide standard curve (different concentration of KCN solution containing 5-50 µg cyanide in 500 ml conical flask received 25 ml of 1 N HCl each).

Phytate determination

The rapid determination of phytate *was* according to the method of Oberlease *et al*. [13].

The plant material was extracted with 0.2 N HCl such that we have 3-30 µg/ml phytate solution. The extract (0.5 ml) was pipetted into a test tube fitted with a ground-glass stopper and 1 ml of ferric solution (0.2 g ammonium iron (111) sulphate $12H_2O$ in 100ml 2 N HCl and made up to 1000 ml with distilled water) was added. The test tube was heated in a boiling water bath for 30 minutes. Sample was cooled in ice water for 15 minutes and allowed to adjust to room temperature. The content of the tube was mixed and centrifuged for 30 minutes at 3000 rpm. The supernatant (1 ml) was transferred to another and 1.5 ml of 2, 2-Bipyridine solution (10g 2, 2-bipyridine and 10ml thioglycollic acid in distilled water and made up to 1000ml). Absorbance of the solution measured at 519 nm against distilled water. The method had to be calibrated with the reference solutions as a substitute for the sample solution with each set of analyses. Preparations of the calibration curve was carried out by plotting the concentrations of the reference solutions against their corresponding absorbance. Then the absorbance of the test sample was used to obtain the concentration from the calibration curve.

Tannin determination

The Folin-Denis spectrophotometric method was used to determine tannin. The method was described by Pearson [14]. A measured weight of each sample (1.0 g) was dispersed in 10 ml distilled water and agitated. This was left to stand for 30min at room temperature, being shaken every 5 minutes. At the end of the 30 minutes, it was centrifuged and the extract gotten. 2.5 ml of the supernatant (extract) was dispersed into a 50 ml volumetric flask. Similarly, 2.5 ml of standard tannic acid solution was dispersed into a separate 50ml flask. A 1.0 ml Folin-Denis reagent was measured into each flask, followed by 2.5 ml of saturated $NaCO_3$ solution. The mixture was diluted to mark in the

flask (50 ml), and incubated for 90min at room temperature. The absorbance was measured at 250 nm in a Genway model 6000 electronic spectrophotometer. Readings were taken with the reagent blanket zero. The tannin content was given as follows.

% Tannin-An/As x C x 100/w x Five

Where, An = absorbance of test sample; As = absorbance of standard solution; C = concentration of standard solution; W= weight of sample used; Vf= total volume of extract; Va= volume of extract analysed

Enzyme inhibitors determination

The trypsin inhibitor activity (TIA) assay via spectrophotometric method described by Arnfield *et al*. [15].

***Extraction of sample*:** Test sample weight of 1.0 g was dispersed in 50ml of 0.5 M NaCl solution, the mixture was stirred for 30 minutes at room temperature and centrifuged. The supernatant was filtered through filter paper (Whatman No. 41) and the filtrate used for the assay. To 10ml of the substrate in a test tube, 2ml of the standard trypsin solution (N - α –Bensoyl –DL arginine – P- nitroanilide [BAPA]) was added and a blank of 10 ml of the same substrate in a test tube with no extract added. The content of the test tubes were allowed to stand for at least 5 minutes and then measured spectrophotometrically at 410 nm wavelength. One trypsin unit inhibited is given by an increase of 0.01 absorbance units at 410 nm, given a 100 ml of the mixture. That is one trypsin unit inhibited (TUI) is equal to an increase of 0.01 in absorbance unit at 410 nm. The trypsin inhibitor activity is expressed as the number of trypsin units inhibited (TUI) per unit weight (g) of the sample analysed.

$$\text{TUI/mg} = \frac{Absorbance\ \ of\ Sample}{Absorbance\ \ of\ Standard} \text{X } 0.01\text{F}$$

$$\text{TUI/mg} = \frac{b-a}{0.01} \text{X F}$$

Where b = absorbance of the test sample solution; a = absorbance of the blank (control);

F =experimental factor, given by

$$\text{F} = \frac{1}{\text{w}} \text{ X } \frac{\text{Vf}}{\text{Va}} \text{ X D}$$

Where w = weight of the sample; V*f* = total volume of extract; V_a = volume of extract used in the assay; D = dilution factor (if any)

Oxalates determination

This determination involves three major steps digestion, oxalate precipitation and permanganate titration.

Digestion: Sample (2 g) was suspended in 190 ml of distilled water in a 250 ml volumetric flask. To this, 10 ml of 6 M HCl was added and the suspension digested at 100˚C for 1 hour, cooled and made up to 250 ml mark before filtration.

Oxalate Precipitation: Duplicate portions of 125 ml of the filtrate were measured into beakers and four drops of methyl red indicator added. This was followed by the addition of conc. NH_4OH solution (drop wise) until the test solution changed from salmon pink colour to a faint yellow colour (pH 4 – 4.5). Each portion is then heated to 90˚C, cooled and filtered to remove precipitate containing ferrous ion. The filtrate is again heated to 90˚C and 10 ml of 5% $CaCl_2$ solution added while being stirred constantly. After heating, it was cooled and left overnight at 5˚C. The solution is then centrifuged at 2500 rpm for 5 minutes. The supernatant was decanted and the precipitate completely dissolved in 10 ml of 20% (v/v) H_2SO_4 solution.

Permanganate Titration: The total filtrate resulting from digestion of 2 g of test sample was made up to 300 ml. Aliquots of 125 ml of the filtrate was heated until near-boiling and then titrated against 0.05 M standardized $KMnO_4$ solution to a faint pink colour which persists for 30 seconds. The calcium oxalate content was calculated using the formula.

$$\text{Oxalate } (\frac{\text{mg}}{100\text{g}}) = \frac{\text{T x (Vme)(Df)x } 10^5}{\text{(ME)x (Mf)}}$$

Where T is the titre of $KMnO_4$ (ml), Vme is the volume – mass equivalent ($1cm^3$ of 0.05 M $KMnO_4$ solution is equivalent to 0.00225 g anhydrous oxalic acid), Df is the dilution of factor V_T/A (2.4 where V_T is the total volume of titrate (300 ml) and A is the aliquot used (125 ml), ME is the molar equivalent of $KMnO_4$ in oxalate ($KMnO_4$ redox reaction) and Mf is the mass of the test sample used.

Flavonoids (extraction and determination)

Plant sample (1.5g) was weighed into a set of extraction tube(s) and 20ml of boiled ultra-pure water dispensed into each extraction tubes. The setup was allowed to stand for 1.5 hours and voltexed for 5 minutes. The solution was transferred to a set of centrifuge tubes, shaked for 15 minutes and centrifuged for 5 minutes at 3000rpm. Thereafter, a set of vials were used to collect the supernatants for determination on water 616/626 HPLC. The conditions for the analysis of flavonoids were as follows: (i) An autosampler (ii) An automated gradient controller (iii) Gradient elution HPLC pump (iv) Reverse-phase HPLC column, thermostatically heated in a temperature-controlled room. (v) Detector by fluorescence (vi) Carrier gas: Nitrogen gas at flow rate of 60ml/min. (vii) Temperature: Detector- 147^oC; Injector port- 166^oC and Column: 115^oC (viii) Computer facilities for storing data. (ix) Printer for results reporting

Alkaloids (extraction and determination)

Ten gram (10g) of plant sample was de-fated, out of which 5g was weighed into a flask and 100ml of 12% alcohol added, shaked, filtered and washed with industrial alcohol. The extracted residue was washed into a flask with 50ml of ammonia water (ultrapure water) and heated in boiling water for 20 minutes and allowed to cool. Then, 0.1g of diastase (+ water) added and maintain at 50-55^oC for 2 hrs. It was cooled and made up to 250ml with ultrapure water, swirled and filtered. The filtrate (200ml) was mixed with 20ml hydrochloric acid (sp.g. 1.125) and heated in boiling water for 3 hours. Thereafter, it was allowed to cool, neutralized with sodium hydroxide solution and made up to 250ml. The sample was shaked, centrifuged and supernatant decanted for determination using water 616/626 HPLC. The conditions of HPLC (Water 616/626) for the analysis of alkaloids were as follows: (i) An autosampler (ii) An automated gradient controller (iii) Gradient elution HPLC pump (iv) Reverse-phase HPLC column, thermostatically heated in a temperature-controlled room. (v) Detector by fluorescence (vi) Carrier gas: Nitrogen gas at flow rate of 40ml/mins. (vii) Temperature: Detector- 170^oC; Injector port- 190^oC and Column- 125^oC (viii) Computer facilities for storing data. (ix) Printer for results reporting

Results and Discussion

Anti-nutrients composition

The anti-nutrient composition of *T. occidentalis* leaf grown in three different geoponic media are as shown in Tables 1, 2 and 3. Concentration of the components vary from one medium to another: oxalate (3.814, 5.927 and 4.882 ppm), phytate (7.131, 8.857 and 8.866 ppm), tannin (1.436, 2.127 and 4.348 ppm), saponin (4.175, 7.253 and 5.108 ppm), trypsin-inhibitor (0.959, 2.584 and 1.556 ppm) and hydrogen cyanide (0.007, 0.001 and 0.002 ppm) for white-sand (WS), humus soil (HS) and sawdust (SD), respectively (Table 1). High cyanide possesses health risk to consumers. Nicolau [16] reported that cyanide toxicity affects individuals with low and deficient of iodine. However, the values obtained were below the established standard (10 ppm HCN) for cassava flour by FAO/WHO [17]. The processing of cassava reduces the cyanide content than in other products [18]. This implies that cooked *T. occidentalis* will have reduced hydrogen cyanide content. The WS medium gave the highest hydrogen cyanide content and least values for saponin, tannin, trypsin-inhibitor, phytate and oxalate compared to HS and SD media. Chibueze and Akubugwo [19] reported ranges of some anti-nutrients content of *T. occidentalis* under different fertilizer applications: saponins (4.00 – 6.23%), tannins (0.15 – 0.20%), flavonoids (3.13 – 3.51%), alkaloids (1.17 – 2.09%) and phenols (0.07 – 0.11%). Trypsin inhibitor (TIU/mg) for unprocessed and under-processed seeds of *T. Occidentalis* were 23.18±0.07 and 2.13±0.12, respectively [20]. Studies by Jack and Nna [21] have shown that *T. occidentalis* extracted with methanol had 0.2429 µg/g of phytate content. Aletor and Adeogun [22] earlier recorded 40 mg/100g of oxalic acid for *T. occidentalis* on fresh weight basis. Otitoju *et al.* [23] recorded 0.85, 0.04, 0.06, 0.80

and 0.14 g/100g for alkaloids, flavonoids, oxalate, saponin and tannin, respectively in raw fluted pumpkin leaves. However, lower range (0.1 – 0.3 mg/100g fresh weight) was reported for *Vernonia* species [24]. According to Okunade and Adesina [25], high anti-nutrient content is a possible health menace having intoxicating effect on human. The fatal amount (30 mg/kg) of tannin was reported by Inuwa *et al.* [26]. Nevertheless, studies have shown that any form of processing of vegetable with intense heat greatly decreases the anti-nutrient contents [27, 28]. Again, the consumption of saponins by human beings may be crucial in addressing cardiac related ailments even though it is harmful [29]. Also, tannins decrease plasma fat [30]. Some anti-nutrient constituents of *T. occidentalis* leaves extract reported by Ekpenyong *et al.* [31] were tannin (35.0 mg/100g), cyanide (50.1 mg/100g), phytic acid (85.06 mg/100g) and oxalate (68.20 mg/100g).

Table 1: Anti-nutrient compositions of *T. occidentalis* grown in three different medium

Phytochemicals (ppm)	**Growth medium**		
	White Sand	**Humus soil**	**Sawdust**
Oxalate	3.814	5.927	4.882
Phytate	7.131	8.857	8.866
Tannin	1.436	2.127	4.348
Saponin	4.175	7.253	5.108
Trypsin-inhibitor	0.959	2.584	1.556
Hydrogen cyanide	0.007	0.001	0.002

Flavonoids

The flavonoids content of *T. occidentalis* leaves grown in three different solid media are presented in Table 2. The total flavonoids were 7.679, 8.061 and 13.389 g/100g for the media (white-sand, humus soil and sawdust), respectively. The most abundant of the flavonoids subgroups was flavan-3-ols (4.648, 3.918 and 7.226 g/100g) while anthocyanin (0.083, 0.083 and 0.082 g/100g) was the least in that same order. The percentage of flavan-3-ols in total flavonoids content of *T. occidentalis* were 0.61, 0.49 and 0.54%. Daidzein (1.436, 2.308 and 4.348 g/100g), catechin (1.558, 1.555 and 3.090 g/100g), epicatechin (1.074, 1.200 and 0.959 g/100g) and epigallocatechin (1.806, 0.991 and 2.708 g/100g) were the most concentrated among the thirty seven (37) flavonoids assessed in the three media, in that sequence. Flavanones subgroups were more in number (12) compared to other flavonoids subgroups. However, the concentration of the individual members of the subgroups were low. Salau*et al*. [32] reported flavonoids content of some (10) fresh leafy vegetable with the range 54.70 – 222.36 mg/100g dry weight. Iweala and Obidoa [33] reported the presence of flavonoids, alkaloids, oxalates, saponins and resins in *T. occidentalis.* According to Irina and Mohamed [34] (2012), the level of flavonoids in plants are affected by genetic and environmental factors. Studies have shown that flavonoids derived from food have revealed to play crucial parts in tumour inhibition [35, 36]. Natural flavonoids and their derivatives have been an important source of bioactive molecules in medicines much before the advancement of other modern therapeutics in the post-genomic period [37]. Flavonoids have the defensive capacity against biological contaminants from microorganisms [38, 39] and inorganic contaminants agents from the environment is a key factor in the adaptive features of plants [32].

Table 2: Flavonoids present in *T. occidentalis* grown in different medium

Subgroups of flavonoids	**Flavonoids (g/100g)**	**Growth medium**		
		White Sand	**Humus soil**	**Sawdust**
Flavones	Acacetin	0.027	0.010	0.013
	Nobiletin	0.001	0.001	0.000
	Apigenin	0.004	0.000	0.003
	Luteolin	0.100	0.007	0.095
	Neodiosmin	0.019	0.019	0.014
	Tangeretin	0.007	0.005	0.003

	Rhoifolin	0.018	0.020	0.024
	Diosmin	0.002	0.002	0.003
	Sinensetin	0.018	0.018	0.022
Isoflavones	Daidzein	1.436	2.308	4.348
	Genistein	0.694	1.222	1.134
	Glycetin	0.191	0.201	0.069
Flavanones	Hesperidin	0.009	0.006	0.009
	Nanirutin	0.013	0.007	0.011
	Eriocitrin	0.003	0.003	0.002
	Naringin	0.017	0.022	0.018
	Naringenin	0.003	0.003	0.002
	Eriodictyol	0.098	0.053	0.075
	Didymin	0.017	0.008	0.016
	Neoeriocitin	0.019	0.011	0.016
	Poncirin	0.009	0.009	0.009
	Raxifolin	0.009	0.009	0.010
	Taxifolin	0.002	0.004	0.003
	Hesperetin	0.009	0.009	0.009
Flavan-3-ols	Catechin	1.558	1.555	3.090
	Epicatechin	1.074	1.200	0.959
	Theaflarins	0.115	0.090	0.398
	Thearubigins	0.046	0.047	0.043
	Epigallocatechin	1.806	0.991	2.708
	Epicatechingallate	0.015	0.009	0.008
	Epigallocatechingallate	0.014	0.011	0.008
	Proanthocyanidins	0.020	0.015	0.012
Flavanols	Isorharmnetic	0.181	0.075	0.116
	Myricetrin	0.002	0.002	0.002
	Kaempferol	0.003	0.000	0.002
	Quercetin	0.037	0.026	0.053
Anthocyanin	Anthocyanin	0.083	0.083	0.082
Total flavonoids (g/100g)		7.679	8.061	13.389

Alkaloids

The detected alkaloids and their concentrations in *T. occidentalis* grown in white-sand, humus soil and sawdust are shown in Table III. Total of forty five (45) alkaloids were determined from twelve (12) groups/classes of alkaloids. Total alkaloids in *T. occidentalis* grown in three different solid media were 17.848 g/100g, 9.077 g/100g and 12.445 g/100g, respectively. The abundant groups of alkaloidpresent in *T. occidentalis* were quinoline (4.493 g/100g, 4.507 g/100g and 5.041 g/100g) and indole/benzypyrole (5.959 g/100g, 1.059 g/100g and 3.885 g/100g) with percentage (quinoline - 25.17%, 49.65% and 40.51%; indole/benzypyrole - 33.39%, 11.67% and 31.22%) of total alkaloids, in that sequence. Among the alkaloids determined in *T. occidentalis*, reserpine is the most abundant (5.897 g/100g, 3.610 g/100g and 4.366 g/100g) while psychotrine, theobromine and narcotine were the least (0.001 g/100g, 0.001

g/100g and 0.001 g/100g) for the media – white-sand, humus soil and sawdust, respectively. Alkaloids content in *T. occidentalis* varied from one growth medium to another. Enujiugha *et al*. [40] reported that the alkaloid content of *T. occidentalis* aqueous and ethanol extracts were 0.350 mg/g and 0.450 mg/g, respectively. The total alkaloid contents obtained in this study were higher compared to the work of Odufuwa *et al.* [41] on *T. occidentalis* during processing.

Table 3: Alkaloids present in *T. occidentalis* grown in different medium

Group of alkaloids	Alkaloids (g/100g)	Growth medium		
		White Sand	Humus soil	Sawdust
Indole/benzypyrole alkaloids	Eserine	0.002	0.001	0.002
	Rauwolfia	0.009	0.009	0.009
	Reserpine	5.897	0.950	3.814
	Strychnine	0.030	0.074	0.035
	Ergotamine	0.017	0.017	0.019
	β-carboline	0.004	0.008	0.006
Purine (Pseudo) alkaloids	Caffeine	0.036	0.009	0.008
	Theobromine	0.001	0.001	0.001
	Theophylline	0.007	0.005	0.005
Vinca alkaloids	Vincristine	0.103	0.054	0.097
	Vinblastine	2.774	0.426	1.131
Colchicine alkaloids	Colchicine	0.042	0.011	0.009
Quinoline alkaloids	Cinchonine	0.062	0.016	0.014
	Quinine	0.000	0.001	0.000
	Quinidine	1.022	0.877	0.659
	Quinoline	3.406	3.610	4.366
	Cinchonidine	0.003	0.003	0.002
Isoquinoline alkaloids	Morphine	0.133	0.014	0.010
	Narcotine	0.001	0.001	0.001
	Codeine	0.008	0.005	0.014
	Papaverine	0.056	0.015	0.011
	Tubocurarine	0.005	0.085	0.005
	Heroin	0.092	0.082	0.085
	Emetine	2.744	2.014	1.671
	Apomorphine	0.015	0.003	0.002
	Psychotrine	0.001	0.001	0.001
	Berberine	0.003	0.004	0.004
	Cephaline	0.007	0.007	0.007
Tropane alkaloids	Atropine	0.087	0.015	0.005
	Apoatropine	0.002	0.005	0.001
	Cocaine	0.012	0.012	0.013
	Hyoscine	0.023	0.022	0.012
Pyridine alkaloids	Nicotine	0.765	0.287	0.088

	Ricinine	0.005	0.005	0.003
	Nornicotine	0.012	0.012	0.010
	Peletrevine	0.216	0.211	0.155
	Pyridine	0.048	0.048	0.035
Piperidine alkaloids	Coniine	0.032	0.029	0.016
	Piperine	0.022	0.022	0.016
	Lobeline	0.105	0.059	0.060
Acridine alkaloids	Acridine	0.008	0.009	0.011
Imidazole alkaloids	Pilocarpine	0.001	0.003	0.001
β-Phenylethylamine alkaloids	Ephedrine	0.001	0.003	0.001
	Norpseudoephedrine	0.009	0.009	0.009
	Phenylethylamine	0.020	0.023	0.021
	Total Alkaloids	17.848	9.077	12.445

Conclusion

There was variation in the anti-nutrient contents of flute pumpkin plant grown in three different solid media. The growth medium that had the concentrations of tannin, saponin, phytate and total flavonoids was sawdust while oxalate and trypsin inhibitor were high in humus soil medium. Total alkaloids and hydrogen cyanide content of *T. occidentalis* increased in white-sand as growth medium. The study revealed that the bioactive content of *T. occidentalis* changes depending on the growth medium. This suggest that plants anti-nutrients can be reduced or increased by altering growth medium, thereby, harnessing the desired anti-nutrient concentration. Further studies on other solid growth medium should be tested with a view of getting better yield and increased bioactive compounds.

Reference

1. Croteau R, Kutchan TM, Lewis, NG. *Natural products (secondary metabolites)*. Biochemistry and molecular biology of plants, 2000: 1250-1318.
2. Cuin TA, Shabala S. Amino acids regulate salinity-induced potassium efflux in barley root epidermis. *Planta*, 2007, 225: 753-761.
3. Wang W, Qiao S, Li D. Amino acids and gut function. *Amino Acids*, 2009, 37: 105-110.
4. Rhoads MJ, Wu G. Glutamine, arginine, and leucine signaling in the intestine. *Amino Acids*, 2009, 37: 111-122.
5. Ogunlesi M, Okiei W, Azeez L,Obakashi V, Osunsanmi M, Nkenchor G.Vitamin C contents of tropical vegetables and foods determined by voltametric and titrimetric methods and their relevance to the medicinal uses of the plants. *International Journal of Electrochemical Science*, 2010, 5: 105-115.
6. Hotta H, Nagano S, Ueda M, Tsujino Y, Koyama J,Osakai T. Higher radical scavenging activities of polyphenolic antioxidants can ascribe to chemical reactions following their oxidation. *Biochem Biophys Acta*, 2002, 1572: 123-132.
7. Ito N, Fukushima S, Hasegawa A, Shibata M,Ogiso T. Carcinogenicity of butylated hydroxy anisole in F344 rats. *J Natl Cancer Inst*., 1983, 70: 343-347.
8. Namiki M. Antioxidants/antimutagens in food. *Food Science and Nutrition*, 1990, 29: 273-300.
9. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Mortality inrandomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. *J Amr Med Ass (JAMA)*, 2007, 297(8): 842-857.

10. Hue S, Boyce AN, Somasundram C. Antioxidant activity, phenolic and flavonoid contents in the leaves of different varieties of sweet potato (*Ipomoea batatas*). *Australian Journal of Crop Science,* 2012, 6(3): 375-380.
11. Kacharava N, Chanishvili S, Badridze G, Chkhubianishvili E, Janukashvili N. Effect of seed irradiation on the content of antioxidants in leaves of Kidney bean, Cabbage and Beet cultivars. *Australian Journal of Crop Science*, 2009, 3(3): 137-145.
12. Nantitanon W, Yotsawimonwat S, Okonogi S. Factor influencing antioxidant activities and total phenolic content of guava leaf extract. *LWT-Food Science Technology*, 2010, 43: 1095-1103.
13. Oberleas D.*Phytates. In: Toxicants occurring naturally in foods* (Ed: Strong, F.). National Academy of Science, Washington D.C., 1973: 363-371.
14. Pearson DD. *Chemical analysis of foods, 7th ed.* Churchill, Livingstone, London, 1976.
15. Arnfield SD, Ismond MAH,Murray ED. The fate of anti-nutritional factors during the preparation of faba bean protein isolate using micellizationtechnique. *Can. Inst. Food Sci. technol. J.*, 1985, 18: 137-143.
16. Nicolau AI. Safety of fermented cassava products. In: Regulating safety of traditional and ethnic foods. Eds. V. Prakash, Olga Martin-Belloso, Larry Keener, Sian Bethan Astley, Susanne Braun, Helena McMahon and HuubLelieveld. Academic press, 2016: 319-335.
17. FAO/WHO. Food standards programme. Codex AlimentariusCommissio, XII, Suppl. 4, FAO, Rome, Italy, 1991.
18. CCDN (Cassava Cyanide Disease Network) News. Processing of cassava to remove cyanide content, 2004, 3: 3-4.
19. Chibueze U, Akubugwo EI. Nutritive values and phytochemical contents of some leafy vegetables grown with different fertilizers. *Agriculture and Biology Journal of North America,* 2011, 2(12): 1437-1444.
20. Kuku A, Etti UJ, Ibironke IS. Processing of fluted pumpkin seeds, *Telfairia occidentalis* (Hook F) as ytaffects growth performance and nutrient metabolism in rats. *African Journal of Food, Agriculture, Nutrition and Development*, 2014, 14(5): 1992-2014.
21. Jack IR, Nna PJ. Comparative studies of the phytochemical analysis of the methanolic extract of two Nigerian leaves – *Telfairia occidentalis* and *Gongronema latifolium. European Journal of Biomedical and Pharmaceutical Sciences*, 2015, 2(5): 38-45.
22. Aletor VA, Adeogun OA. Nutrient and anti-nutrient components of some tropical leafy vegetables. *Food Chemistry*, 1995, 53: 375-379.
23. Otitoju GTO, Nwamarah JU,Otitoju O, Odoh EC, Iyeghe LU. Phytochemical composition of some underutilized green leafy vegetables in Nsukkaurban LGA of Enugu State. *Journal of Biodiversity and Environmental Sciences*, 2014, 4(4): 208-217.
24. Ejoh RA, Nkonga DV, Innocent G, Moses MC. Nutritional components of some non-conventional leafy vegetables consumed in Cameroon. *Pakistan Journal of Nutrition*, 2007, 6: 712–717.
25. Okunade OA, Adesina K. Preliminary study on the nutritional, anti-nutritional and elemental composition of Bishops vegetable (*Jatropha tanjorensis*) and cashew shoot (*Anarcadium occidentale*) leaves. *International Journal of Advanced Research in Chemical Science*, 2014, 1(7): 43-46.
26. Inuwa HM, Aina VO, Aimola BGI, Amao T. Comparative Determination of Antinutrient Factors in Groundnut Oil, Palm Oil. *Advanced Journal of Food Science and Technology*, 2011, 3(4): 275-279.
27. Akwaowo EU, Ndon BA,Etuk EU. Minerals and Anti- Nutrients of Fluted Pumpkin (*Telfairia occidentalis*), *Food Chemistry*, 2002, 70(2): 235-240.
28. Enechi OC, Odonwodu I. An assessment of the phytochemical and nutrient composition of the pulverized root of *Cissus quadrangularis*, Bioresearch, 2003, 1: 63-68.
29. Price KR, Johnson IT, Fenwick GR.The chemistry and biological significance of saponins in foods and feed stuffs. *CRC Critical Reviews Food Sci. Nut*., 1987, 26: 27-35.
30. Basu SK, Thomas JE, Acharya SN. Prospects for Growth in Global Nutraceutical and Functional Food Markets: A Canadian Perspective. *Aust J Basic ApplSci*, 2007, 1(4): 637-649.

31. Ekpenyong CE, Akpan EE, Udoh NS. Phytochemistry and toxicity studies of *Telfairia occidentalis* aquoues leaves extract on liver biochemical indices in WistarRats. *American Journal of Medicine and Medical Sciences,* 2012, 2(5): 103-110.
32. Salau BA, Odufuwa KT, Adeosun CB, Atunnise AK. Blanching and juicing effect on flavonoids contents in commonly consumed leafy vegetables in south west Nigeria. *International Journal of Biochemistry Research and Review,* 2015, 5(3): 207-203.
33. Iweala EEJ, Obidoa O. Some biochemical, haematological and histological responses to a long term consumption of *Telfairia occidentalis* supplemented diet in rats. *Pak. J. Nut*., 2009, 8: 1199-1203.
34. Irina I, Mohamed G. Biological activities and effects of food processing on flavonoids as phenolic antioxidants. Advances in Applied Biotechnology, Prof. Marian Petre (Ed.), In Tech., 2012, 5:101– 125.
35. Ren W, Oiao Z, Wang H, Zhu L, Zhang L. Flavonoids: Promising anticancer agents. *Med. Res. Rev.*, 2003, 23: 519-534.
36. Aggarwal BB,Shishodia S. Molecular targets of dietary agents for prevention and therapy of cancer. *Biochem. Pharmacol.*, 2006, 71: 1397-1421.
37. Torres-Piedra M, Ortiz-Andrade R, Villalobos-Molina R, Singh N, Medina-Franco JL, Webster SP. A comparative study of flavonoid analogues on streptozotocin-nicotinamide induced diabetic rats: quercetinas a potential antidiabetic agent acting via 11β-hydroxysteroid dehydrogenase type 1 inhibition. *Eur J Med Chem.*, 2010, 45: 2606-2612.
38. Grace PA. Ischaemia-reperfusion injury. *British Journal of Surgery*, 1994, 81: 637– 647.
39. Treutter D. Significance of flavonoids in plant resistance and enhancement of their biosynthesis. *Plant Biology*, 2005, 7(6):581–591.
40. Enujiugha VN, Oluwole TF, Talabi JY,Okunlola AI. Selected bioactive components in fluted pumpkin (*Telfairia occidentalis*) and Amaranth (*Amaranthus caudatus*) leaves. *American Journal of Experimental Agriculture*, 2014, 4(9): 996-1006.
41. Odufuwa KT, Daramola GG, Adeniji PO, Salau BA. Changes in alkaloids content of some selected Nigerian vegetables during processing. *IOSR Journal of Dental and Medical Sciences*, 2013, 6(1): 51-54.