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Anti-nutrient composition of fluted pumpkin leaf grown in different NPK solutions

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ABSTRACT: The study evaluated the anti-nutrient composition of fluted pumpkin, *Telfairia occidentalis* Hooker fil. leaf grown in different NPK 20-10-10 hydroponic solutions. The solutions varied in the amount of NPK (20-10-10) granules (25, 50, 75, 100g, 125 and 150 g) dissolved in water containing micronutrients. The growth media were designated as: M^{25}NPK, M^{50}NPK, M^{75}NPK, M^{100}NPK, M^{125}NPK, M^{150}NPK, and Control. Two-week old seedlings of fluted pumpkin raised using River-sand were transferred into the growth media in four replicates. The hydrogen cyanide, oxalate, phytate, tannin, saponin, trypsin-inhibitor, alkaloid, and flavonoids were determined 5 weeks after planting following standard procedures. Waters 616/626 HPLC was used to determine alkaloid and flavonoid contents. The result showed that the proportion of phytochemicals in the leaves among the growth media range thus: phytate (3.445 – 15.837%), tannin (0.729 – 1.974%), oxalate (3.279 – 8.882%), trypsin-inhibitor (1.018 – 2.756%), saponin (6.075 – 7.558%) and hydrogen cyanide (0.014 – 0.020 ppm). Higher values of phytochemical in the leaves were recorded at M^{100}NPK growth media (for tannin, oxalate, saponin and trypsin-inhibitor). The most abundant group of alkaloids present in the leaves was pyridine with percentage occurrence with respect to the total alkaloids across the growth media as thus: 35.72% (Control), 32.36% (M^{25}NPK), 16.80% (M^{50}NPK), 60.83% (M^{75}NPK), 34.68% (M^{100}NPK), 38.69% (M^{125}NPK), and 61.28% (M^{150}NPK). The most abundant flavonoid was luteolin (9.153 g/100g) followed by eriodictyol (7.573 g/100g) at M^{50}NPK medium, while the lowest value (0.004 g/100g) was epicatechin and anthocyanine at M^{150}NPK and M^{125}NPK media, in that order. The study revealed that appropriate proportion of NPK 20-10-10 in solution could be used in growing fluted pumpkin.

Keywords: Anti-nutrient, fluted pumpkin, hydroponic solutions.

INTRODUCTION

Phytochemicals are defined as bioactive non-nutrient plant compounds in fruits, vegetables, grains and other plant foods (Doughari *et al*., 2009). These phytochemicals are produced through primary or secondary metabolism (Harborne *et al.*, 1999; Molyneux *et al*., 2007). They generally have biological activity in the plant host and play a role in plant growth or defense against competitors, pathogens or predators and have been linked to reducing the risk of major chronic diseases (Molyneux *et al*., 2007). They are not required by human body for sustaining life, but recent research findings indicate that they can also protect human against diseases (Saidu and Okorocha, 2013). It has been reported that green leafy vegetables contain anti-nutrients such as oxalate and phytic acid, which reduce the bio-availability of some essential minerals (Grosvenor and Smolin, 2002; Akindahunsi and Salawu, 2005). Health promoting phytochemicals such as alkaloids, flavonoids, saponin and tannins that aid in the reduction of the risk of cancer and other degenerative diseases are contained in green leafy vegetables (Levander, 1990; Okwu, 2005). Andy *et al*. (2008) also reported that from time immemorial plant has been exploited by traditional herbalists for the treatment of various ailments including typhoid fever, diarrhea and candidasis amongst other ailments. Medicinal plants are sources of raw materials for pharmaceutical drug

formulation (WHO, 2013). A significant number of persons in developing countries like Nigeria depend on herbal drugs to meet their health needs (Uzoekwe and Mohammed, 2015).

The presence of these wide range of bioactive phytochemicals and secondary metabolites have made plants, especially vegetables, a promising source of modern synthetic drugs for management of several diseases (Balogun *et al*., 2016). For instance, it is now evident that flavonoids are a group of multifunctional molecules important in a variety of plant physiological responses as they are responsible for the increase in the overall plant productivity such as sunscreen (Dixon and Paiva,1995; Shirley,1996), detoxification of active oxygen (Takahama, 1992; Yamasaki *et al*.,1996; Yamasaki *et al*.,1997; Yamasaki, 1997), pathogen defense (Rivera-Vargas *et al*.,1993), modulation of root development (Jacobs and Rubery,1988), pollen development (Ylstra *et al*.,1992; Dumontbeboux and Vonaderkas, 1997), regulation of nodulation (Maxwell *et al*.,1991; Djordjevic and Weinman, 1991), and attraction of microsymbionts (Dharmatilake and Bauer, 1992; Pandya *et al*.,1999).

This study evaluated the anti-nutrient composition of fluted pumpkin, *Telfairia occidentalis* Hooker fil. leaf grown in different NPK 20-10-10 hydroponic solutions.

MATERIALS AND METHODS

Source of materials and planting

The seeds of fluted pumpkin were obtained from a farm in Choba, Port Harcourt, and authenticated by a Taxonomist in the University of Port Harcourt Herbarium. The seeds were planted in white sand from the Choba River, Port Harcourt, as a medium for germination. The two-week-old seedlings were transferred into a non-circulating hydroponic nutrient system.

Formulation of hydroponic solutions

The method of Kratky (2002) was used with modification in nutrient formulation and container used. NPK 20-10-10 granular fertilizers were weighed (25, 50, 75, 100, 125 and 150g) and transferred into black plastic bowls with the dimensions of 29 cm width, 41 cm length, and 23 cm depth. The same was dissolved with 20 litres of tap water in the plastic bowls leaving space for aeration with the addition of 20 ml micronutrients stock solution (0.6 g H_3BO_3; 0.4 g $MnCl_2.4H_2O$; 0.05 g $ZnSO_4$; 0.5 g $CuSO_4.5H_2O$; 0.02 g $Na_2MoO_4.2H_2O$) and Epsom salt (9.8 g MgSO4). The Control medium (water) was setup without the addition of NPK, micronutrients and Epsom salt. These formulations were replicated four times and designated as Control, M^{25}NPK, M^{50}NPK, M^{75}NPK, M^{100}NPK, M^{125}NPK, and M^{150}NPK.

Analyses of parameters

The plants in the hydroponic media were allowed to stand for a month. The mature leaves were harvested and rinsed with distilled water to remove dirt and prepared differently to be used for respective analyses: hydrogen cyanide, oxalate, phytate, tannin, saponin, trypsin-inhibitor, alkaloid, and flavonoids. The analyses were carried out at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. The hydrogen cyanide, oxalate, phytate, tannin, saponin, trypsin-inhibitor, flavonoid and alkaloid contents of fluted pumpkin leaves were determined following the method used by Okonwu *et al.* (2017a, b) and Okonwu and Muonekwu (2019). Waters 616/626 liquid chromatography was used in determining flavonoids, alkaloids and organic acids content.

Alkaloids (extraction and analysis)

Ten grams (10 g) of plant sample was de-fated, out of which 5 g was weighed into a flask and 100 mL of 12% alcohol added, shaken, filtered and washed with industrial alcohol. The extracted residue was washed into a flask with 50 ml of ammonia water (ultrapure water), heated in boiling water for 20 minutes and allowed to cool. Then, 0.1 g of diastase (+ water) was added and maintained at 50 to 55^{o}C for 2 hours. It was cooled and made up to 250 mL with ultrapure water, swirled and filtered. The filtrate (200 mL) was mixed with 20 mL hydrochloric acid (sp.g. 1.125) and heated in boiling water for 3 hours. Thereafter, it was allowed to cool, neutralised with sodium hydroxide solution and made up to 250 mL. The sample was shaken, 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 40 mL/mins (vii) Temperature: Detector- 170^{o}C; Injector port- 190^{o}C and Column- 125^{o}C (viii) Computer facilities for storing data (ix) Printer for results reporting.

Flavonoids (extraction and analysis)

Plant samples (1.5 g) each were weighed into a set of extraction tubes and 20 ml of boiled ultra-pure water dispensed into each extraction tube. The set-up was allowed to stand for 1.5 hours and voltexed for 5 minutes. The solution was transferred to a set of centrifuge tubes, shaken for 15 minutes and centrifuged for 5 minutes at 3000 rpm. Thereafter, a set of vials was used to collect the

Table 1. Phytochemical composition of *T. occidentalis* leaves grown in different NPK growth media at 5 weeks after planting.

Growth medium	Phytate (%)	Tannin (%)	Oxalate (%)	Saponin (%)	Trypsin-inhibitor (%)	HCN (ppm)
Control	4.344	0.801	3.605	6.333	1.119	0.020
M^{25}NPK	3.445	0.729	3.279	7.063	1.018	0.016
M^{50}NPK	4.543	0.815	3.670	6.075	1.139	0.019
M^{75}NPK	15.837	1.809	8.140	6.119	2.526	0.014
M^{100}NPK	15.435	1.974	8.882	7.558	2.756	0.017
M^{125}NPK	6.970	1.046	4.709	5.267	1.461	0.020
M^{150}NPK	8.788	1.321	5.945	6.264	1.845	0.014
Mean	8.480	1.214	5.461	6.383	1.695	0.017
Std. dev.	5.209	0.506	2.276	0.738	0.706	0.003
%CV	61.43	41.70	41.68	11.57	41.67	15.22

Std. dev. = Standard deviation; CV = Coefficient of variation; HCN = Hydrogen cyanide.

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 60 ml/min (vii) Temperature: Detector- 147°C; Injector port- 166°C and Column: 115°C (viii), Computer facilities for storing data. (ix) Printer for results reporting.

Data analyses

The data collected were subjected to descriptive statistical analyses using Microsoft Excel 2013.

RESULT

Phytochemicals content of fluted pumpkin leaves grown in varying NPK solutions at 5 WAP

Screening of fluted pumpkin leaves grown in different media of varying NPK concentrations revealed varied proportions of phytochemicals (Table 1). The proportion of phytochemicals in the leaves among the growth media range thus: phytate (3.445 – 15.837%), tannin (0.729 – 1.974%), oxalate (3.279 – 8.882%), trypsin-inhibitor (1.018 – 2.756%), saponin (6.075 – 7.558%) and hydrogen cyanide (0.014 – 0.020 ppm). Higher values of phytochemical in the leaves were recorded at M^{100}NPK growth media (for tannin, oxalate, saponin and trypsin-inhibitor), and M^{75}NPK medium (phytate) while most of the lowest values were observed at M^{25}NPK and M^{150}NPK growth media, respectively. The percentage content of phytate, tannin, oxalate and trypsin-inhibitor in the leaves fluctuates with increased concentration of NPK. However, the higher values of phytochemicals content were recorded at M^{75}NPK and M^{100}NPK growth media. The highest HCN (0.020 ppm) content of the leaves was recorded at the Control and M^{125}NPK media while the lowest HCN (0.014 ppm) was recorded at M^{75}NPK and M^{150}NPK growth media, respectively.

Alkaloid content (g/100g) of fluted pumpkin leaves grown in NPK solutions at 5 WAP

The alkaloid content of fluted pumpkin leaves grown in varying NPK concentrations is shown in Table 2. Forty-six alkaloids were detected and quantified in the leaves from twelve groups/classes of alkaloids. The group of alkaloids range thus: purine (0.265 – 0.966 g/100g), colchicine (0.187 – 0.229 g/100g), quinoline (0.270 – 0.744 g/100g), vinca (0.028 – 0.055 g/100g), indole/benzypyrole (0.298 – 0.418 g/100g), isoquinoline (0.405 – 1.166 g/100g), pyridine (0.873 – 7.208 g/100g), imidazole (0.108 – 0.212 g/100g), tropane (0.243 – 0.342 g/100g), piperidine (1.080 – 1.820 g/100g), acridine (0.006 – 0.024 g/100g), and β-phenylethylamine (0.236 – 0.266 g/100g). The most abundant groups of alkaloids present in the leaves was pyridine with percentage occurrence with respect to the total alkaloids across the growth media as thus: 35.72% (Control), 32.36% (M^{25}NPK), 16.80% (M^{50}NPK), 60.83% (M^{75}NPK), 34.68% (M^{100}NPK), 38.69% (M^{125}NPK), and 61.28% (M^{150}NPK). Values of individual alkaloids in the leaves varied in different growth media. Among the alkaloids present in the leaves, the most concentrated was pyridine, which ranged from 0.521 to 7.139 g/100g while cephaline (0.007 – 0.008 g/100g), tubocurarine (0.007 – 0.009 g/100g), and berberine (0.005 – 0.010 g/100g) were the least. The highest total alkaloid (12.181 g/100g) of the leaves was recorded at M^{150}NPK medium and the lowest (5.196 g/100g) at M^{50}NPK medium.

Flavonoid content of fluted pumpkin leaves grown in NPK solutions at 5 WAP

The flavonoid content of fluted pumpkin leaves in varying growth media with different NPK concentrations is presented in Table 3. A total of 39 flavonoids from 6 sub-groups (9 flavones, 3 isoflavones, 12 flavanones, 9 flavan-

Table 2. Alkaloid content of *T. occidentalis* leaves grown in different NPK growth media at 5 WAP.

Group of alkaloids	Alkaloid (g/100g)	Growth medium							Mean	%CV
		Control	M^{25}NPK	M^{50}NPK	M^{75}NPK	M^{100}NPK	M^{125}NPK	M^{150}NPK		
Purine	Caffeine	0.588	0.638	0.559	0.766	0.234	0.524	0.931	0.605	35.66
	Theobromine	0.024	0.022	0.023	0.023	0.021	0.022	0.023	0.023	4.32
	Theophylline	0.010	0.011	0.012	0.012	0.010	0.012	0.012	0.011	8.43
Quinoline	Cinchonine	0.229	0.218	0.166	0.238	0.174	0.168	0.212	0.201	15.23
	Quinidine	0.031	0.033	0.040	0.039	0.041	0.041	0.042	0.038	11.37
	Quinine	0.010	0.011	0.011	0.012	0.012	0.011	0.013	0.011	8.54
	Quinoline	0.128	0.113	0.033	0.429	0.119	0.181	0.446	0.207	78.93
	Cinchonidine	0.021	0.017	0.020	0.026	0.023	0.023	0.021	0.022	13.07
Vinca	Vinblastine	0.023	0.024	0.022	0.023	0.023	0.022	0.023	0.023	3.02
	Vincristine	0.027	0.019	0.033	0.008	0.005	0.009	0.009	0.016	68.81
Indole/benzypyrole	Reserpine	0.010	0.006	0.010	0.010	0.008	0.010	0.010	0.009	17.21
	Strychine	0.018	0.023	0.065	0.067	0.047	0.057	0.042	0.046	42.51
	Eserine	0.216	0.230	0.206	0.199	0.229	0.212	0.198	0.213	6.140
	Ergotamine	0.022	0.039	0.019	0.109	0.012	0.025	0.024	0.036	93.31
	β-Carboline	0.012	0.012	0.013	0.012	0.011	0.012	0.012	0.012	4.81
	Rauwolfia	0.020	0.022	0.019	0.021	0.020	0.019	0.021	0.020	5.49
Isoquinoline	Morphine	0.186	0.206	0.200	0.217	0.210	0.198	0.230	0.207	6.87
	Apomorphine	0.633	0.470	0.038	0.166	0.272	0.851	0.306	0.391	71.84
	Tubocurarine	0.007	0.007	0.007	0.008	0.009	0.008	0.008	0.008	9.80
	Heroin	0.012	0.013	0.013	0.012	0.013	0.010	0.012	0.012	8.80
	Narcotine	0.011	0.008	0.012	0.011	0.012	0.013	0.012	0.011	14.21
	Codeine	0.011	0.010	0.060	0.011	0.011	0.010	0.010	0.018	106.5
	Papaverine	0.046	0.041	0.040	0.042	0.050	0.039	0.026	0.041	18.44
	Emetine	0.011	0.012	0.011	0.011	0.012	0.011	0.011	0.011	4.32
	Berberine	0.008	0.005	0.007	0.010	0.009	0.008	0.008	0.008	20.03
	Psychotrine	0.010	0.010	0.010	0.006	0.010	0.010	0.010	0.009	16.03
	Cephaline	0.007	0.007	0.007	0.008	0.008	0.008	0.008	0.008	7.06
Pyridine	Nicotine	0.059	0.058	0.061	0.059	0.059	0.050	0.051	0.057	7.67
	Ricinine	0.011	0.011	0.013	0.013	0.015	0.069	0.011	0.020	105.10
	Peletrevine	0.050	0.049	0.059	0.059	0.049	0.059	0.049	0.053	9.78
	Pyridine	2.040	1.801	0.521	6.859	1.900	2.900	7.139	3.309	79.09
	Nornicotine	0.230	0.198	0.219	0.218	0.218	0.208	0.215	0.215	4.64

Table 2. Contd.

Group of alkaloids	Alkaloid (g/100g)	Growth medium							Mean	%CV
		Control	$M^{25}NPK$	$M^{50}NPK$	$M^{75}NPK$	$M^{100}NPK$	$M^{125}NPK$	$M^{150}NPK$		
Imidazole	Pilocarpine	0.212	0.116	0.137	0.108	0.136	0.128	0.117		
Tropane	Atropine	0.010	0.008	0.010	0.010	0.010	0.008	0.008	0.009	11.69
	Apoatropine	0.023	0.022	0.020	0.021	0.023	0.023	0.020	0.022	6.36
	Cocaine	0.023	0.076	0.034	0.086	0.086	0.023	0.047	0.054	63.35
	Hyoscine	0.187	0.205	0.213	0.225	0.186	0.218	0.209	0.206	7.21
Piperidine	Conine	0.801	1.001	1.481	0.921	1.381	1.501	0.861	1.135	27.02
	Piperine	0.050	0.063	0.093	0.058	0.086	0.094	0.054	0.071	26.92
	Lobelline	0.217	0.231	0.207	0.200	0.230	0.213	0.199	0.214	6.11
	Piperidine	0.012	0.013	0.011	0.011	0.013	0.012	0.011	0.012	7.59
Acridine	Acridine	0.013	0.006	0.010	0.024	0.016	0.013	0.024	0.015	44.86
Colchicine	Colchicine	0.185	0.205	0.199	0.216	0.209	0.197	0.229	0.206	6.90
β-Phenylethylamine	Norpseudo-ephedrine	0.186	0.204	0.212	0.224	0.185	0.217	0.208	0.205	7.24
	Ephedrine	0.020	0.021	0.019	0.022	0.024	0.023	0.021	0.021	8.02
	Phenylethyl-amine	0.030	0.027	0.021	0.020	0.031	0.023	0.028	0.026	17.06
Total Alkaloids		6.690	6.542	5.196	11.850	6.462	8.493	12.181	8.202	33.88

3-ols, 5 flavonols and anthocyanin) were detected and quantified in the leaves. The percentage range of the flavonoid subgroups with respect to total flavonoid of the growth media was as follows: flavanones (26.15 – 30.33%), flavones (48.76 – 52.82%), isoflavones (1.85 – 3.92%), flavanols (9.85 – 15.72%), flavan-3-ols (4.10 – 5.44%) and anthocyanin (0.010 – 0.034%). The concentration of the individual flavonoids varies within each growth media. Among the growth media, highest total flavonoids (45.352 g/100g) of the leaves was recorded at $M^{75}NPK$ medium while the lowest (21.343 g/100g) was obtained at $M^{150}NPK$ medium. The most abundant flavonoid was luteolin (9.153 g/100g) followed by eriodictyol (7.573 g/100g) at $M^{50}NPK$ medium, respectively while the lowest value (0.004 g/100g) was epicatechin and anthocyanine at $M^{150}NPK$ and $M^{125}NPK$ media, in that order.

DISCUSSION

The phytochemical composition (tannin, oxalate, saponin and trypsin-inhibitor) of fluted pumpkin leaf was predominant in the $M^{100}NPK$ growth media. Secondary metabolism is key to plant adaptation to stress (Croteau *et al.,* 2000). However, some molecules produced during primary metabolism can participate in stress responses and provide plant defense properties.

The phytate content of fluted pumpkin obtained in the leaves was higher than the works of others. According to Idris (2011), fluted pumpkin contains 5.11 mg/g of phytate. Jack and Nna (2015) observed and reported 0.24 µg/g of phytate in fluted pumpkin. It has been reported that phytates occur naturally as mixed K, Mg and Ca salts in complex diets (Zhou and Erdman, 1995). It also delays glucose absorption (Philip and Owen, 2014). The tannin contents obtained in fluted pumpkin grown in different NPK solutions were

Table 3. Flavonoid content of *T. occidentalis* leaves grown in different NPK growth media at 5 WAP.

Sub-groups of flavonoid	Flavonoid (g/100g)	Growth medium							Mean	%CV
		Control	$M^{25}NPK$	$M^{50}NPK$	$M^{75}NPK$	$M^{100}NPK$	$M^{125}NPK$	$M^{150}NPK$		
Flavanones	Hesperidin	0.097	0.057	0.104	0.071	0.054	0.090	0.048	0.074	30.33
	Nanirutin	0.494	0.290	0.527	0.361	0.276	0.455	0.241	0.378	30.34
	Neoeriocitin	0.159	0.093	0.169	0.116	0.089	0.146	0.078	0.121	30.17
	Poncirin	0.234	0.137	0.249	0.171	0.131	0.215	0.114	0.179	30.29
	Didymin	0.806	0.874	0.931	0.283	0.397	0.486	0.205	0.569	52.30
	Eriocitrin	0.420	0.247	0.448	0.307	0.235	0.387	0.205	0.321	30.29
	Naringin	0.299	0.176	0.319	0.219	0.167	0.276	0.146	0.229	30.30
	Naringinenin	0.056	0.033	0.060	0.041	0.032	0.052	0.028	0.043	29.76
	Raxifolin	0.424	0.249	0.453	0.310	0.237	0.391	0.207	0.324	30.36
	Eriodictyol	3.098	4.170	7.573	5.188	3.969	6.541	3.465	4.858	34.26
	Hesperetin	2.432	1.429	2.595	1.778	1.360	2.241	1.187	1.860	30.31
	Taxifolin	0.305	0.179	0.325	0.223	0.170	0.281	0.149	0.233	30.33
Flavones	Rhoifolin	3.262	1.916	3.480	2.384	1.824	3.006	1.592	2.495	30.31
	Diosmin	0.158	0.093	0.169	0.116	0.089	0.146	0.077	0.121	30.27
	Sinensetrin	0.204	0.120	0.217	0.149	0.114	0.188	0.099	0.156	30.33
	Nobiletin	3.424	2.012	3.653	2.502	1.914	3.155	1.671	2.619	30.32
	Acacetin	0.290	0.170	0.309	0.212	0.162	0.267	0.141	0.222	30.38
	Tangeretin	2.466	1.449	2.632	1.803	1.379	2.273	1.204	1.887	30.32
	Apigenin	3.680	2.162	3.926	2.689	2.058	3.391	1.796	2.815	30.31
	Neodiosmin	0.389	0.229	0.415	0.284	0.218	0.359	0.190	0.298	30.26
	Luteolin	2.578	5.040	9.153	6.270	4.797	7.905	4.188	5.704	39.46
Anthocyanin	Anthocyanine	0.005	0.009	0.006	0.005	0.008	0.004	0.007	0.006	28.63
Isoflavones	Daidzein	0.957	0.613	0.825	0.470	0.245	0.940	0.326	0.625	46.52
	Genistein	0.090	0.053	0.096	0.066	0.051	0.083	0.044	0.069	30.05
	Glycitein	0.275	0.162	0.293	0.201	0.154	0.253	0.134	0.210	30.24
Flavanols	Quercetin	0.797	0.507	0.792	0.778	0.662	0.458	0.547	0.649	22.36
	Myricetin	0.471	0.754	0.161	0.866	0.139	0.004	0.598	0.428	77.77
	Kaempferol	0.793	0.042	0.051	0.735	0.509	0.318	0.175	0.375	83.07
	Isorharmnetic	2.366	1.390	2.525	1.730	1.323	2.181	1.155	1.810	30.32
	Rhamnazin	0.878	0.516	0.937	0.642	0.491	0.809	0.429	0.672	30.30

Table 3. Contd.

Sub-groups of flavonoid	Flavonoid (g/100g)	Growth medium							Mean	%CV
		Control	$M^{25}NPK$	$M^{50}NPK$	$M^{75}NPK$	$M^{100}NPK$	$M^{125}NPK$	$M^{150}NPK$		
	Catechin	1.100	0.646	1.174	0.804	0.615	1.014	0.537		
	Epicatechin	0.009	0.005	0.009	0.006	0.005	0.008	0.004	0.007	31.50
	Theaflarins	0.037	0.022	0.040	0.027	0.021	0.034	0.018	0.028	30.32
	Thearubigins	0.025	0.014	0.026	0.018	0.014	0.023	0.012	0.019	30.68
Flavan-3-ols	Epigallocatechin	0.058	0.034	0.062	0.043	0.033	0.054	0.028	0.045	30.32
	Epicatechin gallate	0.027	0.016	0.028	0.019	0.015	0.025	0.013	0.020	30.16
	Epigallocatechin gallate	0.055	0.032	0.058	0.040	0.031	0.050	0.027	0.042	29.89
	Proantho-cyanidins	0.023	0.014	0.025	0.017	0.013	0.022	0.011	0.018	30.75
	Fisetin	0.501	0.294	0.535	0.366	0.280	0.462	0.245	0.383	30.34
Total Flavonoids		33.742	26.248	45.350	32.310	24.281	38.993	21.341	31.75	26.85

comparable with the results of other researchers. Several researchers had reported varying proportions of tannin in fluted pumpkin: 184 µg/100g (Idris, 2011), 0.20 g/100g (Chibueze and Akubugwo, 2011), 0.035 g/100g (Ekpenyong *et al.*, 2012), 0.14 g/100g (Otitoju *et al.*, 2014) and 18.72 µg/g (Jack and Nna, 2015) in that order. The lethal dose of tannin was reported to be 30 mg/kg (Inuwa *et al.,* 2011).

Contrary to the report of Jack and Nna (2015) on the absence of oxalate in fluted pumpkin, several researchers have reported oxalate content of fresh fluted pumpkin leaves at 2.60 g/100g (Akoroda, 1990), 0.04 g/100g (Aletor and Adeogun, 1995), 3.4 µg/100g (Idris, 2011), 0.068 g/100g (Ekpenyong *et al*., 2012) and 0.06 g/100g (Otitoju *et al.*, 2014). These observations were lower compared to the values obtained for the same plant grown in varying concentrations of NPK solutions. The levels of oxalate in the human diet either as vegetables or fruits are important due to the associated health risks. The presence of saponin had been observed in some underutilized plants in Nigeria and reported (Akundu, 1984; Edeoga *et al.*, 2006; Belewu *et al.*, 2009). Saponin content of fluted pumpkin reported by some workers is 0.80% (Aletor and Adeogun, 1995) and 4.00 – 6.23% (Chibueze and Akubugwo, 2011). These values were not consistence with saponin contents of fluted pumpkin grown in hydroponic solutions.

The cyanide content of fluted pumpkin leaves was lower (0.001 – 0.020 ppm) compared to the ranged (6.0 – 6.4 ppm db) reported by Kajihausa *et al.* (2010), who worked on the nutrient contents and thermal degradation of vitamins in organically grown fluted pumpkin leaves. The cyanide contents obtained were below the safe limit of cyanide content (10 ppm DM) reported for garri (IITA, 1990) and established standard for cassava flour (FAO/WHO, 1991). This makes fluted pumpkin a safe vegetable for consumption, even in large amounts. Fluted pumpkin contains a considerable amount of anti-nutrients like phytic acid, tannin, oxalic acids, hydrocyanic acid and saponins (Ladeji *et al*., 1995; Akwaowo *et al*., 2000; Ajibade *et al*., 2006).

The highest total alkaloid was obtained in $M^{150}NPK$ growth media. Pyridine was the most concentrated alkaloid subgroup. The total alkaloid content of fluted pumpkin grown in varying proportion of NPK was higher compared to other works (Onyeka and Nwambekwe, 2007; Odufuwa *et al.*, 2013; Otitoju *et al.*, 2014). The highest total flavonoid was obtained in $M^{125}NPK$ growth media. Flavones were the most concentrated flavonoid subgroup. The results of total flavonoids content recorded in fluted pumpkin grown in varying concentrations of NPK solutions were higher compared to previous reports by other researchers (Chibueze and Akubugwo, 2011; Aminu *et al.*, 2012; Otitoju *et al.*, 2014), who reported flavonoids content of 6.67, 0.84 and 0.07 g/100g, respectively on the same plant. According to Stefova *et al.* (2003), flavonoids play an essential part in the biochemistry and physiology of plants. The flavonoid content of plants is limited by genetic and environmental factors (Irina and Mohamed, 2012).

Conclusion

The percentage content of phytate, tannin,

oxalate and trypsin-inhibitor in the leaves fluctuates with increased concentration of NPK. The concentration of the individual alkaloid and flavonoids varies within each growth media.

Recommendation

The study recommends the use of NPK 20-10-10 in appropriate proportion in growing fluted pumpkin.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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