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Phytochemicals of *Telfairia occidentalis* Leaf Grown in Urea Solutions

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ABSTRACT

Background and Objective: The plant, *Telfairia occidentalis* Hooker fil. contains the varying composition of phytochemicals and has been grown mostly in geoponic media. The study aimed at evaluating the anti-nutrient composition of *T. occidentalis* leaf grown in different urea hydroponic solutions. **Materials and Methods:** The Urea solutions varied in the number of Urea granules (25, 50, 75, 100, 125 and 150 g, respectively) dissolved in water containing micronutrients and designated as $M^{25}U$, $M^{50}U$, $M^{75}U$, $M^{100}U$, $M^{125}U$, $M^{150}U$ and control. The hydrogen cyanide, oxalate, phytate, tannin, saponin, trypsin-inhibitor, alkaloid and flavonoids contents of *T. occidentalis* were determined 5 weeks after planting (WAP) following standard procedures. **Results:** The study showed that the proportion of phytochemicals in *T. occidentalis* ranged thus: Phytate (4.07-16.88%), tannin (0.80-1.96%), oxalate (3.61-8.80%), trypsin-inhibitor (1.12-2.73%), saponin (6.12-8.58%) and hydrogen cyanide (0.014-0.020 ppm). Higher values of phytochemicals in the leaves were recorded at $M^{25}U$ medium (for tannin, oxalate and trypsin-inhibitor), $M^{100}U$ treatment (for phytate) and $M^{125}U$ treatment (for saponin). The group of alkaloids ranged thus: Purine (0.225-0.988 g/100 g), colchicine (0.185-0.220 g/100 g), quinoline (0.313-0.801 g/100 g), tropane (0.217-0.295 g/100 g), vinca (0.025-0.084 g/100 g), indole/benzopyrrole (0.258-0.413 g/100 g), isoquinoline (0.468-1.054 g/100 g), pyridine (1.436-9.262 g/100 g), imidazole (0.099 - 0.212 g/100 g), piperidine (0.919-2.350 g/100 g), acridine (0.009-0.017 g/100 g) and β-phenylethylamine (0.198-0.257 g/100 g). Among the growth media, the highest total flavonoids (45.35 g/100 g) of the leaves were recorded at the $M^{50}U$ medium while the lowest (21.343 g/100 g) was obtained at the $M^{150}U$ medium. The abundant flavonoid was luteolin (7.232 g/100 g) at the $M^{75}U$ medium, followed by eriodictyol (5.746 g/100 g) at the $M^{25}U$ medium. **Conclusion:** The growth media with lower urea content ($M^{25}U$ growth media) had higher tannin, oxalate, saponin, trypsin-inhibitor and *T. occidentalis*.

KEYWORDS

Grown, hydroponic, phytochemicals, urea solutions, *T. occidentalis,* alkaloid content

INTRODUCTION

Hydroponics technology has been explored to make crop production in areas that ordinarily are not suitable for the traditional farming system due to some natural or imposed features like poor soil conditions, lack of fresh water and climatic changes. In modern hydroponic systems, the nutrient solution is typically aerated and the electrical conductivity, temperature, pH and nutrient contents are monitored

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and automatically corrected to optimum levels, whereas, non-circulating hydroponic systems do not require electricity and pump, mechanical ventilation and movement of the nutrient solution making them relatively inexpensive to set up and require little maintenance[1,2]. According to Fallovo *et al*.[3], increased electrical conductivity in the solution reduces the yield of vegetable crops, although, in many cases, it improves their nutritional quality as observed in plants grown in both soil and soilless cultures. The non-circulating system allows an entire crop to be grown with only one stock solution of nutrient medium[2]. The preference of soilless medium to soil medium in several countries' greenhouses is due to soil impurity, uneasiness in pH control, the electrical conductivity of soil, reduced nutrient presence in soil, delayed growth and crop ripening and limited crop yield, amongst others[4]. *Telfairia occidentalis* Hooker fil. is a perennial angiosperm plant with enormous economic significance in Nigeria. It is a dioecious vegetable crop belonging to the family Cucurbitaceae, commonly called fluted pumpkin and is a tropical vine cultivated in West Africa as a leafy vegetable and for its edible seeds[5-8]. *Telfairia occidentalis* is indigenous to West Africa and native to South-East Nigeria[9]. It is a perennial herb, climbing by coiled, often branched tendrils to a height of more than 20 m. Studies have shown that *T. occidentalis* leaf is rich in minerals, antioxidants, vitamins and phytochemicals[10-15]. The seeds contain oil and are used for cooking, also as a potential raw material for local industries, especially, marmalade manufacturing and cookie formulation[16]. Leaves possess free radical scavenging and antioxidant properties[15,17-21]. The study aimed at evaluating the anti-nutrient composition of *T. occidentalis* leaf grown in different Urea hydroponic solutions.

MATERIALS AND METHODS

Study site, climatic condition and duration of study: The study was conducted inside the Abuja campus of the University of Port Harcourt (Latitude N4°54'15'', longitude E6°54'35''). The site was free from any obstructions and was open to sunlight any time of the day. During the period of the experiment, the climatic condition of the University was relatively wet with the daytime temperature that ranges from 24°C in the early morning to 32°C in the middle part of the day. The study was conducted from March through June, 2018.

Source of materials and planting: The seeds of *T. occidentalis* 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 2 weeks old seedlings were transferred into a non-circulating hydroponic nutrient system.

Formulation of hydroponic solutions: The method of Ostrowska and Skrzydlewska[22] was used with modification in nutrient formulation and container used. Urea granular fertilizers were weighed (25, 50, 75, 100, 125 and 150 g, respectively) and transferred into black plastic bowls with the dimensions: 29 cm in width, 41 cm in length and 23 cm in depth. The same was dissolved with 20 L 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 $MnCl_2{\cdot}4H_2O$, 0.05 g $ZnSO_4$, 0.5 g $CuSO_4{\cdot}5H_2O$, 0.02 g $Na_2MoO_4{\cdot}2H_2O$) and Epsom salt (9.8 g $MgSO_4$). The control medium (water) was set up without the addition of NPK, micronutrients and Epsom salt. These formulations were replicated four times and designated as Control, $M^{25}U$, $M^{50}U$, $M^{75}U$, $M^{100}U$, $M^{125}U$ and $M^{150}U$.

Analysis 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 analysis: 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 other researchers[23-25]. Waters 616/626 liquid chromatography was the tool used in determining flavonoids, alkaloids and organic acids content.

Alkaloids (extraction and analysis): Ten grams 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 50-55°C for 2 hrs. It was cooled and made up to 250 mL with ultrapure water, swirled and filtered. The mixture of the filtrate (200 mL) and hydrochloric acid (20 mL) was heated again for 3 hrs in boiling water. The mixture was cooled and neutralized with an alkaline solution, sodium hydroxide and made up to 250 mL capacity of the flask. The mixture was centrifuged and the supernatant decanted for alkaloid determination. The setup for alkaloid analysis using water 616/626 HPLC were:

- An autosampler
- An automated gradient controller
- Gradient elution HPLC pump
- Reverse-phase HPLC column, thermostatically heated in a temperature-controlled room
- Detector by fluorescence
- Carrier gas: Nitrogen gas at flow rate of 40 mL min^{-1}
- Temperature: Detector -170°C, Injector port -190°C and Column -125°C
- Computer facilities for storing data
- 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 was dispensed into each extraction tube. The set-up was allowed to stand for 1.5 hrs and voltexed for 5 min. The solution was transferred to a set of centrifuge tubes, shaken for 15 min and centrifuged for 5 min at 3000 rpm. 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:

- An autosampler
- An automated gradient controller
- Gradient elution HPLC pump
- Reverse-phase HPLC column, thermostatically heated in a temperature-controlled room
- Detector by fluorescence
- Carrier gas: Nitrogen gas at flow rate of 60 mL min^{-1}
- Temperature: Detector -147°C, Injector port- 166°C and Column: 115°C
- Computer facilities for storing data
- Printer for results reporting

RESULTS

Phytochemicals content of *T. occidentalis* leaves grown in Urea solutions at 5 WAP: The phytochemicals content of *T. occidentalis* leaves grown in different media of varying Urea concentrations are presented in Table 1. The proportion of phytochemicals in *T. occidentalis* leaves ranged thus: Phytate (4.070-16.877%), tannin (0.801-1.956%), oxalate (3.605-8.803%), trypsin-inhibitor (1.119-2.732%), saponin (6.116-8.575%) and hydrogen cyanide (0.014-0.020 ppm). Higher values of phytochemicals in the leaves were recorded at $M^{25}U$ medium (for tannin, oxalate and trypsin-inhibitor), $M^{100}U$ treatment (for phytate) and $M^{125}U$ treatment (for saponin). The values recorded for the phytochemicals fluctuates across growth media. The highest HCN (0.020 ppm) was recorded at the control medium while the lowest HCN (0.014 ppm) was recorded at $M^{75}U$ and $M^{150}U$ media, respectively.

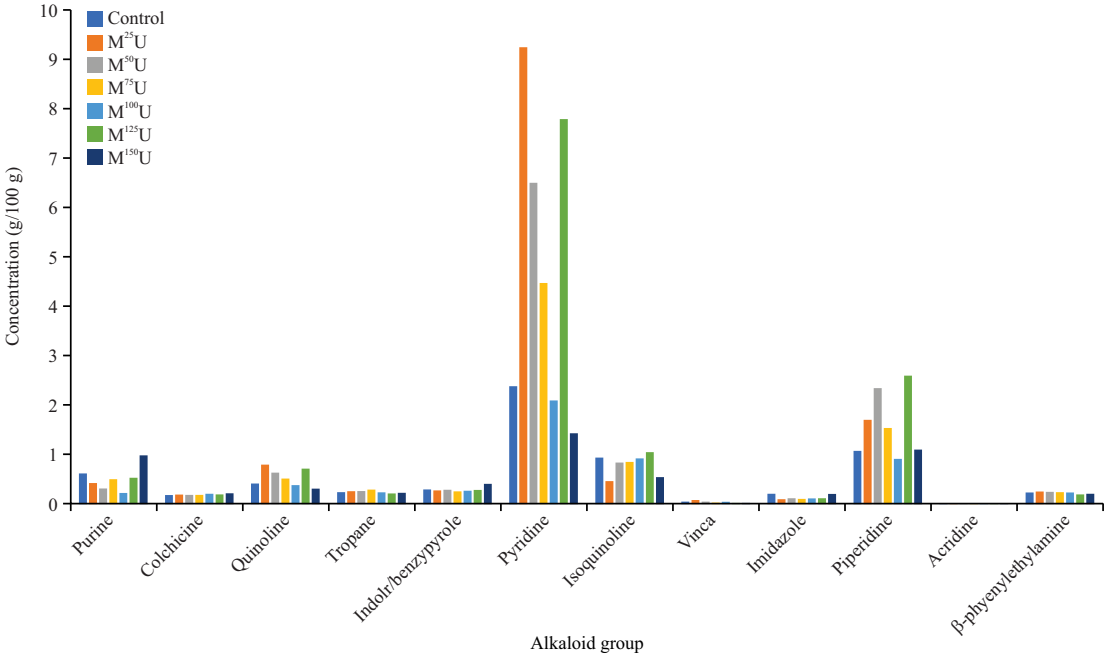

Fig. 1: Alkaloid content of *T. occidentalis* leaves in different urea growth media at 5 WAP

Table 1: Phytochemical composition of *T. occidentalis* leaves grown in different urea growth media at 5 WAP

Growth medium	Phytate (%)	Tannin (%)	Oxalate (%)	Saponin (%)	Trypsin-inhibitor (%)	HCN (ppm)
Control	4.344	0.801	3.605	6.333	1.119	0.020
$M^{25}U$	6.146	1.956	8.803	8.277	2.732	0.019
$M^{50}U$	8.217	1.380	6.211	7.169	1.928	0.019
$M^{75}U$	4.070	1.855	8.349	6.116	2.591	0.014
$M^{100}U$	16.877	1.665	7.490	7.520	2.325	0.017
$M^{125}U$	8.65	0.899	4.045	8.575	1.255	0.019
$M^{150}U$	9.321	1.535	6.906	6.373	2.143	0.014
Mean	8.232	1.442	6.487	7.195	2.013	0.017
Std. dev.	4.338	0.448	2.015	0.980	0.625	0.003
CV (%)	52.69	31.06	31.06	13.62	31.06	14.39

Std. dev.: Standard deviation, CV: Coefficient of variation and WAP: Weeks after planting

Alkaloid content of *T. occidentalis* leaves grown in urea solutions at 5 WAP: The alkaloid content of *T. occidentalis* leaves grown in varying urea concentrations is shown in Fig. 1. Forty-six alkaloids were detected and quantified in the leaves from twelve groups of alkaloids: Purine (caffeine, theobromine, theophylline), colchicine, quinoline (cinchonine, quinine, quinidine, quinolone, cinchonidine), tropane (atropine, apoatropine, cocaine, hyoscine), indole/benzylpyrrole (strychnine, eserine, reserpine, rauwolfia, ergotamine, β-carboline), pyridine (nicotine, ricinine, peletrevine, pyridine, nornicotine), isoquinoline (morphine, apomorphine, narcotine, codeine, papaverine, tubocurarine, heroin, emetine, berberine, psychotrine, cephaline), vinca (vinblastine, vincristine), imidazole (pilocarpine), piperidine (coniine, piperine, piperidine, lobeline), acridine, β-phenylethylamine (ephedrine, norpseudo-ephedrine, phenylethylamine). The highest and lowest purine concentration were recorded at $M^{150}U$ and $M^{100}U$ treatments, respectively. Colchicine concentrations were 0.220 g/100 g and 0.185 g/100 g for treatments $M^{150}U$ and control, in that order. For quinoline and vinca, $M^{25}U$ and $M^{150}U$ treatments had the highest concentration of 0.801 and 0.084 g/100 g with the least concentration of 0.313 and 0.025 g/100 g, respectively, while $M^{75}U$ and $M^{125}U$ treatments had 0.295 g/100 g and 0.217 g/100 g concentrations for tropane. Indole/benzylpyrrole content was high at 0.413 g/100 g in $M^{150}U$ treatment and low at 0.258 g/100 g in $M^{75}U$ treatment. The pyridine contents of the leaf were 9.262 g/100 g and 2.103 g/100 g for $M^{25}U$ and $M^{100}U$ treatments, respectively, while isoquinoline contents were 1.054 and 0.468 g/100 g for $M^{125}U$ and $M^{25}U$ treatments. The imidazole contents were 0.212 and 0.099 g/100 g for Control and $M^{25}U$ treatments. The most abundant group of

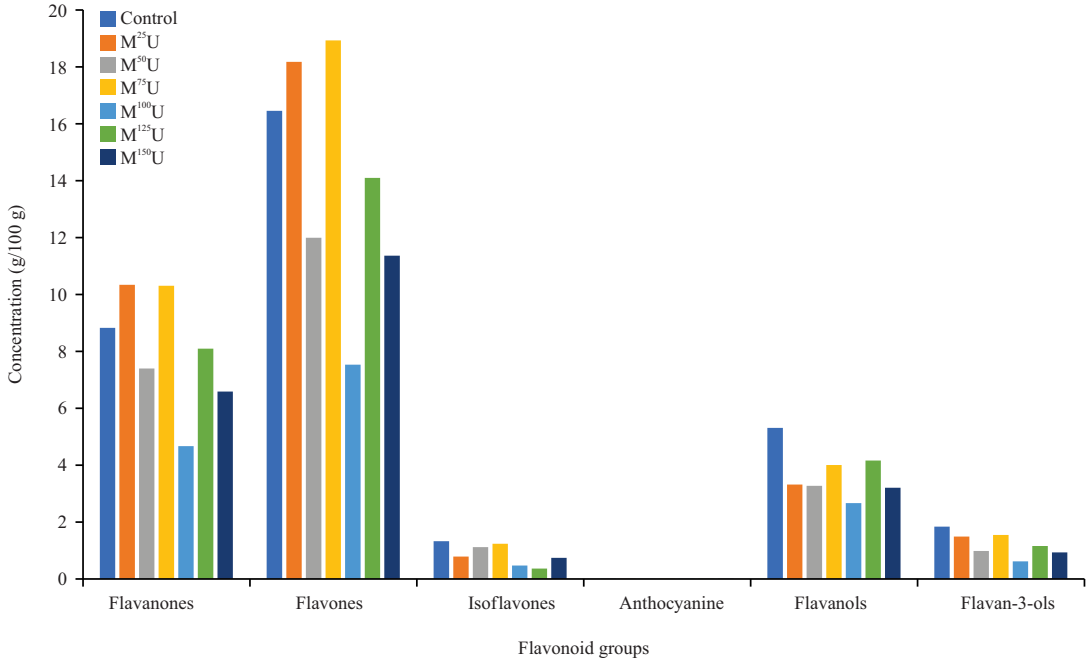

Fig. 2: Flavonoid content of *T. occidentalis* leaves grown in different urea growth media at 5 WAP

alkaloids present in the leaves was pyridine with percentage occurrence concerning the total alkaloids across the growth media as follows: 35.72% (Control), 66.86% ($M^{25}U$), 55.02% ($M^{50}U$), 49.57% ($M^{75}U$), 36.92% ($M^{100}U$), 56.63% ($M^{125}U$) and 25.14% ($M^{150}U$). Values of individual alkaloids in the leaves varied in different growth media. Among the alkaloids, the most concentrated was pyridine which ranged from 1.101-8.959 g/100 g while cephaline and berberine (0.007-0.008 g/100 g) were the least. The value recorded for tubocurarine was 0.007 g/100 g across the growth media. The highest total alkaloid (13.853 g/100 g) of the leaves was obtained at $M^{25}U$ growth media and the lowest (5.696 g/100 g) at $M^{100}U$ growth media.

Flavonoid content of *T. occidentalis* leaves grown in Urea solutions at 5 WAP: The flavonoid content of *T. occidentalis* leaves in varying growth media with different urea concentrations is presented in Fig. 2. A total of 39 flavonoids from 6 sub-groups (9 flavones, 3 isoflavones, 12 flavanones, 9 flavan-3-ols, 5 flavonols and anthocyanin) were detected and quantified in the leaves. These flavonoids sub-groups were: flavanones (hesperidin, nanirutin, neoriocitin, poncirin, didymin, eriocitrin, taxifolin, naringin, naringenin, eriodictyol, hesperetin, taxifolin), flavones (rhoifolin, diosmin, sinensetin, nobiletin, acacetin, tangeretin, neodiosmin, luteolin, apigenin), isoflavones (daidzein, genistein, glycitein), anthocyanin (anthocyanine), flavanols (quercetin, myricetin, kaempferol, isorhamnetin, rhamnazin) and flavan-3-ols (catechin, epicatechin, theaflavins, thearubigins, epigallocatechin, epicatechin gallate, epigallocatechin gallate, proanthocyanidins, fisetin). The highest flavanones and flavones contents of 10.334 g/100 g and 18.173 g/100 g were recorded in $M^{25}U$ treatment while the least content -4.666 and 7.528 g/100 g were obtained in $M^{100}U$ treatment, respectively. For isoflavones, the Control treatment had a high content of 1.322 g/100 g while the $M^{125}U$ treatment had 0.363 g/100 g. Again, the Control treatment had the highest flavanols (5.305 g/100 g) and flavan-3-ols (1.835 g/100 g) contents of *T. occidentalis* leaves while the $M^{100}U$ treatment had the least content -2.662 and 0.615 g/100 g, in that order. For anthocyanin, the $M^{150}U$ treatment gave the highest anthocyanin content (0.008 g/100 g) while the least content (0.003 g/100 g) was recorded at the $M^{75}U$ treatment. The percentage range of the flavonoid subgroups concerning total flavonoids of the growth media was as follows: Flavanones (26.15-30.31%), flavones (47.22-53.29%), isoflavones (1.30-4.50%), flavanols (9.73-16.70%), flavan-3-ols (3.95-5.44%) and anthocyanin (0.008-0.0376%). The concentration of the individual flavonoids varied within each medium. Among the

growth media, the highest total flavonoids (45.35 g/100 g) of the leaves were recorded at the $M^{50}U$ medium while the lowest (21.343 g/100 g) was obtained at the $M^{150}U$ medium. The most abundant flavonoid was luteolin (7.232 g/100 g) in the $M^{75}U$ medium, followed by eriodictyol (5.746 g/100 g) in the $M^{25}U$ medium while epicatechin and anthocyanine had the lowest value (0.003 g/100 g) at both $M^{75}U$ and $M^{100}U$ growth media.

DISCUSSION

The phytochemical composition (tannin, oxalate, saponin and trypsin-inhibitor) of *T. occidentalis* was predominant in the $M^{25}U$ growth media. Natural food sources are the greatest sources of phytochemicals[26,27]. According to Zhang *et al.*[28], some phytochemicals can act as an antioxidant. The phytate content of *T. occidentalis* obtained in the leaves was higher than the works of others. Verla *et al.*[29] observed and reported 12.20±2.10 mg/100 g of phytate in fluted pumpkin. For best health, phytate should be lowered as much as possible, ideally to 25 mg and less per 100 g or about 0.03% of the phytate containing food eaten. Inuwa *et al.*[30] stated that the lethal dose of phytate is 50-60 mg kg^{-1}. It has been reported that leaves containing tannins can be used for the treatment of intestinal disorder[31]. Basu *et al.*[32] stated that the presence of tannin reduces plasma fat. The tannin contents obtained in *T. occidentalis* grown in different Urea solutions were compared with the results of other researchers. Otitoju *et al.*[33] report tannin content of 0.14 g/100 g. The oxalate contents in urea hydroponic solutions were lower compared to the value reported by Ekpenyong *et al.*[34]. Oxalates are anti-nutritive and can form non-absorbable insoluble salts with Ca^{2+}, Fe^{2+} and Mg^{2+} rendering these minerals unavailable[35]. However, a diet with high oxalate content is prone to increase kidney stone formation and may lead to the reduction of Ca absorption[35]. In the body, oxalic acid combines with divalent metallic cations (such as Ca^{2+} and Fe^{2+}) to form crystals of the corresponding oxalates which can form larger kidney stones that can obstruct the kidney tubules. It is estimated that 80% of kidney stones are formed from calcium oxalates[36]. The toxic dose of oxalate was reported to be 2.5 g kg^{-1} [30]. This presupposes close monitoring of the dietary intake of oxalate because of its health implications.

Chibueze and Akubugwo[37] reported saponin range of 4.00-6.23% of *T. occidentalis*. These values were not consistence with saponin contents of *T. occidentalis* grown in hydroponic solutions. The high saponin content is a potential health risk as they are potent human poison but Soetan and Oyewole[38] reported that saponin binds cholesterol making it unavailable for absorption and when in excess causes hypocholestrolaemia. However, research has shown that proper cooking before consumption significantly reduces the levels of these anti-nutrients in leaves or vegetables[39]. The cyanide content of *T. occidentalis* leaves was lower (0.001-0.020 ppm) compared to the work of Njoku *et al.*[40], who reported 17.69 and 38.98 mg/100 g of boiled and fresh *T. occidentalis,* respectively. Nicolau[41] observed a decline in cyanide content of cassava when passed through any form of processing especially heating. This suggests a variation in the cyanide content of fresh and cooked fluted pumpkin leaves. However, cyanide toxicity affects human beings having deficient iodine content[41]. The low content of cyanide permits high consumption of *T. occidentalis* without posing any health risk. It was also reported by Kuku *et al.*[42] that trypsin inhibitor content for unprocessed and under-processed seeds of fluted pumpkin were 23.18 and 2.13 TIU mg^{-1}. The trypsin inhibitors obtained in this study were lower than that reported by Kuku *et al.*[42] on the *T. occidentalis* seeds.

The highest total alkaloid was obtained in $M^{25}U$ growth media. Alkaloids content of vegetable grown in different growth media varied. According to Enujiugha *et al.*[43], aqueous and ethanol extracts of pumpkin contained 0.35 and 0.45 mg g^{-1} of alkaloids, respectively. The bitter taste is mostly associated with high alkaloid content according to Onyeka and Nwambekwe[44]. In higher concentration, alkaloids could be toxic especially when it exceeds the lethal dose of 20 mg/100 g[30]. The highest total flavonoid was obtained in $M^{25}U$ growth media, respectively. Flavones were the most concentrated flavonoid subgroup. The results

of total flavonoid content recorded in *T. occidentalis* grown in varying concentrations of Urea solutions were higher compared to previous reports by other researchers[33,37,45]. Flavonoids from natural sources and their derivatives have been crucial bioactive molecules used in medicine[46] and have health benefits[47]. They have the protective capacity against biological impurities from microbes[48,49]. It has been reported that flavonoids obtained from food materials have the potential to inhibit tumour formation[50,51]. Flavonoids have been reported to play similar roles as vitamins in the human system[51].

CONCLUSION

The growth media with lower urea content ($M^{25}U$ growth media) had higher tannin, oxalate, saponin, trypsin-inhibitor and alkaloid content of *T. occidentalis*. However, the most abundant flavonoid was luteolin in the $M^{75}U$ medium, followed by eriodictyol in the $M^{25}U$ medium while epicatechin and anthocyanine had the lowest value in both $M^{75}U$ and $M^{100}U$ growth media. The study showed a varied trend in the phytochemical composition of *T. occidentalis*.

SIGNIFICANCE STATEMENT

The study showed that the abundant alkaloids and flavonoids are pyridine and luteolin, respectively. The growth media with lower urea content ($M^{25}U$ growth media) had higher tannin, oxalate, saponin, trypsin-inhibitor and alkaloid content of *T. occidentalis*.

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