NJB, Volume 32(1), June, 2019

PROFILE OF ORGANIC ACIDS, GLYCOSIDES AND PHENOLIC COMPOUNDS IN THE LEAVES OF *TELFAIRIA OCCIDENTALIS* CULTIVATED IN HYDROPONIC AND GEOPONIC MEDIA USING WATER 616/626 HPLC AS A TOOL

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Received 27th February, 2019; Accepted 17th March, 2019

ABSTRACT

The Water 616/626 HPLC was used as a tool for identifying and quantifying the organic acids, glycosides and phenolic compounds of *Telfairia occidentalis* pumpkin leaves grown in hydroponic and geoponic media. Standard procedures were adopted for the determination of these secondary metabolites. The study showed that the organic acids, glycosides and phenolic compounds of *T. occidentalis* leaves grown in hydroponic and geoponic media varied in composition and concentration. Also, the total organic acids, total glycosides and phenolic compounds were more in the *T. occidentalis* leaves grown in hydroponic condition compared to geoponic medium. The *T. occidentalis* leaves had total organic acids (6.880 g/100g; 6.508 g/100g), total glycosides (6.762 g/100g; 4.549 g/100g) and phenolic compounds (2.032 g/100g; 1.387 g/100g) for hydroponic and geoponic media, respectively. However, individual components of organic acids, glycosides and phenolics revealed variation in concentration between the media. Shikimic acid was the predominant organic acid among the organic acids assessed in both hydroponic (28.76%) and geoponic (44.73%) media while E-strophanthin acid (21.37%; 23.92%) was the most abundant glycoside out of the total glycosides in that order. Phenolic profiles of *T. occidentalis* leaves showed 45 phenolic compounds, which had some important phenolics such as ferulic, cinnamic acid and *p*-coumaric acid. To effectively harness these secondary metabolites, the study recommends the use of hydroponic system for cultivation of *T. occidentalis*.

Key words: Glycosides, HPLC, organic acids, phenolic compounds, *Telfairia occidentalis*

INTRODUCTION

Plant and animal tissues contain a wide range of organic acids. These acids are organic compounds with acidic properties and they are referred to as weak acids. Organic acids have positive effects on the well-being of man (Ivanova-Petropulos *et al.*, 2015). Organic acids with lower molecular mass like lactic acid and formic acid are miscible in water, whereas those with higher molecular mass like benzoic acid are insoluble in neutral form. The presence of adequate concentrations of organic acids in grape berries has been used as a tool for determining the quality of berries and wines (Conde *et al.*, 2007).

Secondary metabolites are synthesized randomly in plants and have their usefulness (Bernhoft, 2000). They are frequently produced at the highest levels during transition from active growth to stationary phase. Phenolic compounds are among the most widely distributed secondary metabolites and play a crucial role based on their chemical diversities. Tania *et al.* (2012) reported that the absence of secondary metabolites does not result in immediate death of the plant species, but rather in long-term impairment of their survivability. Some of the functions of phenolic compounds include defence against herbivores and pathogens, mechanical support in attracting pollinators and fruit dispensers, in absorbing harmful ultraviolent radiation, or in reducing the growth of nearby competing plants (Forkmann, 1991; Dixon, 1999; Dudareva *et al.*, 2004; Molyneux *et al.*, 2007). The presence of bioactive phytochemicals and secondary metabolites has made plants, especially vegetables, a promising source of modern synthetic drugs for management of several diseases (Balogun *et al*., 2016). It has been reported by Bassey *et al.* (2006) that these phytochemicals occur in tangible quantities in the leaves.

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Telfairia occidentalis Hook f. (fluted pumpkin) is a plant in the family Cucurbitaceae. It is a dioecious perennial plant with enormous economic significance in eastern Nigeria. It is a vegetable crop and tropical vine cultivated in West Africa as a leafy vegetable and for its edible seeds (Irvine, 1969; Esiaba, 1982; Akoroda, 1990; Ogar and Asiegbu, 2005). *Telfairia occidentalis* is a popular cultivated vegetable in West Africa. It is a common vegetable in eastern Nigeria and has been widely accepted as a dietary constituent that has healing properties, being used as blood tonic for the sick or convalescent (Akwaowo *et al.,* 2000; Okoli, 2013). Studies have shown that *T. occidentalis* leaf is rich in minerals, antioxidants, vitamins and phytochemicals (Horsfall and Spiff, 2005; Oboh, 2005; Fasuyi, 2006; Oboh *et al.,* 2006; Ajibade *et al.,* 2006; Kayode *et al.,* 2009). Phytochemicals are defined as bioactive non-nutrient plant compounds in fruits, vegetables, grains and other plant foods (Doughari *et al*., 2009). For instance, it is 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 defence (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 (Dharmartilake and Bauer, 1992; Pandya *et al*.,1999).

The soil has always been used as a medium for growing plants, and to serve as anchorage and a reservoir of nutrients for the plants. Recently, researches have shown that plants especially vegetables can grow in soilless medium (Savage, 1985; Kratky, 1993, 1995, 1996, 2002, 2004, 2005; Kratky *et al*., 2008; Kratky, 2009; Falloro *et al*., 2009; Pelesco and Bentor-Jr., 2013; Okonwu *et al*., 2017a, 2017b, 2018a, 2018b). Several works (Sonneveld, 2000; Silberbush and Ben, 2001; Savvas, 2002; Murali-Mugundhan *et al.,* 2011; Okemwa, 2015) have shown the merits and demerits of these growth media when compared. This study aimed to identify and quantify the organic acids, glycosides and phenolic compounds present in *T. occidentalis* leaves cultivated in hydroponic and geoponic media using Water 616/626 HPLC.

MATERIALS AND METHODS

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 divided into two batches and planted in white sand from the Choba River and top humus soil (0-25 cm) from a garden in University of Port Harcourt, as a medium for germination. The two week-old seedlings from the white sand were transferred into a non-circulating hydroponic nutrient system. The nutrient solution used for the hydroponic was bicfarms concept formulation. The plants in both soilless medium (HM) and soil medium (GM) were allowed to stand for a month, when it could be harvested to prepare food. The mature leaves were harvested and used to determine the organic acids, glycosides and phenolic compounds in both geoponic and hydroponic media. The analysis of organic acids, glycosides and phenolic compounds was carried out at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria.

Organic acids (extraction and analysis): Five (5) g plant sample was weighed into 250 ml extraction bottle, 100 ml of ultrapure water was dispensed to cover the sample and placed in a cool environment at 4^{o}C for 6 days. Then sample materials were squeezed from the extraction bottle through two layers of cheesecloth (or glass wool in a funnel) or the liquid was centrifuged at about 2,000 rpm for 5 minutes. The sample was placed in a corked bottle and stored in a cool environment. Sample solution (1.5 ml) and 1ml working standard were pipetted into a 10 ml centrifuge tube, mixed and allowed to stand for 30 minutes. Thereafter, the content was centrifuged at 3,000 rpm for 10 minutes. The supernatant (1.5 ml) was injected directly on the HPLC column (4% CW-20M 80/120 carbg pack-BDA column) fitted with a flame ionisation detector. The conditions for the analysis of organic acids 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 flame ionisation detector (vi) Carrier gas: Nitrogen gas at flow rate of 65 ml/min (vii) Temperature: Detector- 201^{o}C; Injector port- 195^{o}C; Column- 150^{o}C (viii) Computer facilities for storing data. (ix) Printer for reporting results.

Glycosides (extraction and analysis): Half a gram **(**0.5 g) of plant sample was weighed into a set of digestive tubes, 5 ml of 0.1 M HCl was added, warmed gently for 15 minutes at 105^{o}C and transferred into a 50 ml volumetric flask. The procedure was repeated twice, rinsed with two to three aliquots, allowed to filter completely and the filtrate volume was made up to 100 ml with the extractant solution and mixed thoroughly. Then, 5 ml of extract solution was taken from the 100 ml flask and ran through a 2 cm layer (resin was packed on a macro-pipette tip) cation exchange resin (CEC). Glycoside compound was eluted with 10 ml of absolute ethanol. The ethanol was washed from the column with ultrapure water (10 ml) and the supernatant was transferred to a sample vial and ran on water 616/626 HPLC HPLC. The conditions for the analysis of glycosides 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 38 ml/min. (vii) Temperature: Detector- 167^{o}C; Injector port- 183^{o}C and Column- 130^{o}C (viii) Computer facilities for storing data. (ix) Printer for reporting results.

Phenolics (extraction and analysis): Two **(**2) g plant sample was weighed into a set of test tubes, Three (3) ml of 70% acetone in water was added and the tube was placed in an ultrasonic water bath at 10^{o}C for 5 minutes and stirred occasionally with a glass rod. It was filtered through a 50-60 µ Gooch crucible into a 50 ml Erlenmeyer flask. The extraction was repeated 3 times by adding 3 ml of 70% acetone in water and allowing it to stand in the water bath at 10^{o}C for 5 minutes. The test tube was rinsed with the final 3 ml portion of 70% acetone in water and emptied into the test tubes. Then 2 ml of 0.1M yb-acetate and 15 ml of 0.1M TEA reagent were added into the filtrate. The flask was closed with rubber stopper, swirled and shaken for 20 minutes after transferring the sample solutions to a set of plastic volumetric tubes. This was allowed to settle for 4 hours and the supernatant was collected for analysis using HPLC. The conditions for the analysis of phenolics 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: Argon gas at flow rate of 60 ml/mins (vii) Temperature: Detector- 120^{o}C; Injector port- 155^{o}C and Column- 117^{o}C (viii) Computer facilities for storing data (ix) Printer for reporting results.

RESULTS AND DISCUSSION

Organic Acids: The total organic acids in *T. occidentalis* grown in soilless medium and in the conventional soil medium are shown in Table I. The soilless system had a higher total organic acid than soil medium (6.88 g/100g HM; 6.508 g/100g GM). The results agree with the work of Chandra *et al.* (2014), who observed that plants grown in the aeroponic system had a higher yield and comparable phenolics, flavonoids and antioxidant properties as compared to those grown in the soil. Among the organic acids assessed, the hydroponic system had higher organic acids than geoponic with the exception of butyric acid (0.262 g/100g HM; 0.301 g/100g GM), citric acid (0.022 g/100g HM; 0.092 g/100g GM), gallic acid (0.069 g/100g HM; 0.076 g/100g GM), malic acid (0.262 g/100g HM; 0.301 g/100g GM), succinic acid (0.212 g/100g HM; 0.315 g/100g GM) and shikimic acid (1.979 g/100g HM; 2.911 g/100g GM). From the result, the main organic acid was shikimic acid (28.76%; 44.73% of the total organic acid), followed by hydrobromic acid (21.41%; 11.16%), captopril acid (12.30%; 6.41%) and others such as glucamic acid (6.25%; 5.09%), velaric acid (4.78%; 5.09%) in that order for HM and GM. Flores *et al.* (2012) indicated that leafy vegetables showed a high concentration of malic acid that varied between 0.190, 0.083, 0.081, 0.575 and 0.233 g/100 g fresh weight in green pepper, red pepper, tomato, lettuce and lamb's lettuce, respectively. The values reported by Morales *et al*. (2014) in non-cultivated vegetables, were 51.36 ± 7.41 mg/100 g fresh weight for *Beta marítima* L. and 147.19 ± 92.49 mg/100 g fresh weight for *Papaver rhoeas* L. Uusiku *et al.* (2010) collected data of oxalic acid content on fresh weight basis present in the edible portion of many African leafy vegetables such as *E. hirta* (1.115 g/100g), *Ipomoea involucrata* (0.913 g/100g), *Xanthosoma sp.* (0.654 g/100g), *Amaranthus sp*. (0.04–0.05 g/100g), *M. esculenta* (0.02 g/100g), *Celosia argentea* (0.02 g/100g), *Telfairia occidentalis* (0.04 g/100g) and *Vernonia sp*. (1-2 g/100g).

Table I: Organic acids present in *T. occidentalis* grown in soilless and soil media

Organic acid (g/100g)	Growth medium	
	HM	GM
18-beta-glycyrrhetinic acid	0.007	0.003
Acetic acid	0.179	0.061
Ameodipine acid	0.015	0.013
Atenonol acid	0.104	0.051
Butyric acid	0.262	0.301
Captopril acid	0.846	0.417
Citric acid	0.022	0.092
Digitoxin acid	0.013	0.012
Digoxin acid	0.042	0.021
Enalapril acid	0.047	0.028
E-strophanthin acid	0.002	0.002
Fumaric acid	0.004	0.004
Galacturonic acid	0.110	0.145
Gallic acid	0.069	0.076
Glucamic acid	0.430	0.331
Acrylic acid	0.004	0.002
Glycolic acid	0.038	0.033
Hydrobromic acid	1.473	0.726
Lisinopril acid	0.019	0.016
Malic acid	0.262	0.301
Metoprolol acid	0.303	0.261
Nifedipine acid	0.027	0.013
OLeandrin acid	0.019	0.011
Propranolol acid	0.011	0.006
Pyruvic acid	0.043	0.022
Shikimic acid	1.979	2.911
Succinic acid	0.212	0.315
Valeric acid	0.329	0.331
Varapamil acid	0.009	0.005
Total Organic acid	6.880	6.508

HM represents Soilless medium; GM represents Soil medium

Glycosides: The glycoside composition of *T. occidentalis* grown in soilless and soil media is presented in Table 2. The values of glycosides content were low in GM medium compared to HM medium. The six main glycosides were E-strophanthin acid (21.37%; 23.92% of total glycosides), glycyrhizic acid (16.25%; 11.58%), OLeandrin acid (11.96%; 13.39%), enalapril acid (8.62%; 7.06%), varapamil acid (8.30%; 9.28%) and glycyrrhetinic acid (7.39%; 8.27%); the other 12 detected such as digoxin acid, digitoxin acid and amlodipine acid were less than 6% of the total glycosides for HM and GM, respectively. Total glycosides of *T. occidentalis* were higher in HM (6.762 g/100g) than in GM (4.549 g/100g). The total glycosides of *T. occidentalis* were higher than that reported for *Sansevieria liberica* (Ikewuchi *et al*., 2011).

Table 2: Glycosides composition of *T. occidentalis* grown in soilless and soil media

Glycoside (g/100g)	Growth medium			
	HM		GM	
	Conc. (g/100g)	%	Conc. (g/100g)	%
18-beta-glycyrrhetinic acid	0.324	4.79	0.244	5.36
Amlodipine acid	0.065	0.96	0.036	0.79
Atenolol acid	0.151	2.23	0.130	2.86
Captopril acid	0.159	2.35	0.088	1.93
Digitoxin acid	0.073	1.08	0.055	1.21
Digoxin acid	0.120	1.77	0.090	1.98
Enalapril acid	0.583	8.62	0.321	7.06
E-strophanthin acid	1.445	21.37	1.088	23.92
Furosemide acid	0.054	0.80	0.030	0.66
Glycyrhizic acid	1.099	16.25	0.527	11.58
Glycyrrhetinic acid	0.500	7.39	0.376	8.27
Hydrochlorathiazide acid	0.083	1.23	0.046	1.01
Lisinopril acid	0.135	2.00	0.074	1.63
Metoprolol acid	0.262	3.87	0.226	4.97
Nifedipine acid	0.250	3.70	0.138	3.03
OLeandrin acid	0.809	11.96	0.609	13.39
Propranolol acid	0.089	1.32	0.049	1.08
Varapamil acid	0.561	8.30	0.422	9.28
Total glycosides	6.762		4.549	

HM and GM represent Soilless and Soil media, respectively;% represents percentage of total glycosides

Phenolic compounds: Phenolic profiles of *T. occidentalis* showed 45 phenolic compounds (Table 3). It contains important phenolics such as ferulic, cinnamic acid and *p*-coumaric acid. Mussatto *et al.* (2007) reported that ferulic acid has anti-microbial, anti-inflammatory, anti-cancer activities and that it lowers cholesterol level in serum and increases sperm viability. Also, cinnamic acid and *p*-coumaric acid are widely distributed in food stuff and well documented for antioxidant properties. They are also believed to reduce the formation of carcinogenic nitrosamines in the stomach (Ramadoss *et al.,* 2015). The HPLC analysis of phenolics in *T. occidentalis* showed a range from 0.054-9.619 g/100g and 0.030-6.896 g/100g for HM and GM, respectively. The study with the aid of HPLC showed that *p*-hydroxybenzoic acid (23.37% and 22.71% for HM and GM of total phenolics studied) was predominant among the phenolic compounds, followed by aesculetin acid (16.62% and 16.20% for HM and GM, respectively). There were variations between phenolic compounds within the same plant when grown in different media. Total phenolics obtained in this study was higher than the one reported by Aminu *et al.* (2012) on the same plant. The hydroxycinnamic acids were more abundant than the hydroxybenzoic acids and consisted chiefly of *p*-coumaric, caffeic, ferulic and sinapic acids. These acids are rarely found in the free form, except in processed food that has undergone freezing, sterilization, or fermentation (Manach *et al*., 2004). Caffeic and quinic acid combine to form chlorogenic acid, which is found in many types of fruit and in high concentrations in coffee; a single cup may contain 70–350 mg chlorogenic acid (Clifford, 1999). The hydroxybenzoic acid content of edible plants is generally very low, with the exception of certain red fruits, black radish and onions, which can have concentrations of several tens of milligrams per kilogram fresh weight (Shahidi and Naczk, 1995). The amount of flavonoids and phenolics recorded in *T. occidentalis* leaf confirmed its antioxidant effect. These corroborated the results of Ganiyat *et al.* (2011) and Anokwuru *et al.* (2011). Several studies have shown that phenolics are biological active agents, which possess antioxidants and properties of free radical scavengers (Rice-Evans *et al*., 1995; Kahkonen *et al*., 1999; Sugihara *et al*., 1999). The antioxidant potential of phenolics is mainly due to their redox properties, which permit them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Rice-Evans *et al*., 1995; Rice-Evans *et al*., 1996; Ramarathnam *et al.*, 1997).

Plant extracts that contain a high amount of polyphenols also exhibit high antioxidant activity (Wong *et al*, 2006). Biological activities such as promotion of bile secretion and reduction in blood loss have been reported for some phenolic acids (Ghasemzadeh and Ghasemzadeh, 2011). The amount of phenolic compounds in a plant is strongly dependent on several factors such as climate, soil composition, geographic location and storage conditions (Tiwari *et al*., 2011). Phenolic acids also hinder iron absorption as tannin and flavonoids (Brune *et al.*, 1989; Serrano *et al.*, 2009).

Table 3: The phenolic composition of *T. occidentalis* grown in soilless and soil media

Phenolics (g/100g)	Growth medium	
	HM	GM
Aesculetin acid	6.841	4.918
Astringin acid	0.262	0.226
Benzoic acid	0.116	0.096
Cafein acid	0.324	0.244
Caffaric acid	0.561	0.422
Caffeic acid	0.809	0.609
Carreic acid	0.325	0.269
Castarinol C1 acid	0.159	0.088
Castarinol C2 acid	0.083	0.046
Castarinol C3 acid	0.054	0.030
Castarinol C4 acid	0.089	0.049
Catechin acid	0.079	0.069
Cinnamic acid	0.703	0.480
Contaric acid	0.065	0.036
Coumaric acid	0.250	0.138
Cutissin acid	0.151	0.130
Cyanidin 30-glucoside	0.135	0.074
Cyanidin coumaroyl 30-glucoside	0.583	0.321
Ethy/ gallon acid	0.440	0.380
Ethyl/caffeati acid	2.641	2.281
Ferteric acid	0.559	0.483
Ferulic acid	0.175	0.145
Galic acid	0.500	0.376
Gallic acid	1.637	1.125
Genticitic acid	0.102	0.070
Homogentisic acid	0.757	0.627
Homovanilic acid	0.207	0.171
Homovanillic acid	0.754	0.573
Izoferulic acid	0.258	0.196
Mendelic acid	0.448	0.340
M-OH-benzoic acid	4.526	3.436
P-cumaric acid	0.505	0.383
Piperonic acid	0.455	0.311
P -OH-benzoic acid	0.073	0.055
P -OH-Phenyloacetic acid	9.619	6.896
Protocatechic acid	0.958	0.727
Pyrogallic acid	0.108	0.090
Salicitic acid	0.136	0.103
Salicylic acid	0.789	0.539
Sinapic acid	1.445	1.088
Sinamic acid	0.084	0.070
Singlic acid	0.120	0.090
Syringic acid	0.070	0.058
Vanillic acid	0.169	0.115
Veratric acid	2.032	1.387

HM represents Soilless medium; GM represents Soil medium

CONCLUSION

The individual organic acids, glycosides and phenolic compounds of *T. occidentalis* leaves cultivated in hydroponic and geoponic media varied in concentrations. The study revealed that total organic acids, total glycosides and phenolic compounds were higher in the *T. occidentalis* leaves cultivated in hydroponic condition compared to geoponic medium. To effectively harness these secondary metabolites, the study recommends the use of hydroponic system for the cultivation of *T. occidentalis*.

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