Elemental Micronutrients, Antioxidant Activity, Total Polyphenol, and Total Flavonoid Content of Selected Spider Plant Accessions (Cleome gynandra) Grown in Eastern Africa and the Eastern United States

Elemental Micronutrients, Antioxidant Activity, Total Polyphenol, and Total Flavonoid Content of Selected Spider Plant Accessions (*Cleome gynandra*) Grown in Eastern Africa and the Eastern United States

Bernard Somers[1,2], Fekadu F. Dinssa[3], Qingli Wu[1,2], and James E. Simon[1,4*]

[1]New Use Agriculture and Natural Plant Products Program, Department of Plant Biology, Rutgers University, 59 Dudley Road, New Brunswick, NJ 08901, USA
[2] Department of Medicinal Chemistry, Rutgers University, 65 Dudley Road, New Brunswick, NJ 08901, USA
[3] World Vegetable Center, Arusha, Tanzania
[4] Center for Agricultural Food Ecosystems, The New Jersey Institute for Food, Nutrition, and Health, Rutgers University, 61 Dudley Road, New Brunswick, NJ 08901, USA

***Corresponding author:** jimsimon@rutgers.edu

Manuscript received: April 26, 2020

Keywords: African indigenous vegetable, spider plant, micronutrient

ABSTRACT

African spider plant, *Cleome gynandra,* is an economically important source of highly nutritious leafy greens and traditional phytopharmaceuticals in sub-Saharan Africa. In this study, five genetic accessions of African spider plant were field grown in New Jersey, USA (2015) and Arusha, Tanzania (2016 and 2017). When the spider plant was at full flower and ready for the market, the leaves were manually sampled for nutritional and phytochemical investigation. Elemental analysis was conducted via inductively coupled plasma mass spectrometry to characterize the mineral nutrients of spider plant samples. Spectrophotometric determination of total polyphenol content, ferric reducing antioxidant power, and total flavonoid content provided additional characterization of the phytochemistry. Results showed that spider plant has high antioxidant activity and results from elemental analysis indicate the spider plant is a "source" of iron and a "high source" vegetable for calcium, manganese, and magnesium.

INTRODUCTION

In many sub-Saharan African countries, lack of healthful dietary variety and deficiencies of calcium, iron, and vitamin A is common (Hoffman et al., 2017). African indigenous vegetables (AIVs), can contribute toward filling this nutritional gap as a source of vegetable-derived nutrients. The AIVs, such as African nightshade, amaranth, moringa, and spider plant, are traditional foods that are collected in the wild, or grown and utilized by many African communities. These indigenous or naturalized plants are often relatively easy to produce under adverse agricultural conditions and contribute to local economies. Crops that can reduce the risk of crop failure due to environmental conditions, promote food security, and provide local income generating opportunities to improve health and nutrition are needed and AIVs can serve in these roles (Weller et al., 2015).

African spider plant (*Cleome gynandra*) is an AIV and greatly underutilized crop that has a diverse nutrient profile and promising traditional medicinal properties (Chweya and Mnzava, 1997; Neugart et al., 2017). Decoctions of the leaves are used as an anti-inflammatory agent and to treat arthritis, relieve pneumonia, cure recurrent malaria, and serve as an antibacterial agent (Chweya and Mnzava, 1997; Omondi et al., 2017). In the present study, we report the micronutrient content and spectrophotometric secondary metabolite screening of five *Cleome gynadra* accessions each grown in New Jersey in 2015, and Tanzania in 2016 and 2017.

A food that meets or exceeds "high source" thresholds for one or more micronutrients may be used to quantitatively evaluate health status improvement in populations deficient in micronutrients (Codex Alimentarius, 1997). The key quantities of micronutrients in this study are defined in *Codex Alimentarius* Guidelines for Use of Nutrition and Health Claims (Codex Alimentarius, 1997). "High source" thresholds are defined as the content necessary to provide at least 30% of the Nutrient Reference Value (NRV) per micronutrient if 100 g fresh weight is consumed: 4.2 mg/100g Fe, 90 mg/100g Mg, 300 mg/100g Ca, and 3.3 mg/100g Zn, fresh weight basis (Codex Alimentarius, 1997; Codex Alimentarius, 2015). "Source" thresholds are defined as half the value of respective high source thresholds (Codex Alimentarius, 1997). Given spider plant's reported nutritional, medicinal and drought resistant properties, we sought to evaluate the phytochemical profile of select populations with good field performance. The nutritional traits of these advanced populations can be utilized as a tool to combat micronutrient deficiency in low-income Sub-Saharan African populations, and to further understand the purported medicinal potential.

MATERIALS AND METHODS

Collection and preparation of plant material. Seeds of five different genotypes (ML-SF-17, ML-SF-29, PS, UG-SF-15, and UG-SF-23) of *Cleome gynandra* were obtained from World Vegetable Center (WorldVeg) in Arusha, Tanzania. These genotypes were each botanically authenticated as to their genus and species by the taxonomists and plant breeders at the WorldVeg. The accessions (Table 1) were grown in a randomized block design at the Clifford E. and Melda C. Snyder Research Farm in Pittstown, NJ (Longitude: 40.55762, Latitude: -74.960574) in Northern New Jersey in 2015 and in Arusha, Tanzania in 2016 and 2017 (Longitude: 36.682995. Latitude: -3.386925). The entire above ground biomass of the plant was harvested when at full flowering and dried using a walk-in, forced air commercial Powell Tobacco dryer converted for the drying of herbs and botanicals at 37°C, while solar dried in Africa. Once dried, the plant materials were grinded to a powder, and stored at ambient temperature until analysis.

Table 1. List of spider plant (*Cleome gynandra*) genetic accessions

Variety	NJ 2015	Tanzania 2016	Tanzania 2017
UG SF 23	NJ 15 3	Tz 16 19	Tz 17 10
ML SF 17	NJ 15 4	Tz 16 20	Tz 17 7
PS	NJ 15 5	Tz 16 18	Tz 17 9
UG SF 15	NJ 15 6	Tz 16 16	Tz 17 6
ML SF 29	NJ 15 7	Tz 16 17	Tz 17 8

Preparation of extracts. Approximately 200 mg of dried powder was extracted in 5 ml 70% methanol with 0.1% formic acid at ambient temperature. The extract was then vortexed for 3 minutes, sonicated for 15 minutes, vortexed a second time for 3 minutes, then filtered to obtain the working extracts.

Elemental Analysis. Elemental content was quantified on ground powder by inductively coupled plasma mass spectrometry (ICP-MS) analysis at Penn State Agricultural Analytical Services (Huang and Schulte, 1985).

Folin's Total Polyphenol, Ferric Reducing Antioxidant Power (FRAP) and Total Flavonoid. Total phenolic content of the extract was determined by the Folin–Ciocalteu method. The standard curve was generated using gallic acid.

Ferric reducing antioxidant power was determined using a colorimetric method (Benzie and Strain, 1999). The standard curve was generated using L-ascorbic acid. Total flavonoid content of crude extract was determined by an aluminum chloride colorimetric method (Mammen and Daniel, 2012). The standard curve was generated using catechin (Jimenez-Aguilar and Grusak, 2015; Moyo et al., 2013).

RESULTS AND DISCUSSION

Micronutrients. The results of the elemental analysis were reported on a fresh weight basis by converting from an average moisture content of 10% for the dry samples to 90% moisture content, the approximate water content for raw spider plant leaves (Matenge et al., 2017; Uusiku et al., 2010). This conversion was done to conservatively estimate the micronutrient content as it would typically be used for cooking. Furthermore, this conversion to units of "mg/100g fresh weight basis" allowed for direct comparisons with data reported by the USDA Standard Nutrient Database and Codex Alimentarius Guidelines for Use of Nutrition and Health Claims (Codex Alimentarius, 1997; Codex Alimentarius, 2015).

Tanzania 2016 samples were contaminated with rust during the postharvest handling as the drying rack was rusted. This lead to excessive levels of iron and aluminum. For this reason, Fe and Al content for Tz16 were removed from data analysis (Fig. 2). The iron in spider plant samples tested ranged from 2.1 – 3.5 mg/100 g FW. All samples were below the "high source" threshold (4.2 mg/100 g) but above the "source" threshold (2.1 mg/100 g) (Fig. 2, Table 2). These values were analogous to other works with *C. gynandra* accessions (Jimenez-Aguilar and Grusak, 2015; Uusiku et al., 2010) while significantly less than concentrations reported elsewhere (Nesamvuni et al., 2001; Omondi et al., 2017; Schönfeldt and Pretorius, 2011). Another work by Omondi et al. (2017) used the same genetic line (ML-SF-17) as we report herein. However, they reported very high Fe content ranging from 12.1 – 65.5 mg/100 g FW. This may be attributable to surface contamination from soil. However, Fe, Al, and Cr levels were all very high and may point to contamination as a reason for the discrepancy (Omondi et al., 2017). Phylogenetic relatives of *C. gynandra* show comparable iron accumulation levels: *C. viscosa* (3.3 mg/100 g FW Fe content) levels were analogous to our results while *C. burmanni* (1.1 mg/100g FW Fe content) levels were lower (Pillai and Nair, 2013). Compared to other commonly consumed leafy greens on the USDA Food Database, our results suggest spider plant accumulates more iron than *Brassica oleracea L.* (1.47 mg/100 g FW), *Spinacia oleracea* (2.71 mg/100 g FW), *Brassica oleracea var. viridis* (0.47 mg/100 g FW), and *Dioscorea spp*. (0.54 mg/100 g FW). Spider plant can be considered a "source" of iron (USDA, 2019).

The calcium content across all of the samples tested ranged from 209 – 423 mg/100 g FW. The NJ15 samples were all above the "high source" threshold (300 mg/100 g FW) while the Tanzania 2016 and Tanzania 2017 samples were below "high source" but above the "source" threshold (150 mg/100 g FW) (Fig. 3, Table 2). These results align with other publications on spider plant calcium content, that concluded spider plant to be above the "high source" threshold (Nesamvuni et al., 2001; Schönfeldt and Pretorius, 2011; Uusiku et al., 2010). *C. viscosa* (180 mg/100 g FW Ca content), *Cleome burmanni* (81 mg/100 g FW), and *Cleome monophylla* (1.9 mg/100 g FW) all accumulated a lesser amount of calcium relative to our *C. gynandra* samples (Nesamvuni et al., 2001; Pillai and Nair, 2013). Spider plant samples tested accumulated higher calcium levels than other well-known, calcium-rich vegetables such as *S. oleracea* (99 mg/100 g FW), *B. oleracea var. viridis* (232 mg/100 g FW), *Brassica juncea* (115 mg/100 g FW), and *Brassica rapa* (105 mg/100 g) (USDA, 2019). These results suggest spider plant is a "source" of calcium regardless of genotype and environment and can aid in combatting nutrient deficiencies for those lacking calcium in their diets.

The manganese content in all spider plant samples ranged from 1.0 – 2.1 mg/100 g FW. All samples tested were above the "high source" threshold (0.9 mg/100 g FW) (Fig. 2, Table 2). In contrast, other works showed less manganese accumulation in spider plant (Jimenez-Aguilar and Grusak, 2015) while others showed manganese levels slightly greater than or analogous to our results (Omondi et al., 2017). Relatives to spider plant, *C. viscosa* (0.86 mg/100 g FW Ca content) and *C. burmanni* (1.36 mg/100 g FW) both accumulated manganese levels comparable to our samples (Pillai and Nair, 2013). Spider plant accumulated greater manganese content than *Dioscorea spp*. (0.4 mg/100 g FW), but less than the African leafy vegetables *Solanum nigrum* (2.1 mg/100 g FW) and *Vigna unguiculata* (2.7 mg/100g FW) (USDA, 2019; Van Rensburg et al., 2007). These results suggest that some vegetables contain a greater amount of manganese than spider plant, despite its "high source" classification for this micronutrient.

The magnesium content in all spider plant samples ranged from 41 – 111 mg/100 g FW. All NJ15 samples were above the "high source" threshold (90 mg/100 g FW) while the Tanzania 2017 samples were below "high source" but above the "source" threshold (45 mg/100 g) (Fig. 3, Table 2). Tanzania 2016 samples were below the "source" threshold, by a small margin (Table 2). Previous publications show analogous magnesium levels in their spider plant samples (Jimenez-Aguilar and Grusak, 2015; Omondi et al., 2017; Uusiku et al., 2010; Van Jaarsveld et al., 2014). *C. viscosa* (160 mg/100 g FW) contains a greater amount of magnesium while *C. burmanni* (80 mg/100 g FW) contains similar magnesium levels relative to our samples. Other leafy green vegetables such as: *B. juncea* (32 mg/100 g FW), *Dioscorea spp*. (79 mg/100 g FW), and *B. oleracea L.* (47 mg/100 g) contain significantly less than or similar magnesium levels while *Chenopodium album* (155-211 mg/100 g FW) and *Amaranthus sp.* (105-224 mg/100 g) can contain magnesium levels comparable to or much greater than our spider plant samples. These results suggest spider plant magnesium levels can be variable and can depend on genotype and environmental effects (Byrnes, 2018). Nonetheless, *C. gynandra* can be considered a "source" of magnesium and even a "high source" in the case of NJ15 samples.

The zinc content in all spider plant samples ranged from 0.6 – 1.0 mg/100 g FW and were below the "source" threshold (1.7 mg/100 g). Other works on spider plants also report values below the "source" threshold support this result (Jimenez-Aguilar and Grusak, 2015; Omondi et al., 2017; Schönfeldt and Pretorius, 2011; Uusiku et al., 2010). *C. monophylla* (0.43 mg/100 g FW) and *C. maxima* (0.61 mg/100 g FW) relatives also accumulate analogous zinc levels (Nesamvuni et al., 2001). However, the levels of zinc in spider plant is greater than some other leafy vegetables such as: *V. unguiculata* (0.42 mg/100 g FW), *Amaranthus esculentus* (0.36 mg/100 g FW), *Corchorus tridens* (0.28 mg/100 g), and *S. oleracea* (0.53 mg/100 g FW), but less than other leafy vegetables such as: *C. album* (1.4-18.5 mg/100 g FW), *S. nigrum* (0.8-3.5 mg/100 g FW), and *Amaranthus* sp. (0.02-8.4 mg/100 g FW). (Nesamvuni et al., 2001; USDA, 2019; Uusiku et al., 2010). These results further solidify the levels of zinc accumulation in spider plants as corroborated by other works and suggests that those deficient in zinc can find greater levels in other vegetables but can still benefit from lower levels within spider plant.

Despite environmental variations, all spider plant samples reliably exceeded the "source" threshold levels for iron, calcium, and manganese. Reasons for the observed differences between locations are difficult to ascertain but generally may be attributable to the soil chemistry, soil fertility, amount of sun and rain, and time of harvest. Also, as the actual tools used, such as cutting knives, drying racks, we found that inadvertent contamination from the soil, and even from the drying rack can affect results by unintentionally introducing 'contamination'. For this reason, we eliminated Tanzania 2016 iron and aluminum data as we detected a rust contamination. Those initial results, which we observed and that led to the decision to not use such results, do suggest that when researchers find or literature reports excessively high levels in plant tissue additional confirmation is suggested. The data suggests that NJ field conditions led to higher nutrient accumulation in spider plant samples. In particular, calcium and magnesium levels were substantially higher in the NJ grown material relative to Tanzania grown material.

Total Polyphenol. Spider plant samples' total phenol content ranged from 0.54 – 1.03 g/100 g. NJ15 samples showed the highest concentration of total phenolics represented by NJ-15-3, NJ-15-5, and NJ-15-7 all greater than 1% polyphenol content by dry mass. Spider plant accessions tested in a previous work showed similar levels to our Tanzania 2016 samples but less phenolic content relative to NJ15 samples (Jimenez-Aguilar and Grusak, 2015). Spider plant samples collected in Eastern Botswana showed total phenol content analogous to our NJ15 samples of ~1% dry weight content (Matenge et al., 2017).

Spider plant contains comparable total phenol content to other African vegetables such as: *C. album* (860 mg/100 g), *Solanum asper* (1.05 g/100 g), *S. nigrum* (458 mg/100 g), *Urtica urens* (668 mg/100 g), *Cnidoscolus aconitifolius* (566 mg/100 g), and *Solanum scabrum* (317 -342 mg/100 g) (Afolayan and Jimoh, 2009; Jimenez-Aguilar and Grusak, 2015). Polyphenols are known for their nutraceutical properties, antibiotic activity, and disease prevention characteristics (Chen et al., 2017; Herald and Davidson, 1983). Spider plant contains diverse coumaric, ferulic, and caffeic hydroxycinnamic acid derivatives which can contribute to the observed total phenol content and traditional medicinal uses (Chweya and Mnzava, 1997; Neugart et al., 2017).

Antioxidant Power. The tested spider plant samples' antioxidant power ranged from 236 – 623 mg ascorbic acid equivalents (AAE) /100 g. NJ15 and Tanzania 2017 samples showed the highest antioxidant capacity (Fig. 1). In 2015, another group characterized *C. gynandra* samples which ranged from 386 – 824 mg/100g trolox equiv. (TE) and were comparable to our obtained results (Jimenez-Aguilar and Grusak, 2015). Another team reported 390 mg TE/100 g in *C. gynandra*, most similar to our Tanzania 2016 samples and less than NJ15 and Tanzania 2017 samples (Stangeland et al., 2009). This report also examined the antioxidant activity in thirty-five Ugandan fruits and vegetables and found spider plant to have the greatest antioxidant power (390 mg TE/100 g) of the green leafy vegetables included in their study. In comparison to their fruits, our experimental results suggests spider plant possessed similar antioxidant power to *Tamarindus indica L*. (470 mg TE/100 g), *Mangifera indica L.* (405 mg TE/100 g), and *Cyphomandra betacea* (Cav.) Sendth. (405 mg TE/100 g).

Antioxidants protect cells from oxidative damage caused by free radicals which can accumulate in the body as a result of disease, chemical exposure, the sun, etc. Several works reveal a close link between oxidative stress and the onset of various conditions such as cancer, AIDS, hypertension, inflammatory conditions, and neurodegenerative disorders (Adefegha and Oboh, 2011; Kamble and Gacche, 2018; Stangeland et al., 2009). Our results suggest spider plant is a source of antioxidants and supports its use as a traditional medicinal vegetable.

Total Flavonoid. The tested spider plant samples' flavonoid content ranged from 113 – 585 mg catechin equivalents (CE)/100 g. NJ15 and Tanzania 2017 samples showed the greatest flavonoid content (Fig. 1). A previous publication on eight unique spider plant accessions showed a range of 513 – 823 mg CE/100 g (Jimenez-Aguilar and Grusak, 2015). These values were comparable to and slightly greater than our observed flavonoid content. Other leafy greens such as *Crotalaria longirostrata, C. aconitifolius, C. album, S. asper, S. nigrum, and U. urens* showed less flavonoid content while *S. scabrum* showed a greater flavonoid content (Afolayan and Jimoh, 2009; Jimenez-Aguilar and Grusak, 2015).

Flavonoids are a type of polyphenol and are well-known phytochemicals. Pharmacologically, flavonoids are involved in combatting obesity, inflammatory conditions, and Alzheimer's disease (Ganeshpurkar and Saluja, 2017). Rutin is a flavonoid found in spider plant and accumulates at very high concentrations (Neugart et al., 2017). This flavonoid was proven to be involved in a plethora of disease applications and possesses pharmacological potential (Ganeshpurkar and Saluja, 2017). Some of these pharmacological actions include: prevention of inflammation, antibacterial activity, antiviral activity, and CNS protectant activity, to only name a few (Ganeshpurkar and Saluja, 2017). Rutin is only one of many flavonoids found in spider plant. These results further support spider plant's use as a traditional medicine.

Spider plant varieties grown in both Northern New Jersey and Tanzania exhibited a significant amount of nutritional diversity. According to Codex Alimentarius International Food Standards, *C. gynandra* can be considered a "high source" of manganese, calcium, and magnesium. There are currently no other leafy green vegetables characterized by the USDA Food Nutrition Database that are regarded as "high source" for any two essential elemental micronutrients (Byrnes, 2018). This makes the spider plant accessions tested herein, in particular the NJ15 samples, a strong and promising leafy green vegetable currently defined for delivering essential micronutrients related to human health.

Table 2. African spider plant (*Cleome gynandra*) micronutrient content (mg/100g FW)

Genotype	Sample ID	P	K	Ca	Mg	S	Mn	Fe	Cu	B	Al	Zn	Na
UG SF 23	**NJ 15 3**	76.1	304.9	382.7	96.3	81.1	1.2	2.4	0.2	0.4	1.0	0.7	2.3
ML SF 17	**NJ 15 4**	77.6	284.5	381.3	111.1	74.3	1.2	2.1	0.2	0.3	0.8	0.7	2.7
PS	**NJ 15 5**	75.7	251.2	423.3	107.6	85.7	1.5	3.3	0.2	0.3	1.7	1.0	3.0
UG SF 15	**NJ 15 6**	77.0	292.0	358.4	99.4	91.9	1.2	3.1	0.2	0.4	1.8	1.0	3.4
ML SF 29	**NJ 15 7**	77.9	328.3	346.5	88.9	88.0	1.2	2.5	0.2	0.3	0.8	0.8	2.8
UG SF 15	**Tz 16 16**	84.3	412.8	246.2	42.6	59.1	2.1	C[1]	0.2	0.3	C	0.6	6.9
ML SF 29	**Tz 16 17**	95.6	422.8	227.2	43.8	59.8	1.7	C	0.2	0.3	C	0.6	6.7
PS	**Tz 16 18**	92.3	409.9	209.0	43.5	61.2	1.8	C	0.2	0.3	C	0.6	8.0
UG SF 23	**Tz 16 19**	86.7	433.9	222.8	40.9	63.4	1.8	C	0.2	0.3	C	0.7	6.4
ML SF 17	**Tz 16 20**	93.8	437.4	214.0	42.1	64.1	1.6	C	0.2	0.3	C	0.7	7.6
UG SF 15	**Tz 17 6**	107.5	354.2	252.9	54.0	75.1	1.2	3.0	0.1	0.7	2.4	0.8	5.7
ML SF 17	**Tz 17 7**	105.3	355.9	227.3	48.8	71.4	1.1	2.9	0.1	0.5	2.2	0.7	6.1
ML SF 29	**Tz 17 8**	111.6	383.1	225.5	45.0	69.5	1.0	2.9	0.1	0.6	2.3	0.7	6.2
PS	**Tz 17 9**	111.4	363.8	210.7	47.8	72.5	1.1	3.3	0.1	0.5	2.5	0.8	5.5
UG SF 23	**Tz 17 10**	109.8	370.1	233.4	49.9	76.2	1.3	3.5	0.1	0.6	2.9	0.7	5.7

[1]: "C" values removed due to rust contamination

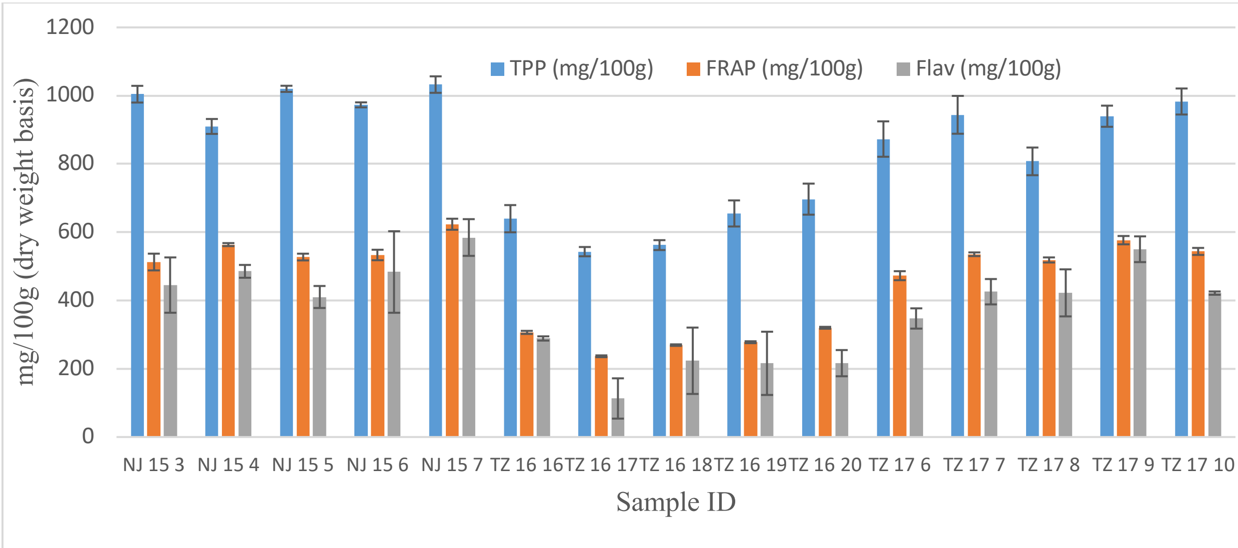

Figure 1. Total Polyphenol (TPP) expressed in mg GAE/100g, Ferric Reducing Antioxidant Power (FRAP) expressed in mg AAE/100g, and Total Flavonoid (Flav) expressed in mg catechin equivalents/100g, content of *Cleome gynandra* accessions, dry weight basis.

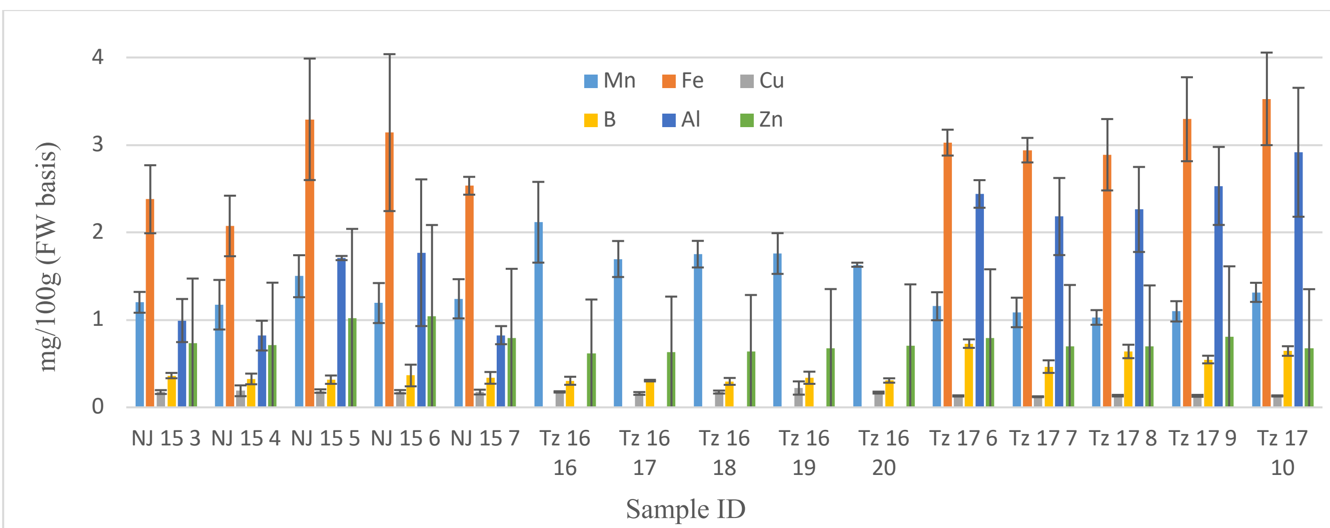

Figure 2. Manganese, Iron, Copper, Boron, Aluminum, and Zinc content of *Cleome gynandra* accessions.

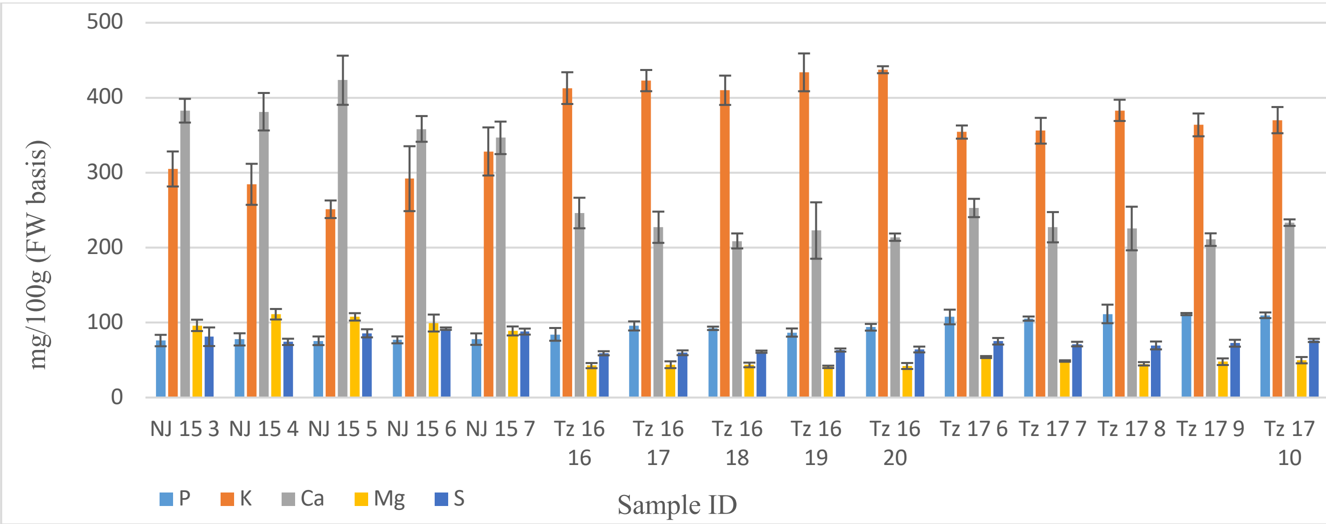

Figure 3. Phosphorus, Potassium, Calcium, Magnesium, and Sulfur content of *Cleome gynandra* accessions.

ACKNOWLEDGEMENTS

This research was supported by the Horticulture Innovation Lab with funding from the U.S. Agency for International Development (USAID EPA-A-00-09-00004), as part of the U.S. Government's global hunger and food security initiative, Feed the Future, for project titled "Improving nutrition with African indigenous vegetables" in eastern Africa. We also thank the New Jersey Agriculture Experiment Station (HATCH project 12158) and the World Vegetable Center (WorldVeg), Arusha, Tanzania. Special thanks to David Byrnes for his logistical and technical support with the AIV samples. We thank John Bowman, USAID-Washington, D.C., and Beth Mitcham, UC-Davis for support and leadership in the Horticulture Innovation Lab.

REFERENCES

Adefegha, S., and Oboh, G. 2011. Cooking enhances the antioxidant properties of some tropical green leafy vegetables. African Journal of Biotechnology 10(4): 632-639.

Afolayan, A., and Jimoh, F. 2009. Nutritional quality of some wild leafy vegetables in South Africa. International Journal of Food Sciences and Nutrition 60(5): 424-431.

Benzie, I.F. and Strain, J.J. 1999. Ferric reducing/antioxidant power assay: direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods in Enzymology 299:15-27.

Byrnes, D.R. 2018. Improvement of African indigenous vegetables for stable delivery of micronutrients. Doctoral dissertation, Rutgers University, New Brunswick-School of Graduate Studies.

Chen, Z., M. Digiacomo, Y. Tu, Q. Gu, S. Wang, X. Yang, J. Chu, Q. Chen, Y. Han, J. Chen and Nesi, G. 2017. Discovery of novel rivastigmine-hydroxycinnamic acid hybrids as multi-targeted agents for Alzheimer's disease. European Journal of Medicinal Chemistry 125:784-792.

Chweya, J.A. and Mnzava, N.A. 1997. Cat's Whiskers, *Cleome Gynandra L* (11). Biodiversity International.

Codex Alimentarius, International Food Standards. 2015. Guidelines on Nutrition Labeling. CAC/GL 2-1985.

Codex Alimentarius. 1997. Guidelines for Use of Nutrition and Health Claims. CAC/GL 23-1997.

Ganeshpurkar, A. and Saluja, A.K. 2017. The pharmacological potential of rutin. Saudi Pharmaceutical Journal 25(2):149-164.

Herald, P.J. and Davidson, P.M. 1983. Antibacterial activity of selected hydroxycinnamic acids. Journal of Food Science 48(4):1378-1379.

Hoffman, D., T. Cacciola, P. Barrios, and Simon, J. 2017. Temporal changes and determinants of childhood nutritional status in Kenya and Zambia. Journal of Health, Population and Nutrition 36(1):27. https://doi.org/10.1186/s41043-017-0095-z

Huang, C.Y.L. and Schulte, E.E. 1985. Digestion of plant tissue for analysis by ICP emission spectroscopy. Communications in Soil Science and Plant Analysis 16(9):943-958.

Jiménez-Aguilar, D.M. and Grusak, M.A. 2015. Evaluation of minerals, phytochemical compounds and antioxidant activity of Mexican, Central American, and African green leafy vegetables. Plant Foods for Human Nutrition 70(4):357-364.

Kamble, S.S. and Gacche, R.N. 2019. Evaluation of anti-breast cancer, anti-angiogenic and antioxidant properties of selected medicinal plants. European Journal of Integrative Medicine 25:13-19.

Mammen, D. and Daniel, M. 2012. A critical evaluation on the reliability of two aluminum chloride chelation methods for quantification of flavonoids. Food Chemistry 135(3):1365-1368.

Matenge, S.T.P., J. Li, S. Apau and Tapera, R. 2017. Nutritional and Phytochemical contents of indigenous leafy vegetables consumed in Botswana. Frontiers in Food and Nutrition Research 3(1):1-7.

Moyo, M., S.O. Amoo, B. Ncube, A.R. Ndhlala, J.F. Finnie, J. Van Staden. 2013. Phytochemical and antioxidant properties of unconventional leafy vegetables consumed in southern Africa. South African Journal of Botany 84:65-71.

Nesamvuni, C., N. Steyn, and Potgieter, M. 2001. Nutritional value of wild, leafy plants consumed by the Vhavenda. South African Journal of Science 97(1-2):51-54.

Neugart, S., S. Baldermann, B. Ngwene, J. Wesonga and Schreiner, M. 2017. Indigenous leafy vegetables of Eastern Africa—A source of extraordinary secondary plant metabolites. Food Research International 100:411-422.

Omondi, E.O., C. Engels, G. Nambafu, M. Schreiner, S. Neugart, M. Abukutsa-Onyango, and Winkelmann, T. 2017. Nutritional compound analysis and morphological characterization of spider plant (*Cleome gynandra*)-an African indigenous leafy vegetable. Food Research International *100*: 284-295.

Pillai, L.S. and Nair, B.R. 2013. Proximate composition, Mineral elements and Anti-nutritional factors in *Cleome viscosa L.* and *Cleome burmanni W.* and A. (*Cleomaceae*). Magnesium 14343:7238.

Schönfeldt, H.C. and Pretorius, B., 2011. The nutrient content of five traditional South African dark green leafy vegetables - A preliminary study. Journal of Food Composition and Analysis 24(8): 1141-1146.

Stangeland, T., S.F. Remberg, and Lye, K.A. 2009. Total antioxidant activity in 35 Ugandan fruits and vegetables. Food Chemistry 113(1): 85-91.

USDA, Agricultural Research Service. FoodData Central, 2019. fdc.nal.usda.gov.

Uusiku, N.P., A. Oelofse, K.G. Duodu, M.J. Bester, and Faber, M. 2010. Nutritional value of leafy vegetables of sub-Saharan Africa and their potential contribution to human health: A review. Journal of Food Composition and Analysis 23(6):499-509.

Van Jaarsveld, P., M. Faber, I. Van Heerden, F. Wenhold, W.J. van Rensburg, and Van Averbeke, W. 2014. Nutrient content of eight African leafy vegetables and their potential contribution to dietary reference intakes. Journal of Food Composition and Analysis 33(1):77-84.

Van Rensburg, W.J., W. Van Averbeke, R. Slabbert, M. Faber, P. Van Jaarsveld, I. Van Heerden, F. Wenhold, and Oelofse, A. 2007. African leafy vegetables in South Africa. Water 33(3):317-326.

Weller, S.C., E. Van Wyk and Simon, J.E. 2015. Sustainable production for more resilient food production systems: Case study of African Indigenous Vegetables in Eastern Africa. International Horticultural Congress**,** Brisbane, Australia. Acta Horticulturae 1102:289-298.