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Original Article

Phytochemical, Vitamin and Antioxidant Assessments of the Stems and Leaves of Four Commonly Consumed Vegetables in Nigeria

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ABSTRACT

Background and Objectives: Nutrient composition of vegetables is critical for diet supplementation and disease management in phytomedicine. This study analyzed phytochemical and vitamin contents and antioxidant potentials of methanolic extracts of leaves and stems from *Ocimum gratissimum* (OG), *Telfairia occidentalis* (TO), *Vernonia amygdalina* (VA) and *Gongronema latifolium* (GL) in Abia State, Nigeria.

Materials and Methods: This study analyzed phytochemicals, antioxidants and vitamins using standard analytical methods. Moreover, 2,2-diphenyl-1-picrylhydrazyl and ferric reducing antioxidant power were assessed using photometric assays.

Results: Phytochemical analyses showed the highest concentrations in phenolic contents of the leaf extracts and flavonoid contents of the stem extracts of OG (6.16 ±0.02 mg/100 g and 5.40 ±0.01 mg/100 mg, respectively). The 2,2-diphenyl-1-picrylhydrazyl assay indicated that the leaf extracts of TO and OG were consistently higher for scavenging activities at 100, 200 and 400 µg/ml concentrations, respectively. In contrast, stem extracts of VA and GL were higher at similar concentrations. The ferric reducing antioxidant power results demonstrated the highest values in the leaves and stems of OG (1.55 ±0.02 and 0.77 ±0.24 µm/fe(II)/l, respectively). Vitamin A and C compositions (mg/100 g) in the stem extracts were higher than those in the leaves; whereas, their leaf extracts included higher compositions of vitamin E (mg/100 g).

Conclusions: This study suggests conscious incorporation of stems and leaves as vegetables in diets to boost diet and supplement biological antioxidant defenses against oxidative stress-induced diseases, developing SDGs two(2) and three(3) with focusing on malnutrition and promoting health and well-being of all ages.

Keywords: Phytochemicals, Antioxidants, Vegetables, Oxidative Stress

Introduction

Recently, the quest for improved health care, disease management and delivery systems through the use of dietary supplements and phytomedicines in the health sector has been significant. (1–3). Furthermore, the present increases in food prices resulting from insurgencies, wars, climate changes and natural and artificial disasters have led to food scarcity, hidden hunger and starvation. These can be lessened by consuming vegetables to ameliorate nutrient deficits (4–7). The selected green vegetables (*Ocimum gratissimum*, *Telfairia occidentalis*, *Vernonia amygdalina* and *Gongronema latifolium*) cultivated indigenously in Southeastern Nigeria are low in calories and fats. These vegetables are rich in antioxidants such as phytochemicals and vitamins, which form the basis of their therapeutic values (8, 5).

Nutritional benefits of the leaves and stems of green vegetable plants in protective health include decreases of cardiovascular diseases (CVDs), congenital disabilities, osteoporosis, cancers, cellular/tissue damages, neurodegenerative disorders, acidities in cells and organs and aging/age-related diseases (7, 9, 10–12). Additionally, phytochemical components in these plants are involved in detoxifying enzymes, immune system stimulations, inflammation reductants, steroidal metabolisms and antibacterial, antihelminthic and antiviral effectors in biological systems (13, 14). However, many people do not maximize benefits of these vegetables as they consume plant leaves alone, with a little regards to stems or vice versa. Several researchers have attributed the therapeutic values of vegetables to various bioactive compounds

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present in various parts of the plants, acting singularly or synergically with others (15–18). Neglected consumption of the edible plant parts may be attributed to poor availability and non-edibility of vegetables and majorly as a result of the public poor awareness of the nutritional values and its health benefits (4, 19–21). Hence, this study investigated the phytochemical constituents, vitamin compositions and antioxidant potentials of the methanolic extracts from the leaves and stems of four commonly consumed green vegetables of bitter leaf (VA), scent leaf (OG), bushbuck leaf (GL) and pumpkin leaf (TO) in Umuahia, Abia State, Nigeria.

Materials and Methods

Chemicals

Folin-Ciocalteu, DPPH, TPTZ and Catechin were purchased from Sigma, St. Louis, MO, USA. Ferric chloride (BDH), hydrochloric acid (BDH), acetic acid (BDH) and sodium acetate (BDH) were purchased from British drugstores (Poole, Dorset, UK). All other reagents included analytical grades.

Collection of the samples

Fresh leaves and stems of *Ocimum gratissimum* (OG), *Telfairia occidentalis* (TO), *Vernonia amygdalina* (VA*)* and *Gongronema latifolium* (GL) were collected from farmlands in Uhabiri Ossah Ibeku, Umuahia North Local Government Area, Abia State, Nigeria. These plants were identified by Prof. Garuba Omosun of the Department of Plant Science and Biotechnology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Nigeria.

Preparation of the samples

Leaves and stems of the samples were separated via hand-picking and washed with clean water. Then, stems were chopped into smaller pieces using clean stainless-steel knives. Plant samples were dried at room temperature (RT) and blended into a fine powder using blender. Extract was soaked in 80% methanol in conical flasks for 48 h, achieving a hydro-alcoholic crude extract with high yields. This was intermittently agitated every 3 h. Extract was filtered using Whatman no. 1 filter papers. Filtrate was concentrated at 40 °C using electric oven (Surgifriend Medical, UK) to enable complete elimination of methanol from the mixture forming a dark-green viscous extract. This was stored at 4 °C using vials tagged with the name of each plant leaves and stems. All samples were analyzed in triplicate.

$$\text{Yield} = \text{Weight of the sample extract/ weight of plant material} \times 100$$

Qualitative phytochemical screening

Vegetable leaf and stem extracts were subjected to phytochemical spot assay to assess presence of alkaloids, saponins, tannins, flavonoids, terpenes, glycosides and phenolics using the methods (22) described in (23).

Quantitative phytochemical screening

Quantitative analysis of detected phytochemicals was carried out for alkaloids, flavonoids, tannins, saponins, cardiac glycoside, terpenes and phenols using methods described by (24).

Antioxidant study

Assessment of 2,2-diphenyl-2-picryl hydrazyl (DPPH) free radical scavenging activities of the extracts of leaves and stems of OG, TO, GL and VA.

Antioxidant activities of the leaves and stems extracts of OG, TO, GL and VA were studied using methods (25) described by (26). The test extract (2 ml) was collected at various concentrations (25, 50, 100, 200 and 400 µg/ml). Each extract concentration was mixed with 0.5 mM DPPH (in 1 ml of methanol) using cuvettes. The absorbance at 517 nm was measured after 30 min of incubation at RT (27 °C) in dark. Concentrations were prepared in triplicates and the proportion of antioxidant activity was calculated as follows:

$$AA = 100 - [(\text{ABS sample} - \text{ABS blank}) \times 100\} / \text{ABS control}]$$

Where, ABS was the absorbance and AA was the antioxidant activity. Furthermore, 1 ml of methanol and 2.0 ml of the test extract were used as blank, while 1 ml of the 0.5-mM DPPH solution and 2 ml of methanol were used as negative control. Ascorbic acid (vitamin C) was the reference standard (24).

Ferric-reducing antioxidant power

Benzie and Strain (27) described the ferric-reducing antioxidant power (FRAP) in (25). The reagent composition included 1) acetate buffer (300 mM), pH 3.6 (3.1 g of sodium acetate. $3H_2O$ and 16 ml of glacial acetic acid in 1000 ml of buffer solution); 2) 2,4,6-triphridyl-s-triazine (TPTZ) (10 mM) in 40 mM of HCl; and 3) $FeCl3.6H_2O$ (20 mM) in distilled water (DW). A fresh working solution was prepared by mixing Solutions 1, 2 and 3 in a ratio of 10:1:1, respectively. Aqueous solution of a known quantity of ascorbic acid was used for calibration. The FRAP reagent (3 ml) and 100 µl of the sample solution at various concentrations of 25, 50, 100, 200 and 400 µg/ml were mixed together and set for 4 min. Colorimetric reading was recorded at 593 nm at 37 °C. The ascorbic acid standard was assessed as well. Calculations were carried out using calibration curve.

$$\text{FRAP value of sample (µmol/l)} = \frac{\text{Changes in ABS of the sample from 4 min} - \text{0 min}}{\text{changes in ABS of Standard 4 min} - \text{0 min}} \times \text{FRAP value of the std (2 µmol/l)}$$

(Where, ABS was the absorbance)

Vitamin analysis

The antioxidant vitamins (vitamins A, C and E) in the leaves and stems of various vegetables were assessed, using a method by the Association of Official Analytical Chemists (28) detailed by Bosha *et al*. (29).

Statistical analysis

Data were analyzed using Statistical Package and Service Solutions software (SPSS, USA). One-way analysis of variance (ANOVA) was used to show the mean differences between the groups and post hoc Duncan test was used to demonstrate mean differences between the groups. The means were separated using the least significant differences of various plants and significant differences were reported when $p < 0.05$. Pearson's correlation was used to compare the DPPH and FRAP assays at $p < 0.05$. Results of phytochemical and vitamin contents were analyzed using simple paired t-test. Data were reported as mean ±SD (standard deviation).

Results

Results of the mean qualitative phytochemical analysis of the plant samples

Results revealed that all the phytochemicals were present in most vegetable parts with various quantities (Table 1**)**. Terpenes were present in all the vegetables except OG, and alkaloids, saponin, tannins, flavonoids, glycosides and phenolics were present in all the vegetables. Results of the phytochemical comparative study between the two various parts of VA (leaves and stems) showed that glycosides were higher in the leaves (+++) than in the stems (++); tannins were higher in the stems (++) than in the leaves (+); and terpenes included similar levels in the leaves and stems (++). Flavonoids were higher in the stems (+++) than in the leaves (++) and saponins were higher in the stems (+++) than in the leaves (++). In TO, glycosides were higher in the leaves (+++) than in the stems (+); tannins were higher in the leaves (++) than in the stems (+); terpenes were higher in the stems (++) than in the leaves (+); and flavonoids were higher in the leaves (++) than in the stems (+). Saponins included similar levels in the leaves and stems. For the leaf extracts, it was reported that glycosides were strongly detected followed by terpenes, flavonoids and saponins. However, tannins were detected in traces. In stem extracts, constituent were present but not with similar quantities while saponins, tannins and terpenes were present with similar quantities, except for flavonoids and glycosides with trace quantities. The GL leaves and stems included the highest quantities of phenolics (+++) and other phytochemicals were present in moderate quantities (++), except for saponins in the leaves.

Quantitative phytochemical contents in the vegetables

Quantitative phytochemical contents of VA, TO, GL and OG are shown in Table 2. Alkaloids (3.52 ±1.05) and phenolics (3.57 ±0.02) included the highest phytochemicals in VA with the most significant leaf levels. In the stems, flavonoids and tannins were significantly higher, compared to other phytochemicals. Proportions of terpenes (0.41 ±0.01) and phenolics (2.29 ±0.02) were significantly high in TO leaves, while alkaloids, saponins, tannins and flavonoids were significant high in the stems of TO. Values of alkaloids, saponins, tannins, terpenes and phenolics were significantly high in the leaves of GL with the high concentrations of alkaloids (1.65 ±0.01), while flavonoids were significantly high in the stems of GL with a concentration of 2.34 ±0.02. In OG, alkaloids, saponins and phenolics were significantly higher than other phytochemicals with phenolics (6.16 ±0.02) including the highest concentrations in the leaves. However, flavonoids and terpenes were significantly high in the stems of OG. Technically, OG included higher concentrations of phenolics in the leaves, followed by the flavonoid contents in the stems (6.16 ±0.02 mg/100 g and 5.40 ±0.01 mg/100 g, respectively) (Table2).

Table 1. Results of the mean qualitative phytochemical analysis of the plant samples. Results revealed that all the phytochemicals were present in most of the vegetables. Terpenes were present in all the vegetables except OG and alkaloids, saponins, tannins, flavonoids, glycosides and phenolics were present in all the vegetables

Vegetable	Phytochemicals						
	Alkaloid	Saponin	Tannins	Flavonoid	Terpenes	Glycosides	Phenolics
VA							
Leaves	++	++	+	++	++	+++	+++
Stem	+	+++	++	+++	++	++	+++
TO							
Leave	+++	+	++	++	+	+++	++
Stem	+	+	+	+	++	+	+++
GL							
Leave	++	+	++	++	++	++	+++
Stem	++	++	+	++	++	+++	+++
OG							
Leave	+	++	+	++	-	+++	+++
Stem	+++	++	++	++	-	+	+++

Key: + (present), ++ (moderately present), +++ (strongly present), - (absent) **VA:** *Vernonia amygdalina*, **TO:** *Telfairia occidentalis*, **GL:** *Gangronema latifolium*, **OG:** *Ocimum gratissimum*.

Table 2. Quantitative phytochemical contents in the vegetables

Vegetables	Phytochemicals(mg/100g)						
	Alkaloid	Saponin	Tannins	Flavonoid	Terpenes	Glycosides	Phenolics
VA							
Leaves	3.52±1.05*	0.03±0.01	0.005±0.02	0.60±0.01	0.28±0.02	0.030±0.02	3.57±0.02*
Stem	3.14±1.03	0.03±0.01	0.030±0.01*	3.88±0.02*	0.18±0.01	0.030±0.01	1.67±0.02
TO							
Leave	0.87±0.03	0.01±0.00	0.003±0.01	1.21±0.02	0.41±0.01*	0.010±0.00	2.29±0.02*
Stem	1.01±0.01*	0.02±0.01*	0.020±0.01*	3.27±0.01*	0.28±0.01	0.010±0.00	0.52±0.01
GL							
Leave	1.65±0.01*	0.03±0.01*	0.03±0.01*	1.85±0.02	0.51±0.02*	0.010±0.00	1.03±0.01*
Stem	0.83±0.01	0.02±0.00	0.01±0.00	2.34±0.02*	0.13±0.00	0.001±0.00	0.03±0.01
OG							
Leave	3.39±0.01*	0.03±0.01*	0.010±0.00	0.83±0.01	0.38±0.01	0.008±0.00	6.16±0.02*
Stem	2.22±0.01	0.01±0.00	0.040±0.00*	5.40±0.01*	0.78±0.02*	0.003±0.00	4.93±0.02

Results are presented as mean ± standard deviation (n = 3) and values with * differ significantly from the paired sample within the column. **VA:** *Vernonia amygdalina*, **TO:** *Telfairia occidenta*, **GL:** *Gangronema latifolium*, **OG:** *Ocimum gratissimum*

Results for TO in Table 2 show that the plant included a significantly higher concentration of flavonoids (3.27 ±0.01 mg/100 g) in the stems, followed by phenolics in the leaves (2.29 ±0.02 g/100), compared to other phytochemicals. Furthermore, VO leaves (Table 2) included the highest concentration of alkaloids (3.52 + 1.05 mg/100 g), followed by OG leaves (3.39 ±0.01 mg/100 g). The most negligible concentration of alkaloids (0.87 + 0.03 mg/100 g) was reported in leaves of TO. Results for saponins showed high concentrations in the leaves and stems of VA. Recently, VA has been reported by other researchers to contain high concentrations of saponins (30, 31). Terpene concentration was maximum in the stems of OG (0.78 + 0.02 mg/100 g) and minimum in the stems of GL (0.13 ±0.00 mg/100 g). Except for OG, plant leaves included higher concentrations of terpenes than that plant stems did. Tannins and glycosides were included with significantly low concentrations in all the vegetables. However, Tannins were included with the maximum concentration in stems of OG (0.04 + 0.00 mg/100 g) and with the minimum in the leaves of OG (0.006 + 0.00 mg/100 g).

DPPH inhibitory activities of the TO, OG, GL and VA leaf and stem extracts

Results for DPPH assay of VA, TO, GL and OG are shown in Table 3. The VA showed the highest activity in the stems (75.74 ±0.09), while the leaves of OG showed the highest activity at 42.86 ±1.86 at 400 µg/ml. Data for TO and OG demonstrated no significant differences ($p > 0.05$) in leaves and stems at various concentrations, compared to ascorbic acid. However, values of GL and VA showed dose-dependent differences ($p < 0.05$), compared to the ascorbic acid group (Table 3).

FRAP activities of the TO, OG, GL and VA leaf and stem extracts

Results for the FRAP assay of VA, TO, GL and OG are shown in Table 4. Values of OG and GL showed no differences ($p > 0.05$) in the leaves and stems at various concentrations, compared to the control. However, TO and VA demonstrated dose-dependent increases ($p < 0.05$), compared to the control. Results for the FRAP assay present in Table 4 showed the highest values in the leaves and stems of OG (1.55 ±0.02 and 0.77 ±0.24 µm/fe(II)/l, respectively) at 400 µg/ml. However, no significant differences were observed in vegetable activities of stems and leaves at various concentrations. In general, values were significantly ($p < 0.05$) lower than that of ascorbic acid (Table 4).

Table 3. 2,2-Diphenyl-2-picryl hydrazyl inhibitory activities of TO, OG, GL and VA leaf and stem extracts

Conc. (µg/mL)	TO DPPH (%) (inhibition)			OG DPPH (%) (inhibition)			GL DPPH (%) (inhibition)			VA DPPH (%) (inhibition)		
	LEAF	STEM	ASCORBIC ACID	LEAF	STEM	ASCORBIC ACID	LEAF	STEM	ASCORBIC ACID	LEAF	STEM	ASCORBIC ACID
25	7.99±0.09a	9.47±0.15b	95.84+0.38c	9.22±0.14^a	6.16±0.38^b	95.51±0.18^c	6.18±0.45^a	5.82±0.20^a	95.51±1.82^b	9.19±0.25^a	14.55±0.27^b	95.51±0.18^c
50	9.06±0.19a	10.22±0.21b	95.55+0.14c	12.75±0.39^a	8.00±0.04^b	95.55±1.44^c	6.26±0.42^a	7.18±0.10^a	95.55±1.44^b	11.48±0.20^a	18.26±0.16^b	95.55±0.14^c
100	12.58±0.05a	9.69±0.40b	95.85+0.20c	16.04±0.32^a	11.78±0.32^b	95.85±0.20^c	8.55±0.40^a	11.75±0.74^b	95.85±0.20^c	15.99±0.49^a	25.70±0.36^b	95.85±0.20^a
200	17.08±0.17a	10.08±0.30b	95.15+0.86c	27.90±0.19^a	18.62±0.42^b	95.15±0.08^c	8.62±0.33^a	13.41±0.45^b	95.15±0.87^c	23.77±0.13^a	42.04±0.28^b	95.15±0.09^c
400	26.11±0.14a	12.26±0.18b	95.35+0.16c	9.22±0.14^a	6.16±0.38^b	95.51±0.18^c	13.8±0.29^a	21.91±0.11^b	95.35±0.16^c	38.50±0.31^a	75.74±0.51^b	95.35±0.16^c
IC50	12.01±3.30	75.90±0.50	333.06±0.14	11.01±3.46	40.53±2.36	333.06±0.14	26.48±1.39	12.89±2.84	333.06±0.14	7.26±5.30	218.39±11.17	333.06±0.14

The results for the DPPH assay of VA, TO, GL and OG are shown in Table 3. The data for TO and OG showed no significant difference (P>0.05) in the leaf and stem at different concentrations compared to ascorbic acid. However, the values of GL and VA showed a dose-dependent difference (P<0.05) compared to the ascorbic group.

Means on the same row with different letter superscripts are significantly different (*P<0.05*). **VA:** *Vernonia amygdalina*, **TO:** *Telfairia occidentalis*, **GL:** *Gangronema latifolium*, **OG:** *Ocimum gratissimum*

Table 4. Ferric-reducing antioxidant power activities of TO, OG, GL and VA leaf and stem extracts

Conc. (µg/mL)	TO FRAP (µM)			OG FRAP (µM)			GL FRAP (µM)			VA FRAP (µM)		
	LEAF	STEM	ASCORBIC ACID	LEAF	STEM	ASCORBIC ACID	LEAF	STEM	ASCORBIC ACID	LEAF	STEM	ASCORBIC ACID
25	0.11±0.06^a	0.22±0.02^a	0.05±0.00^b	0.26±0.15^b	0.43±0.03^a	0.05+0.00^c	0.24±0.12^b	0.32±0.01^a	0.05+0.00^c	0.15±0.07^a	0.24±0.00^a	0.05+0.00^b
50	0.29±0.01^a	0.37±0.02^b	1.0±0.00^c	0.78±0.29^a	0.32±0.08^b	1.00+0.00^c	0.19±0.01^b	0.41±0.07^a	1.00+0.00^c	0.32±0.02^a	0.25±0.00^b	1.00+0.00^c
100	0.36±0.02^a	0.23±0.03^b	2.00±0.03^c	0.72±0.00^b	0.55±0.03^c	2.00+0.00^a	0.25±0.01^b	0.30±0.16^a	2.00+0.00^c	0.39±0.01^a	0.27±0.03^b	2.00+0.00^c
200	031±0.01^a	0.21±0.03^b	4.00±0.00^c	0.86±0.18^b	0.61±0.00^c	4.00+0.00^a	0.46±0.07^b	0.23±0.02^c	4.00+0.00^a	0.27±0.01^a	0.33±0.02^b	4.00+0.00^c
400	0.24±0.00^a	0.25±0.05^a	8.00±0.00^b	0.26±0.15^b	0.43±0.03^a	0.05+0.00^c	0.49±0.15^a	0.59±0.30^b	8.00+0.00^c	0.47±0.08^a	0.33±0.23^a	8.00+0.00^b
IC50	**	**	27.86±1.41	**	**	27.86±1.41	**	**	27.86±1.41	**	**	27.86±1.41

The result for the FRAP assay of VA, TO, GL and OG are shown in Table 4. The values of OG and GL showed no difference (P>0.05) in the leaf and stem at different concentrations compared to the control. However, TO and VA showed a dose-dependent increase (P<0.05) compared to the control.
Means on the same row with different letter superscripts are significantly different (*P<0.05*). **VA:** *Vernonia amygdalina*, **TO:** *Telfairia occidentalis*, **GL:** *Gangronema latifolium*, **OG:** *Ocimum gratissimum*.** indicates IC50 > 1000

Correlation analysis between the antioxidant activities of DPPH and FRAP assays in stem and leaf extracts of the plants

Pearson correlation coefficient (*r*) was computed to assess linear relationships between DPPH and FRAP. Positive correlations were detected between the free radical, DPPH scavenging ability and FRAP in stem and leaf extracts as follows: $r(5) = 0.82$, $p = 0.09$ and $r(5) = 0.77$, $p = 0.13$ for OG; $r(5) = 0.87$, $p = 0.05$ and $r(5) = 0.72$, $p = 0.17$ for VA; $r(5) = 0.85$, $p = 0.12$ and $r(5) = 0.76$, $p = 0.18$ for TO; $r(5) = 0.60$, $p = 0.29$; $r(5) = 0.56$ and $p = 0.35$ for GL in stems and leaves of all vegetable samples, respectively.

Table 5. Vitamin contents of the selected vegetable samples

Vegetables	Vitamin contents (mg/100g)		
	Vitamin A	Vitamin C	Vitamin E
V. amygdalina			
Leaf	75.88±7.20	443.62±1.62*	50.24±1.28*
Stem	80.16±15.92*	394.31±6.72	18.71±0.29
T. occidentalis			
Leaf	84.21±3.37	439.12±11.36	54.08±1.05*
Stem	99.21±6.59*	447.30±2.45*	15.61±0.44
G. latifolium			
Leaf	82.23±17.86	395.21±84.44	20.12±0.16
Stem	101.94±11.33*	431.77±3.948*	24.40±0.24
O. gratissimum			
Leaf	63.29±48.10	529.00±25.70	50.95±1.42
Stem	84.42±11.20*	649.00±13.08*	52.30±0.64

Results are presented as mean ± standard deviation (n = 3) and values with * are significantly different from the paired sample within the column. The result for the vitamin assay of VA, TO, GL and OG are shown in Table 5. The values of Vitamin C were significantly higher (P>0.05) than Vitamin A and Vitamin E analyzed in all the samples. Vitamin C content were highest in the stems than the leaves, with very significant amounts(P>0.05) in stem of GL. Vitamin A in stems of VA, TO, GL and OG showed significant difference (P>0.05). However, Vitamin E had the least amounts indicating no significant difference in the leaves and stems of GL and OG.

Vitamin contents of the selected vegetable samples

Antioxidant vitamins (A, C and E) are well detected in fruits and vegetables. Antioxidant vitamins play roles in fight against oxidative damages caused by free radicals in biological systems. Stems of GL provided better sources of vitamin A (101.94 ±11.33). Generally, plants possessed high quantities of vitamin A, compared to vitamins C and E. Vitamin C was predominant in stems (649.00 ±13.08) and leaves (529.00 ±25.70) of OG followed by TO stems (447.30 ±2.45). The minimum quantity was detected in GL leaves (395.21 ±84.44) of OG showed high concentration of vitamin E (Table 5).

Discussion

Researchers have reported phytochemicals as good players in fight against deleterious effects of free radicals in biological systems, showing great potentials against chronic diseases such as diabetes and cancers (32–34). These therapeutic effects are attributed majorly to antioxidant effects of these phytochemicals, enabling them able to modulate activities of free radicals and other drivers of physiological alterations in several pathological conditions, including cancers (35), diabetes (36) and viral diseases (37). In the present study, leaf and stem samples were analyzed in triplicate (Table 1), showing qualitative phytochemical compositions of VA, GL, OG and TO as mean ±SE (standard error). Alkaloids, flavonoids, tannins, glycosides, terpenes, saponins and phenolics were present in leaves and stems of the four vegetables at various concentrations. Variations in contents might be due to the certain factors such as the plant parts, ages and seasonal variations. This is because the plant growth and development as well as its nature and quantity of secondary metabolites are naturally affected by temperature, rainfall, length of day (quantity of light) and altitude. For example, sunlight regulates quantities of glycosides and alkaloids in plants. Furthermore, continuous rain can lead to leak of water-soluble substances from leaves and roots by leaching

in plants producing alkaloids, glycosides and volatile oils (38).

Quantitative phytochemicals in Table 2 were correlated with qualitative phytochemicals in Table 1. In Table 2, the highest concentration of phytochemicals included phenolics in the leaves of OG, followed by the flavonoids in the OG stems. Generally, phenolics and flavonoids are the major antioxidant components and directly proportional to antioxidant activities in several studies (39). Although, flavonoid results were in contrast to the results of Anthony and Ojeifo (40), who reported absence of flavonoids in the stems of aqueous extracts of TO. It is noteworthy that several factors can affect the phytochemical composition of plant components, including processing methods (41), extraction solvents (42) and extreme temperatures (43). Phenolic compounds in leaves are generally not transported by phloem channels due to the alkalinity of the sap, which makes them quickly oxidized and accumulated as quinones, instead of moving into the stems in their original forms (44). This could be the reason for the lower phenol levels in the stems. Phenols include strong antioxidant capacities in living systems. They participate in oxidation-reduction reactions, which help quench the ravaging actions of unstable oxygen radicals on biological systems, hence protecting human health (45). In addition to their free radical scavenging characteristics, phenols prompt beneficial pharmacological effects by selectively modulating intracellular signaling pathways necessary for cell development, proliferation and apoptosis (46). The high flavonoid content of this plant stems shows that incorporating stems of TO as part of the vegetables used in preparing dishes may help manage CVDs and other chronic diseases (47). These benefits appear to be resulted from improved nitric oxide homeostasis, endothelial function, platelet aggregation and oxidative stress reductions. Flavonoids include great significance in synthesizing vitamins B and K and the breakdown of xenobiotics, bile acids and sterols. These characteristics may be responsible for the beneficial antipathogenic roles of flavonoids and their significance during intestinal epithelium development and antibody production (47). The comparatively higher concentration of flavonoids in plant stems compared to leaves is linked to the reports of Bassey *et al.* (48) on ethanol extract *Sida* L. and Ani and Okolie (49) on ethanol and aqueous extracts of *Azadirachta indica*; as shown in the current study.

In this study, alkaloid concentrations were significantly highest in leaves than stems in all the vegetables. The high concentration of alkaloids in the leaves of plants in this study was similar to those available in the literature on the alkaloids content of the leaves of OG (50, 51), VA (52, 53), TO (54, 55) and GL (56). Alkaloids have been reported to form in higher concentrations in leaves than in stems of other plants. A review by Rajbir and Saroj (57) discussed biological roles of alkaloids, secondary plant metabolites that play essential roles in pharmacological activities, including antimalarial, antiasthma, analgesic anticancer, vasoconstriction, hallucinogenic, anti-acetylcholinesterase, antipyretic and aphrodisiac activities (58). Saponin has shown antioxidant characteristics against such stressors, as well as potencies in antimicrobial effects. After comparing results of saponins for leaves and stems of the vegetables, it can be concluded that they might be beneficial in modulating optimally biological mechanisms (59). Although TO leaves and OG stems indicated the lowest concentration of saponin, most secondary metabolites are synthesized and stimulated upon challenge by biotic or abiotic stresses (60).

Results of research on terpenes are in contrast to those by Mgbemena and Amako (61), who reported higher concentrations of terpenes in VA compared to OG. Terpenes are polymers of isoprenoid units. Cox-Georgian *et al.* (62) explained biomedical roles of terpenes to involve various health-restoring benefits to humans and animals in exerting calming effects in wound healing, anti-inflammatory responses and anti-cancer activities. These chemicals seem to include antiplasmodial effects as well; thus, they may play similar roles in slowing malaria onset (62, 63). Tannins are a group of polyphenols and their nutritional and antinutritional characteristics as blood-clotting agents, blood pressure regulators, hypolipidemic agents and modulators of immune responses have been reported (64).

In this study, glycosides were detected at the highest values in leaves and stems of VA (0.03 + 0.02 g/100 g and 0.03 + 0.01 g/100 g), respectively. Their low occurrence in leaves showed that these chemicals might not be good sources of glycosides as they are further available in fruits and grains (65). Onaolapo and Onaolapo (65) reported in their epidemiological study of patients with heart failure that VA positively affected cardiac crisis retardation and demonstrated its potential as an anticancer agent. *In vitro* antioxidant activity study using DPPH and FRAP assays (Tables 3 and 4 respectively) indicated that the plant materials included dose-dependent antioxidant characteristics. Hence, the higher consumption of the vegetable parts in this study (stems and leaves), the stronger the antioxidant potential of the biological systems to inhibit oxidizing effects of free radicals.

For the DPPH assay, radical scavenging characteristics of the samples were improved with higher concentrations, especially in the stem methanolic extracts. However, leaves of VA and GL were higher than those of stems at similar concentrations. Results of the current study were in contrast to the results of Ganguly *et al.* (66), who reported that the extracts from the stems of OG included more radical scavenging characteristics than those from the leaves did. This might be due to their high flavonoids,

saponins and alkaloids contents and other phytochemicals, which synergistic effects are able to boost antioxidant ability of the vegetable samples (67, 68) (Tables 1 and 2). Furthermore, strong radical DPPH inhibition effects of the methanol extracts were demonstrated at its lower IC50 value, compared to the standard. This effect is technically depend on the solvent extraction types, solvent and extract polarities, separation methods and active component purification and detection methods (41, 69, 70).

In general, FRAP is characterized by the reduction of ferric to ferrous ions (2). Therefore, activity of OG could be attributed to its rich phenolic and flavonoid contents. In this study, OG and VA leaves and stems could be adopted based on their antioxidant and phytochemical abilities, they might be effective in stimulating releases of endogenous antioxidants and thus combat free radical-induced diseases (2, 70). The present study has revealed that the vegetable extracts certainly include proton-donating abilities and can act as radical scavengers or inhibitors.

In this study, Pearson correlation analysis between the antioxidant assays for the stem and leaf extracts at various concentrations reported positive relationships, showing strong antioxidant abilities of the leaves and stems from the vegetable samples. However, the coefficient of determination (R_2) for FRAP values against the concentrations indicated weak linear correlations, reflected in their IC50 > 1000 which was significantly higher than that of ascorbic acid as the standard. This indicated that no single assay can accurately explain the mechanism of oxidant/radical sources or antioxidants present in a test sample (71).

Antioxidant vitamins (A, C and E) are present in vegetables, including great effects on human health. They are essential for biometabolism because of their oxidation/reduction reactions and key roles as enzymatic cofactors. Table 5 shows that vitamin C was the most available vitamin in the stems and leaves, followed by vitamins A and E. Leaves of OG, GL and VA have been reported to include high vitamin C concentrations (51), similar to the present study. Stems of GL included the highest vitamin A concentration (101.94 + 11.33 mg/100 g). Vitamin C included the maximum concentration in the stems (649.00 + 13.08 mg/100 g) and leaves (529.00 + 25.70 mg/100 g) of OG. Stems and leaves of OG showed high concentrations of vitamin E, which further boosted high DPPH radical scavenging activities. This could be linked to the high phenolic contents of the leaves and stems (72).

Antioxidant vitamins play significant roles in fight against oxidative damages caused by free radicals in biological systems. It has been reported that edible plants containing vitamins E, A and C can prevent or delay the onset of cancers (24, 73, 74). Vegetables contain significant quantities of vitamins in various leaf and stem parts and hence consumption of vegetables is essential in human diets as antioxidant agents. Thus, vegetable consumption should be recommended for the consumption by people to improve nutrition and derive health benefits. This study did not include other solvent extracts, antioxidant assays, detection methods and characterization of the bioactive compounds in leaves and stems of TO, OG, VA and GL. However, further studies are necessary to verify the current results.

CONCLUSION

The four vegetable leaves and stems significantly included phenolic, alkaloid and flavonoid concentrations. The DPPH in leaves of TO and OG respectively showed higher activities at 100, 200 and 400 µg/ml than that in stems did, in contrast to GO and VA. The FRAP indicated the highest OG values in leaves and stems at various concentrations. Stems and leaves included high concentrations of antioxidant vitamins, especially vitamin C, which was the highest in stems and leaves of OG. Hence, combining stems and leaves as vegetables in dishes can boost nutritional and antioxidant values of the human diets. Moreover, synergistic use of stems and leaves in modification of phyto-supplements by pharmacological industries can boost its potential antioxidant activity via fortification of the biological antioxidant systems against oxidative stress-induced chronic diseases. Further characterizations of the bioactive components are recommended, using various detection methods in various solvent extracts of these rich vegetables.

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