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Full Length Research Paper

Total antioxidant activity, phenolic, flavonoid and ascorbic acid contents of Nigerian vegetables.

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Antioxidant activities, total phenolic, flavonoid and ascorbic acid contents of different vegetables commonly consumed in Nigeria were determined. The antioxidant activities of vegetables ranged from 22.15% (*Talinum triangulare*) to 92.30% (*Capsicum frutesceus*). *Solanum macrocarpon,* with the lowest IC_{50}, is the most potent vegetable of the samples analyzed, that could scavenge most free radicals; while *Cucumis sativus,* with the highest IC_{50}, is the least potent. Total phenolic, flavonoid and ascorbic acid contents of vegetables ranged from 22.1 to 370.68 mg quercetin g^{-1}; 10.23 to 215.39 mg quercetin g^{-1} and between 16.67 to 150.67 mg ascorbic acid g^{-1}, respectively. A high and significant correlation existed between antioxidant activity and total phenolic content of vegetables (r^2 = 0.861, p < 0.05), indicating that total phenolic content is the major contributor to the antioxidant activity of vegetables. However, flavonoids, which belong to the phenolic compounds, were not significantly correlated with antioxidant activity (r^2 = 0.143, p < 0.05). Ascorbic acid fairly correlated (r^2 = 0.546, p < 0.05) with antioxidant and phenolic content (r^2 = 0.591, p < 0.05).

Keywords: Antioxidant activity, total phenolics, total flavonoids, ascorbic acid, vegetables,

INTRODUCTION

Our body is exposed to a large number of foreign chemicals everyday (Santhakumari *et al,* 2003). The most of which are man-made and our inability to properly metabolize them negatively affects our health by the generation of free radicals. Free radicals are also generated during normal metabolism of aerobic cells (Carmen and Florin, 2009; Ghaseme *et al,* 2009; Li *et al,* 2008; Hunag *et al,* 2005; Zaporozhets *et al*, 2004; Odukoya *et al*, 2007). The oxygen consumption inherent in cells growth leads to the generation of series of oxygen free radicals. Highly active free radicals and their uncontrolled production are responsible for numerous pathological processes such as cell tumour (prostate and colon cancers) and coronary heart diseases (Karadenz *et al,* 2005; Barros *et al,* 2007; Chanwitheesuk *et al,* 2005; Marinova *et al,* 2005; Jagadish *et al*, 2009).

Antioxidants can significantly delay or prevent the oxidation of easily oxidizable substances (Atrooz, 2009; Kim *et al,* 2009). Natural antioxidants are classified according to their mechanism of action as chain-breaking antioxidants which scavenge free radicals or inhibit the initiation step or interrupt the propagation step of oxidation of lipid and as preventive antioxidants which slow the rate of oxidation by several actions but do not convert free radicals (Ou *et al,* 2002; Thaipong *et al*, 2006; Ebrahinzadeh *et al*, 2008; Semalty *et al,* 2009; El-Qudah, 2008; Hodzic *et al*, 2008; Othman *et al,* 2007; Temraz and Hel-Tantawy, 2008; Ahmad and Beigh, 2008). However; there have been concerns about synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) because of their possible activity as promoters of carcinogenesis (Rahman *et al*, 2008). There is growing interest toward natural antioxidants from herbal sources (Larson, 1998; Gazzani *et al*, 1988; Velioglu *et al*, 1988). Epidemiological and *in vitro* studies on medicinal plants and vegetables strongly have supported the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in

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biological systems (Cao *et al*, 1996; Block and Patterson, 1992; Ness and Powles 1997).

Vegetables and fruits contain high concentration of numerous redox-active antioxidants such as polyphenols, carotenoids, ascorbic acids, tocopherol and flavonoids which fight against hazardous oxidative damage of plant cells (Odukoya, 2007; Karadenz *et al,* 2005; Ou *et al,* 2002; El-Qudah, 2008). In animals, antioxidants production is much more limited and generation of free radicals during metabolism beyond the antioxidant capacity has been implicated in the pathogenesis of most diseases. Thus, the consumption of dietary antioxidants from vegetables and fruits is beneficial in preventing these diseases (Sumazian *et al,* 2010; Faujam *et al*, 2009; Magdalena *et al,* 2009).

Owing to the relationship between free radical scavenging capacity of vegetables and fruits, many analytical methodologies have been published for the determination of antioxidant ability. Phenolics in fruits have been monitored by HPLC (Tung *et al,* 2007) or colorimetrically using Folio-ciocalteu reagent (Faujam *et al,* 2009; Magdalena *et al,* 2009). Several assays have been used to evaluate total antioxidant capacity of foods and food products including spectrophotometric methods using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) (Ghaseme *et al,* 2009; Li *et al,* 2008; Odukoya *et al,* 2007; Jagadish *et al*, 2009; Atrooz, 2009; Kim *et al,* 2009; Semalty *et al,* 2009; Ahmad and Beigh, 2008); 2, 2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^+$) (Ou *et al,* 2002; Thaipong *et al,* 2006); ferric reducing power (FRAP) (Atrooz, 2009; Kim *et al,* 2009; Ou *et al,* 2002; Thaipong *et al,* 2006); oxygen radical absorbance capacity (ORAC) (Atrooz, 2009; Kim *et al,* 2009; Ou *et al,* 2002; Thaipong *et al,* 2006; Ebrahinzadeh *et al,* 2008; Semalty *et al,* 2009; El-Qudah, 2008); the β-carotene linoleate model (Barros *et al,* 2007); voltammetry and amperometic methods (Magdalena *et al,* 2009).

Our objectives were to (1) determine the total antioxidant activity, phenolic, flavonoid and ascorbic acid contents of commonly consumed vegetables in Nigeria and identify which of these vegetables has the highest free radical scavenging activity; (2) to determine level of correlation of these measured parameters.

MATERIALS AND METHODS

Sampling procedures

We used in this study fifteen vegetables, *Vernonia amygdalina, Brassica oleracea, Cucumis sativus, Murraya koenigii, Telfaria occidentalis, Basella alba, Amaranth caudatus, Corchorus olitorius, Ocinum gratissimum, Capsicum frutesceus, Spinacia oleracea, Talinum triangulare, Solanum macrocarpon, Allium cepa and Lycopersicon esculentum* bought from various markets in Osogbo and identified by Dr Awodoyin from Botany Department (Fountain University, Osogbo).

Chemicals

Standards: BHA (butylated hydroxyanisol), α-tocopherol, L-ascorbic acid, Quercetin, Folin-ciocalteu's phenol, 2,2-diphenyl-1-picrylhydrazyl (DPPH) were all purchased from Sigma-Aldrich, Germany. Sodium carbonate, Aluminium chloride, 2, 6-dichlorophenolindophenol and methanol were purchased from BDH Poole, England. All the chemicals used were of analytical grade. Deionized distilled water (ddH_2O) was used throughout the experiment. Jenway 6405 UV-Visible Spectrophotometer by Buch Scientific Inc.USA was used for analysis.

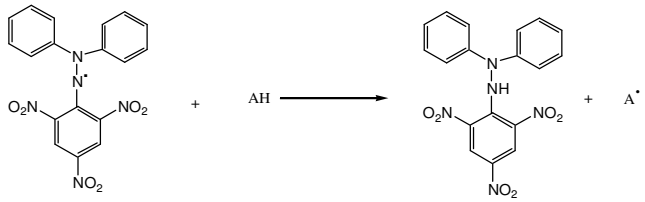
Extraction

The samples were cut into pieces and lyophilized with Lyotrap freeze drying machine (LTE Scientific Ltd UK) to remove the moisture content. Lyophilization was used to give the samples uniform moisture removal and submit the products for analysis in similar form. Resulting dried samples were powdered using Moelinux blender.

Precisely, 1g of ground lyophilized sample was weighed and extracted twice with a total volume of 100 mL of 70% aqueous methanol. The mixture was shaken on an orbital shaker (Stuart SSLI, Barlword Scientific Ltd Britain) for 75 min at 300 rpm and then filtered through Whatman No. 4 filter paper. The combined methanolic extract was then evaporated at 40°C using rotary evaporator (R205D, Shensung Biological Science & Technology, China) to dryness and then dissolved in absolute methanol for analysis. The plant's parts used in this study and their uses are given in Table 1.

DPPH Radical Assay

The hydrogen atom or electron donating abilities of the corresponding extracts and some pure compounds were measured from the bleaching of the purple-coloured methanolic solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as shown in the equation below.



Purple Bleached

One mL of various concentrations of the extracts in methanol was added to 4 mL of 0.1 mmol L^{-1} methanolic solution of DPPH. A blank probe was obtained by mixing 4 mL of 0.1 mmol L^{-1} methanolic solution of DPPH and 200 µL of deionized distilled water (ddH_2O). After 30 min. of incubation in the dark at room temperature, the absorbance was read at 517 nm against the prepared blank. Inhibition of free radicals by DPPH in percent (I %) was calculated using this formula:

$$I(\%) = \left[\frac{(A_{blank} - A_{sample})}{A_{blank}}\right] x\ 100 \qquad (1)$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compund and A_{sample} is the absorbance of the test compound

Table 1: Plants parts used in this study and their uses

Botanical name	Vernacular/Common name	Part used for this study	Uses
Vernonia amygdalina	Ewuro/ Bitter leaf	Leaves	Soup making
Brssica oleracea	Cabbage	Leaves	Eaten raw as salad
Cucumis sativus	Cucumber	Leaves	Eaten raw as salad
Murraya koenigii	Curry leaf	Leaves	As condiment
Telfaria occidentalis	Ugu/Pumpkin leaf	Leaves and stem	Soup making
Basella alba	Amunututu/Green leaf	Leaves and stem	Soup making
Amaranth caudatus	Tete/ Green Amaranth	Leaves and stem	Soup making
Corchorus olitorius	Ewedu/ Jute mallow	Leaves and stem	Soup making
Ocinum gratissimum	Efinrin/ Mint leaf	Leaves	As condiment and herb
Capsicum frutesceus	Ata rodo/Red pepper	Fruit	Soup making
Spinacia oleracea	Soko/Spinach	Leaves and stem	Leaves used in soup making
Talinum triangulare	Gbure/Water leaf	Leaves and stem	Leaves used in soup making
Solanum macrocarpon	Gbagba/Egg plant leaf	Leaves and stem	Leaves used in soup making
Allium cepa	Alubosa pupa/Onion	Bulb	Eaten raw and soup making
Lycopersicon esculentum	Tomato	Fruit	Eaten raw and soup making

L-ascorbic acid, Quercetin, BHA and α-tocopherol were used as standard controls. IC_{50} values denote the concentration of sample which is required to scavenge 50% of DPPH free radicals.

Total phenolics, total flavonoids and ascorbic acids

Total phenolics were determined using Folin-Ciocalteu method of Jagadish *et al.,* (2009) with slight modification. The methanolic extracts (0.5 mL) were added to a 25 mL volumetric flask filled with 10 mL ddH_2O and 2.5 mL of 0.2 N Folin-Ciocalteu phenol reagent. A reagent blank using ddH_2O instead of sample was prepared. After 5 min., 2 mL of 2% Na_2CO_3 solution were added with mixing. The solution was diluted to the volume (25 mL) with ddH_2O and then allowed to stand for 90 min., and the absorbance was measured at 780 nm versus the prepared blank. Quercetin was used as standard for the calibration curve. Total phenolic contents were calculated as mg quercetin g^{-1} dry weight of sample.

The $AlCl_3$ method (Jagadish *et al,* 2009) was used for the determination of the total flavonoid content of the sample extracts. The methanolic extracts (1.5mL) was added to 10 mL volumetric flask filled with 5 mL ddH_2O and 0.3 mL 5% $NaNO_2$ and mixed. A reagent blank using ddH_2O instead of sample was prepared. After 5 min., 1.5ml of 2% methanolic $AlCl_3$ solution was added. Two mL of 1 mol dm^{-3} NaOH was added 5 min. later and then the volume was made up to 10 mL with ddH_2O. The mixture was vigorously shaken on orbital shaker for 5 min. at 200 rpm and after 10 min. of incubation the absorbance was read at 367nm. Flavonoid contents were calculated using a standard calibration curve, prepared from Quercetin. The flavonoid contents were expressed as mg quercetin g^{-1} of extract.

Ascorbic acid was determined using the method described by Barros *et al* (2007). The methanolic extract was diluted with 10 mL of 0.5% oxalic acid and the mixture was shaken for 45 min. on orbital shaker at 200 rpm at room temperature and filtered through Whatman No. 4 filter paper. Precisely 1 mL of the filtrate was mixed with 9 mL of 0.1mol L^{-1} of 2, 6-dichlorophenolindophenol. A reagent blank using ddH_2O instead of sample was prepared. The absorbance was read within 30 min at 515 nm against the prepared blank. The ascorbic acid content was calculated using the calibration curve, prepared from L-ascorbic acid.

Statistical Analysis

Experimental results were expressed as mean ± standard deviation. All measurements were replicated three times. The data were correlated using Pearson correlation coefficient at $p < 0.05$. The IC_{50} values were calculated using linear regression analysis.

RESULTS AND DISCUSSION

Total antioxidant activity

The antioxidant studies of different vegetables in Nigeria have been done. This study focused on total antioxidant activity in Nigerian local vegetables (Table 2). Total antioxidant activity of the vegetables ranged from 22.15% for *Talinum triangulare* to 92.30% for *Capsicum frutesceus*. *Solanum macrocarpon* is the most potent vegetable of all that could scavenge most free radicals as shown by the lowest IC_{50} value while *Cucumis sativus* with the highest IC_{50}, is the least potent (Table 2). The IC_{50} of *Solanum macrocarpon* (6.21 mg mL^{-1}) compared to standards: L-ascorbic acid (IC_{50} = 2.60 mg mL^{-1}), Quercetin (IC_{50} = 1.31 mg mL^{-1}), α-tocopherol (IC_{50} =13.20 mg mL^{-1}) and BHA (IC_{50} = 3.36 mg mL^{-1}), shows that it can scavenge more free radicals than α-tocopherol. The total antioxidant activity obtained in this study were comparable with those obtained by Marinova *et al.,* (2005) but higher than that of Odukoya *et al* (2007). This could be due to methods used for the analysis and the medium of extraction as pointed out by Li *et al.,* (2008).

Relevant antioxidant activities

Relevant antioxidant compounds such as total phenolic, total flavonoid and total ascorbic acid contents were

Table 2: Antioxidant activity, flavonoid, phenolics and ascorbic acid contents of the vegetables studied

Botanical name/Standards	% Yield	Flavonoid content[a]	Phenolic content[a]	DPPH antioxidant[b]	Ascorbic acid[c]	IC_{50}[d]
Vernonia amygdalina	33	216.33±2.89	238.4±5.24	66.73±0.3	98.81±1.54	12.37
Brssica oleracea	63	19.29±2.2	22.1±2.95	31.88±0.27	18.32±1.2	49.62
Cucumis sativus	80	62.43±5.1	101.33±13.05	28.19±0.13	16.67±0.14	71.14
Murraya koenigii	26	243.59±4.44	327.43±7.65	88.43±0.05	150.67±3.21	7.35
Telfaria occidentalis	36	117.25±2.11	251.85±12.83	77.02±0.08	150.34±1.41	11.67
Basella alba	42	26.53±3.57	81.11±6.55	30.49±0.05	47.74±0.4	34.45
Amaranth caudatus	20	69.67±1.15	186.67±67	32.71±2.62	60.81±2.14	15.81
Corchorus olitorius	30	81.38±0.07	200.03±16.07	63.34±0.11	93.93±0.44	11.84
Ocinum gratissimum	25	105.2±5.66	252.2±4.1	72.11±0.04	69.34±2.41	8.67
Capsicum frutesceus	54	24.78±4.2	370.38±6.42	92.30±0.14	135.61±3.11	14.04
Spinacia oleracea	29	139.63±2.71	204.70±5.22	57.3±0.49	38.21±0.04	12.63
Talinum triangulare	36	81.48±6.41	49.26±4.76	22.15±0.17	29.27±1.2	40.51
Solanum macrocarpon	20	215.39±15.5	256.67±13.34	80.59±0.9	111.2±0.01	6.21
Allium cepa	72	10.23±1.93	225.93±8.48	76.92±0.3	35.52±0.02	23.41
Lycopersicon esculentum	48	12.62±0.14	246.88±5.93	70.39±0.08	37.67±0.51	17.05
α-tocopherol				19.07		13.20
L-Ascorbic acid				96.26		2.60
Quercetin				95.24		1.31
BHA				89.28		3.36

Each value is expressed as mean ± standard deviation (n=3); [a]mg quercetin/g of extract; [b]% of methanolic radical scavenging activity; [c]mg ascorbic acid/g of extract; [d]mg /mL of effective concentration at which 50% of DPPH radicals are scavenged;

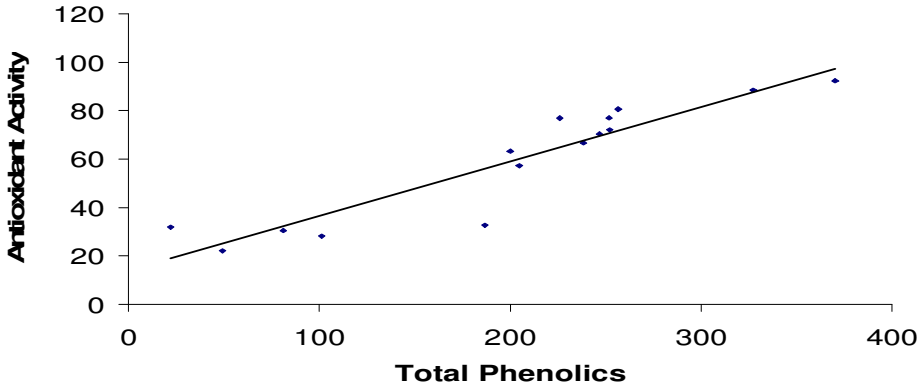


Figure 1. Correlation between antioxidant activity and total phenolics, (r^2 = 0.861).

successfully analyzed from local Nigerian vegetables. The different antioxidant activities of the vegetables can be ascribed to their total phenolic concentrations. When comparing the data in Table 2, *Capsicum frutesceus* had the highest phenolic content (370.68 mg quercetin g^{-1}) followed by *Murraya koenigii, Solanum macrocarpon, Ocinum gratissimum, Telfaria occidentalis*, *Lycopersicon esculentum*, *Vernonia amygdalina*, *Allium cepa, Spinacia olerace, Corchorus olitoriusa, Amaranth caudatus*, *Cucumis sativus, Basella alba, Talinum triangulare* and *Brassica oleracea,* this later had the least phenolic content (22.1 mg quercetin g^{-1}). Several comprehensive works have been done on the effects of phenolic compounds on total antioxidants (Li *et al,* 2008; Magdalena *et al,* 2009; Ghaseme *et al,* 2009; Jagadish *et al,* 2009; Atrooz, 2009; Kim *et al,* 2009; Semalty *et al,* 2009 and Ebrahinzadeh *et al,* 2008), and correlations between phenolic compounds and total antioxidants (Bin Li *et al,* 2008; Barros *et al,* 2007; Chanwitheesuk *et al,* 2005). This same trend was also obtained in our study. There was a good linear correlation (r^2 = 0.861, $p < 0.05$) between the total phenolic content and the scavenging of DPPH radical in each extract (Figure 1). These results indicated that the radical scavenging capacity of each

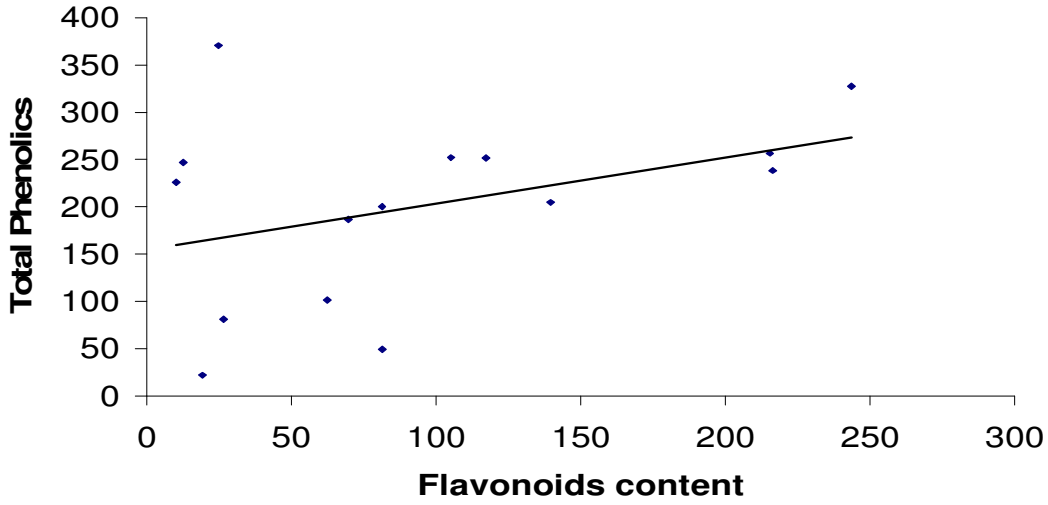


Figure 2. Correlation between total phenol and flavonoid content, (r^2 = 0.1477).

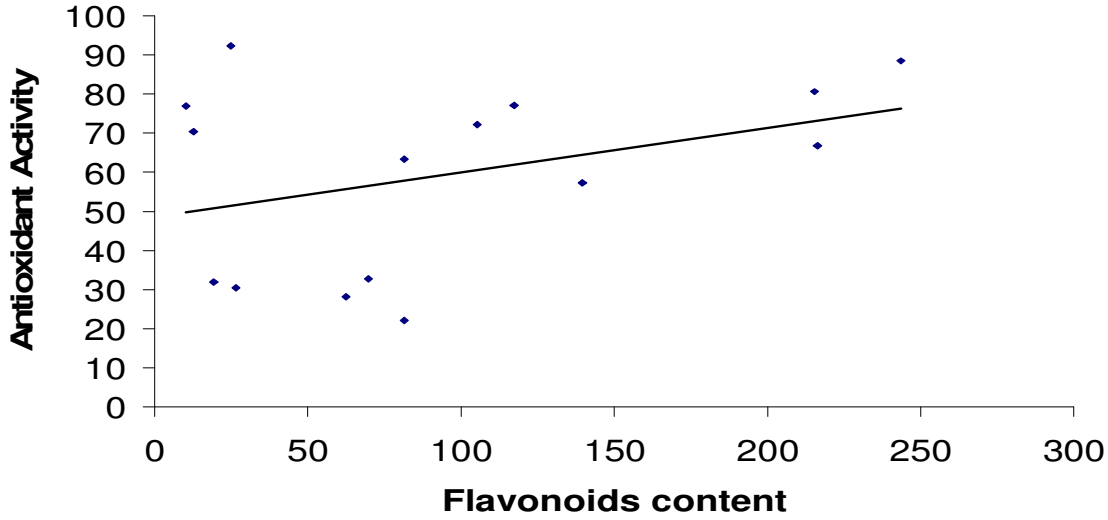


Figure 3. Correlation between antioxidant activity and flavonoid content, (r^2 = 0.1373).

extract might be mostly related to their concentration of phenolic hydroxyl group. The antiradical activity of phenolic compounds depends on their molecular structure, on the availability of phenolic hydrogens and on the possibility for stabilization of the resulting phenoxyl radicals formed by hydrogen donation (Catherine *et al,* 1996; Ramarathnam *et al,* 1997). Flavonoids which belong to the phenolic compounds, poorly correlated (r^2 = 0.145, $p < 0.05$) with phenolic content of the vegetables analyzed (Figure 2).

Flavonoid contents of the vegetables are shown in Table 2. *Solanum macrocarpon* had the highest value of 215.39 mg quercetin g^{-1} and *Allium cepa* had the lowest value of 10.23 mg quercetin g^{-1}. Among the phenolic compounds are flavonoids which possess biological activities such as anti-inflammatory, anti-carcinogenic and anti-atherosclerotic acitivities. There was no correlation between total flavonoids and radical scavenging activity, (r^2 = 0.143) as shown in Figure 3. This lack of relationship is in agreement with other reports (Heinonen *et al*., 1998; Anagnostopoulou *et al.,* 2006; Nickavar *et al.,* 2007); which indicates that flavonoids did not contribute to antioxidant activity of vegetables.

Ascorbic acid contents of vegetables analysed are given in Table 2. *Murraya koenigii* had the highest value of 150.67 mg ascorbic acid g^{-1} and *Cucumis sativus* had the lowest value of 16.67 mg ascorbic acid g^{-1}. The values are in agreement with values obtained by Sumazian *et*

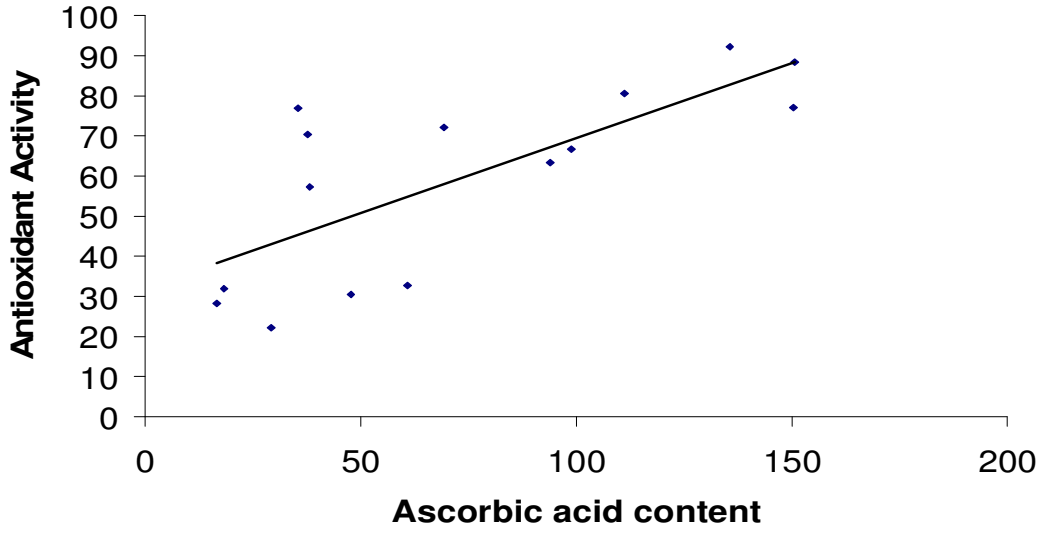


Figure 4. Correlation between antioxidant activity and ascorbic acid, (r^2 = 0.546).

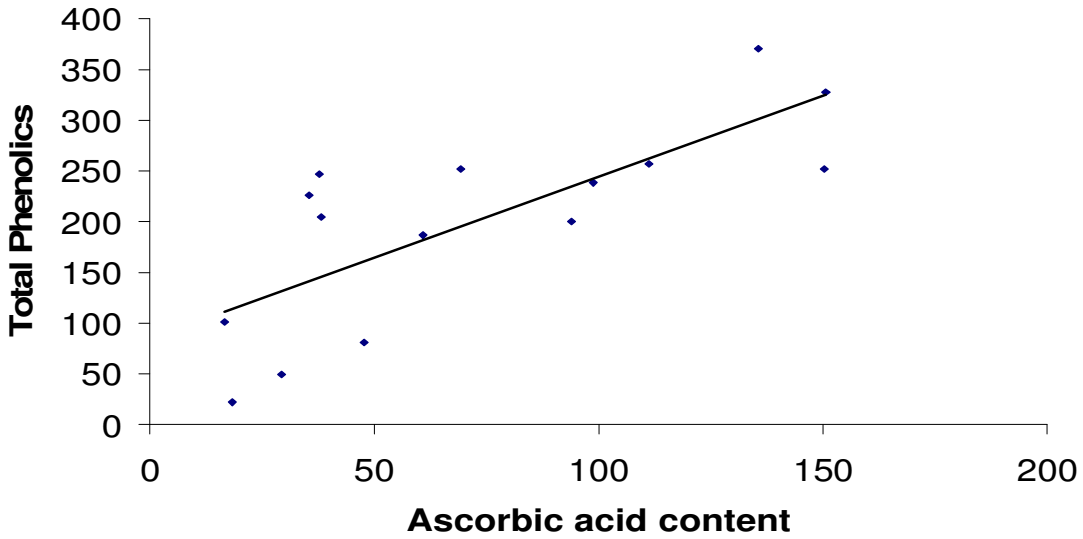


Figure 5. Correlation between total phenolics and ascorbic acid, (r^2 = 0.581).

al., (2010) and Ahmad and Hussain Beigh (2008), but higher than what were obtained by Okiei *et al*., (2009). There is no correlation between total ascorbic acid and total antioxidant activities (r^2 = 0.546, p < 0.05; Figure 4) and phenolic content (r^2 = 0.591, p < 0.05; Figure 5). According to Bahorun *et al.* (2004), it is normal when total ascorbic acid do not correlate with the total antioxidant activities since total ascorbic acid made little or no contribution to the total antioxidant activities of vegetables.

CONCLUSION

The antioxidant capacities, total phenolic, flavonoid and ascorbic acid contents of fifteen vegetables commonly consumed in Nigeria were evaluated. Some of the

vegetables can be considered as good sources of antioxidant as shown by their IC_{50}, anti-cancer and anti-athesclerosis; and as shown by their total phenolic and flavonoid contents and anti-inhibitory agent as indicated by their ascorbic acid content. *Solanum macrocarpon* is the most potent vegetable (lowest IC_{50} value) and with highest total phenolic, flavonoid and good ascorbic acid contents. A significant correlation was obtained between antioxidant activity and phenolic content indicating that phenolic compounds contribute significantly to antioxidant activity of the investigated vegetables.

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