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Antioxidant Activity of Selected Nigerian Green Leafy Vegetables

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Abstract: Antioxidant activity of hot water extracts of 21 Green Leafy Vegetables (GLV): *Amaranthus hybridus* Linn. (Amaranthaceae), *Amaranthus caudatus* (Amaranthaceae), *Beilschmedia manni* (Meisn.) Benth. Et Hook.f. (Lauraceae), *Celosia argentea var argentea* (L.) O. Kuntze (Amarantheceae) *Celosia argentea var cristata* Linn. (Amarantheceae), *Corchorus olitorius* L. (Tiliaceae), *Crassocephalum crepidioides* (Benth). S.Moore (Asteraceae), *Gnetum bucholzianum* Welw. (Gnetaceae), *Gongronema latifolium* Benth. (Asclepiadaceae), *Heinsia crinita* (Afz.) G. Taylor (Rubiaceae), *Hibiscus callyphyllus* Cav. (Malvaceae), *Lasianthera africana* P. Beauv (Icacinaceae), *Myrianthus arboreus* P. Beauv. (Urticaceae), *Pterocarpus mildbraedii* Harms (Papilionaceae), *Pterocarpus santalinoides* DC. (Papilionaceae), *Solanum macrocarpon* L. (Solanaceae), *Solanum melongena* Linn. (Solanaceae), *Struchium sparganophora* (Linn.) O. Ktze (Asteraceae), *Talinum triangulare* (Jacq.) Wild. Portulacaceae, *Telferia occidetalis* Hook (Curcurbitaceae) and *Vernonia amygdalina* Del. (Asteraceae) was investigated. Potential free radical scanvenging activity of these vegetables was confirmed by spraying spots of the extracts with DPPH (yellow color on purple background). Antioxidant activity was assayed in linoleic acid model system. Total polyphenols as Tannic Acid Equivalent (TAE) and ascorbic acid were evaluated spectrophotometrically. The activity of each extract was calculated as %inhibition of lipid peroxidation. The extracts showed marked antioxidant activity in linoleic acid model systems. Antioxidant values (AA) ranged from as low as 3.67% in *A. hybridus* to as high as 68.41% in *C. argentea var cristata*. Phenol content (TAE) varied from 21.83 mg/100 g dry weight in *T. triangulare* to 546.97 mg/100 g dry weight in *G. bucholzianum*. Ascorbic acid content (ASC) was from 13.41 mg/100 g dry weight in *V. amygdalina* to 187.11 mg/100 g dry weight in *G. latifolium*. There was low correlation between AA/TAE (R^2 = 0.432), AA/ASC (R^2 = 0.28) and TAE/ASC (R^2 = 0.35), respectively.

Key words: Green leafy vegetables, antioxidant activity, total phenols, ascorbic acid

INTRODUCTION

Nigeria is blessed with enormous biodiversity resources. In view of the diversity of the habitats and the climate of the country, the biota exhibits considerable diversity. The plant resources are diverse and some of them could be unexpected food or remedy for the natives. In traditional societies, nutrition and health care are interconnected and many plants are consumed as food in order to benefit health (Etkin, 1996; Pieroni, 2000; Pieroni *et al*., 2002a, b; El and Karakaya, 2004; Ansari *et al*., 2005).

Native plant species are used for daily diet in Nigeria as vegetables, spices and condiments. Some of them are also employed for traditional medicine. There are several varieties of these leafy vegetables

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either in the wild or under cultivation in the rural areas. Migration to urban centres has a great influence on the choice of vegetables used as food. High consumption of vegetables has been associated with a lowered incidence of degenerative diseases. These protective effects are considered to be related to the various antioxidants contained in them. The oxidative stress experienced by a tissue, organelle or organ results from the balance between the production and removal of potentially damaging reactive oxygen species (ROS). Since the ROS removal rate is mostly controlled by a variety of low molecular weight antioxidants, there is a great interest in determining their levels and the way they are related to pathological states and whether they can be controlled by an antioxidant-rich diet and/or by the ingestation of an antioxidant supplementation (Urquiaga and Leighton, 2000; Crozier *et al*., 2000).

Antioxidants have become synonymous with good health. They are a class of compounds thought to prevent certain types of chemical damage caused by an excess of free radicals, charged molecules that are generated by a variety of sources including pesticides, smoking and exhaust fumes. Destroying free radicals may help fight cancer, heart disease, stroke and other immune compromising diseases (Yi-Fang *et al*., 2002; Aruoma, 2003). Many of the natural antioxidants, especially flavonoids, seem to be very important in the prevention of these diseases. Fruits and vegetables have long been viewed as a rich source of antioxidant compounds. Health officials have been urging consumers for years to eat more fruits and vegetables in order to gain the health benefits of antioxidants.Epidemiological studies show that the consumption of vegetables and fruits can protect humans against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species (Ames *et al*., 1993). Spices and herbs are recognized as sources of natural antioxidants that can protect from oxidative stress and thus play an important role in the chemoprevention of diseases that have their etiology and pathophyiology in reactive oxygen species (Velioglu *et al*., 1988; Lee and Shibamoto, 2002; Dragland *et al*., 2003; Odukoya *et al*., 2005a-c; Atawodi, 2005). In this study, the potential of 21 GLV in the cooked form as natural antioxidant supplements diets was assessed to offer a cheap but rich source of micronutrients and other phytochemicals having antioxidant properties essential for good health.

MATERIALS AND METHODS

Collection of Plant Material

All the plant materials used were purchased on separate occasions from different open markets in Lagos Nigeria and authenticated at the Herbarium of the Forest Research Institute of Nigeria (FRIN) Ibadan, Nigeria by comparing with herbarium specimens using specific morphological features.

Preparation of Extracts

The vegetables were picked to remove debris, cut into small pieces and air dried. The materials were weighed (20 g) and homogenized with (100 mL) of distilled water using a Moulinex blender. Homogenate was boiled for 3 min and allowed to stand at room temperature for 24 h before filteration. Filterates were diluted to produce a 200 mg L^{-1} of extract needed for the antioxidant assays.

Determination of Antioxidant Activity

The antioxidant activity was assayed in linoleic acid model system using the ferric thiocyanate method as described by Kikuzaki and Nakatani (1993) and used by Odukoya *et al*. (2005a-c). Tocopherol (Sigma) was used as standard antioxidant while a blank of distilled water was ran with each assay. All determinations were carried out in triplicate.

The activity of each extract was calculated as %inhibition of lipid peroxidation by following equation:

$$\% \text{ Inhibition} = \frac{A1 - A2}{A1} X100\%$$

Where A1 was the absorbance of the control reaction and A2 was the absorbance in the presence of the extract sample.

Determination of Total Phenolic Content

Extracts were screened for presence of phenolics with ferric chloride solution before commencement of assay. Total phenolics were determined using the Tannic Acid Equivalent (TAE) method of relative astringency of the plant extracts as a direct measurement of total soluble tannin as earlier described and used by Odukoya *et al*. (2001, 2005a).

Determination of Ascorbic Acid Content

The amount of vitamin C in samples was determined by spectrophotometry using a modified method of Aydogmus and Cetin (2002). About 5-10 g of the weighed sample was soaked for 10-15 min in 25-40 mL 5% metaphosphoric acid in the presence of about 2 g of activated charcoal. The mixture was centrifuged and supernatant used for the test. Standard solutions of ascorbic acid were prepared by dissolving 40-100 mg of ascorbic acid in 100 mL of 2% (w/v) metaphosphoric acid. A solution of DCIP in distilled water was prepared and 1.0 mL of this reagent was mixed with 1.0 mL of ascorbic acid. The reaction mixture was shaken thoroughly and left for 12 min at room temperature. The absorbance of the mixture was measured spectrophotometrically at 265 nm against a blank containing the metaphosphoric acid. The results were used for the calibration curve. Samples were replaced with the standard ascorbic acid solution and corresponding vitamin C content extrapolated from the curve.

Statistical Evaluation

All determinations were carried out in triplicate and results obtained were expressed as Mean±SD.

RESULTS AND DISCUSSION

Interest in the role of antioxidants in human health has prompted research in the fields of food science and horticulture to assess fruit and vegetable antioxidants (Kalt *et al*., 1999). The protective action of fruits and vegetables has been attributed to the presence of anti-oxidants, especially anti-oxidant vitamins including ascorbic acid, α-tocopherol and β-carotene (Cao *et al*., 1996; Grivetti *et al*., 2000).

Some vegetables need to be cooked before they become edible. All the samples studied are eaten as cooked leafy vegetable in soups and 3 species (*Gnetum bucholzianum*, *G. africanum* and *Gongronema latifolium)* are also eaten raw in salads (Table 1). It is well established that many vitamins like vitamin C, are easily destroyed by heat and light, hence the recommendation to cook vegetables in minimal heat.

Many studies have revealed that beta-carotene and antioxidant phenolic compounds are actually more bio-available from cooked vegetables compared with raw. This is probably due to cooking breaking down the tough cell walls, releasing the nutrient content for easier absorption from the small intestine. Carotenoids are present in chloroplasts in the leaves of dark green leafy vegetables, which are not readily digested in the body. It is believed that the fibre of vegetables entraps the beta-carotene, reducing its availability to be incorporated into micelles prior to absorption from the intestines. By comparison, beta-carotene in fruits is contained within the readily digestible cell wall (Dewanto *et al*., 2002). Hence, cooking of the vegetables before eating increases bioavailability.

Table 1: Botanical source, common/vernacular names and uses of indigenous vegetables studied from Nigeria

Botanical source/code used	Common english name	Vernacular name	Uses
Amaranthus hybridus Linn. (Amaranthaceae) AHY	Green amaranth	Tete Abalaye	Leafy vegetable in soups
Amaranthus caudatus (Amaranthaceae) ACU	Slim amaranth	Tete Oyinbo	Leafy vegetable in soups
Beilschmedia manni (Meisn.) Benth et Hook.f (Lauraceae) BMA	Vegetable	Atiokwo	Leafy vegetable in soups
Celosia argentea var argentea (L.) O. Kuntze (Amaranthaceae) CAA	(White flowers/green stem) Cockscomb	Green Soko	Leafy vegetable soups
Celosia argentea var cristata Linn. (Amaranthaceae) CAC	Red variety/Red flowers Cockscomb	Red Soko	Leafy vegetable in soups
Corchorus olitorius L. (Tiliaceae) COL	Jute	Ewedu	Leafy mucilaginous vegetable
Crassocephalum crepidioides (Benth). S. Moore (Asteraceae) CRS	Fireweed, Thickhead	Ebolo	Leafy vegetable in soups
Gnetum bucholzianum Welw. (Gnetaceae) GBU	Koko vine	Okazi/Afang	Leaves eaten raw in salads and also in soups
Gnetum africanum Welw. (Gnetaceae) GAF	African gnetum African jointfir	Okazi/Afang	Leaves eaten raw in salads and also in soups
Gongronema latifolium Benth. (Asclepiadaceae) GLA	Vegetable	Utazi	Leaves eaten raw in salads and flavour in meat and fresh fish pepper soups
Heinsia crinita (Afz.) G. Taylor (Rubiaceae) HCR	Vegetable	Atama	Leafy vegetable in soups
Hibiscus callyphyllus Cav. (Malvaceae) HCC	Rocks hibiscus, Lemon Yellow Rose mallow	Isapa	Leafy vegetable in soups
Lasianthera africana P. Beauv (Icacinaceae) LAF	Vegetable	Editan	Leafy vegetable in soups
Myrianthus arboreus P. Beauv. (Urticaceae) MAR	Giant yellow Mulberry	Ujuju	Leafy vegetable in soups
Pterocarpus mildbraedii Harms (Papilionaceae) PMD	Vegetable	Oha	Leafy vegetable soups
Pterocarpus santalinoides DC. (Papilionaceae) PSD	Red sandalwood	Nturukpa	Leafy vegetable in soups
Solanum macrocarpon L. (Solanaceae) SMP	African egg plant	Igbo	Leafy vegetable in soups
Solanum melongena Linn. (Solanaceae) SMG	Egg plant	Igba	Leafy vegetable in soups
Talinum triangulare (Jacq.) Wild. (Portulacaceae) TTG	Water leaf	Gbure	Leafy vegetable in soups
Telferia occidetalis Hook (Curcurbitaceae) TOC	Fluted pumpkin	Ugu	Leafy vegetable in soups
Vernonia amygdalina Del. (Asteraceae) VAG	Bitter leaf	Ewuro/Onugbu	Leafy vegetable in soups

Antioxidant activity was accessed as a marker of lipid peroxidation in linoleic acid model. Our results demonstrated that there are large differences among total antioxidants in various dietary vegetables.The vegetable extracts showed marked antioxidant activity in linoleic acid model systems. Antioxidant values (AA) ranged from as low as 3.67% in *A. hybridus* to as high as 68.41% in *C. argentea var cristata*. Phenolic content expressed in Tannic Acid Equivalents (TAE), varied from 21.83 mg/100 g dry weight in *T. triangulare* to 546.97 mg/100 g dry weight in *G. bucholzianum*. Ascorbic acid content (ASC) was from 13.41 mg/100 g dry weight in *V. amygdalina* to 187.11 mg/100 g dry weight in *G. latifolium* (Table 2).

Table 2: Antioxidant activity, total phenol and ascorbic acid contents of indigenous vegetables from Nigeria

Botanical source/ code used	AA % inhibition	Tae (mg 100 g^{-1})	Ascorbic acid (mg 100 g^{-1})
Amaranthus hybridus Linn. (Amaranthaceae) AHY	3.67±0.11	406.33±0.17	52.17±0.71
Amaranthus caudatus (Amaranthaceae) ACU	17.89±0.04	312.18±5.19	106.84±3.20
Beilschmedia manni BMA	28.71±1.26	107.72±2.01	42.07±0.11
Celosia argentea var argentea (L.) O. Kuntze (Amaranthaceae) CAA	36.67±3.28	161.91±3.06	102.01±0.08
Celosia argentea var cristata Linn. (Amaranthaceae) CAC	68.41±0.78	216.85±2.11	108.48±0.14
Corchorus olitorius L. (Tiliaceae) COL	47.52±0.64	503.72±1.96	153.63±1.22
Crassocephalum crepidioides (Benth). S. Moore (Asteraceae) CRS	36.11±0.89	182.91±0.48	122.95±4.01
Gnetum bucholzianum Welw. (Gnetaceae) GBU	47.44±0.72	546.97±3.01	168.31±1.74
Gnetum africanum Welw. (Gnetaceae) GAF	45.89±0.06	543.76±0.74	166.96±0.14
Gongronema latifolium Benth. (Asclepiadaceae) GLA	61.04±0.14	316.91±3.16	187.11±0.98
Heinsia crinita (Afz.) G. Taylor (Rubiaceae) HCR	13.85±0.06	42.49±0.95	109.80±1.34
Hibiscus callyphyllus Cav. (Malvaceae) HCC	47.97±0.33	542.36±8.04	142.58±1.69
Lasianthera africana P. Beauv (Icacinaceae) LAF	16.72±0.12	119.62±1.01	89.76±1.05
Myrianthus arboreus P. Beauv. (Urticaceae) MAR	22.34±0.53	74.68±0.06	102.53±2.81
Pterocarpus mildbraedii Harms (Papilionaceae) PMD	54.27±0.08	376.42±0.96	49.18±0.03
Pterocarpus santalinoides DC. (Papilionaceae) PSD	33.49±0.07	216.68±4.31	113.56±1.44
Solanum macrocarpon L. (Solanaceae) SMP	46.85±0.11	190.07±0.01	38.11±0.13
Solanum melongena Linn. (Solanaceae) SMG	34.83±1.63	92.40±1.11	98.22±0.99
Talinum triangulare (Jacq.) Wild. (Portulacaceae) TTG	19.76±0.07	21.83±3.09	116.35±1.08
Telferia occidetalis Hook (Curcurbitaceae) TOC	48.21±0.48	212.44±0.88	137.82±3.11
Vernonia amygdalina Del. (Asteraceae) VAG	45.93±0.01	301.18±9.86	13.41±4.06

It was observed that the correlation coefficient was low between AA/TAE ($R^2 = 0.40$), AA/ASC ($R^2 = 0.28$), TAE/ASC ($R^2 = 0.35$). Variation in antioxidant activity of the vegetables may be due to the differences in the structures of phenolic compounds primarily related to their hydroxylation and methylation patterns and incresed lag time of binding vegetable phenols with lipoproteins which subsequently protects them from oxidation. The human body cannot produce ascorbic acid, so it must be obtained entirely through one's diet. The amount of ascorbic acid in the vegetables studied varied greatly. This might be as a result of factors as the variety, weather, maturity stage and storage. Since vitamin C is easily oxidized, storage and the cooking in air leads to the eventual oxidation of vitamin C by oxygen in the atmosphere. These vegetables were obtained from the open market where they are directly exposed to the effects of sunlight and rain so the degree of freshness is relative. It is concluded that high consumption of vegetables containing phenolic antioxidants may slow down the process of degenerative diseases. For the selection of superior indigenous vegetables, evaluation of antioxidant activity, ascorbic acid content and total phenols can be used as an index. This sturdy recommends more consumption of these vegetables because of their potential health benefits.

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