



***In vitro* comparative studies on antioxidant capacities of *Gnetum africanum* (“Afang”) and *Gongronema latifolium* (“Utazi”) leafy vegetables**

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Abstract

This study sought to investigate the antioxidant potentials of aqueous extracts of *Gongronema latifolium* (“Utazi”) and *Gnetum africanum* (“Afang”) vegetables for the management/prevention of reactive oxygen species (ROS) generated as a result of stress. The total phenol, total flavonoids, ferric reducing property (FRAP), Fe²⁺ chelation and antioxidants activities [1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging ability, and hydroxyl radicals (OH*)] and were determined. Gallic acid was used as standard for total phenol and quercetin as standard for total flavonoid determination. *Gnetum africanum* extract exhibited higher total phenol, total flavonoid, FRAP, DPPH compared to *Gongronema latifolium* with higher OH* scavenging ability. The extract chelated Fe²⁺ in a concentration-dependent manner from the IC₅₀ value. The antioxidant activities correlated with its phenol/flavonoid content. In conclusion, both aqueous extracts of *Gongronema latifolium* (“Utazi”) and *Gnetum africanum* (“Afang”) are rich in phenolic compounds with high antioxidant activity, but *Utazi* had higher antioxidant activity.

Keywords: Vegetables; Antioxidants; Phenols; Reactive Oxygen Species (ROS); Medicinal

Introduction

Many plants in Africa are medicinal including domesticated vegetables. *Gnetum africanum* “Afang” and *Gongronema latifolium* “Utazi” have been used as components of various herbal preparations in ethnomedicine. *Gnetum africanum* “Afang” belongs to the family Gnetaceae, which grows as a wild evergreen climbing plant in the rainforest of Nigeria where it is sought for and highly priced in markets. The distribution of the plant is in the West/Central Africa sub region. It is known by various names in the

following countries: Cameroon (Eru, okok, m’fumbua, or fumbua), Angola (koko), Nigeria (ukase or afang), Gabon (koko), Central African Republic (koko), Congo (koko), and the Democratic Republic of Congo (m’fumbua or fumbua). *Gnetum africanum* has also been referred to as a form of ‘wild spinach’ in English. Primarily, the leaves are used as a vegetable for soups and stews, called “eru” or “afang” soup (Ali *et al.*, 2001). The leaves may further be used as a remedy for nausea, sore throats, or as a dressing for warts. The stem of the plant may

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also be used for medicinal purposes, including the reduction of pain during childbirth. Furthermore, *Gnetum africanum* produces a root tuber that may be used as a source of 'famine food' (Ali *et al.*, 2011).

Gongronema latifolium belongs to the Ascepiadaceae family. Commonly called 'utazi' and 'arokeke' in the South Western and South Eastern parts of Nigeria, it is a tropical rainforest plant primarily used as spice and vegetable in traditional folk medicine (Ugochukwu and Babady, 2003). It is found throughout Nigeria and other tropical countries such as Guinea-Bissau, Western Cameroon and Sierra Leone. It has been used in traditional medicine for treating various gastrointestinal disorders such as diarrhoea, ulcers, dyspepsia and in the management of diabetes mellitus (Nwinyi *et al.*, 2008). The leaves have been reported to have hypoglycaemic effect by decreasing activity of glucokinase enzyme and levels of hepatic glycogen, hepatic and blood glucose. It is rich in fats, proteins, vitamins, minerals and essential amino acids (Etim *et al.*, 2008).

The present study sought to determine the antioxidant potentials of *Gongronema latifolium* and *Gnetum africanum* vegetables. Recent reports show that free radical-mediated lipid peroxidation plays a crucial role in the pathogenesis of many neurodegenerative diseases. In living organisms, various reactive oxygen species (ROS) can be formed in different ways. Normal aerobic respiration appear to be the main endogenous source of most of the oxidants produced by cells (Thenmozi *et al.*, 2011). However, emerging studies have shown that phenolic phytochemicals from plants are cheap and effective sources of AChE inhibitors with little or no side effects and could also serve as dietary intervention. Phenolic phytochemicals are secondary metabolites of plant origin and are an important part of the diet, providing potential antioxidant benefits for managing oxidative

stress related pathologies, such as Alzheimer's disease, diabetes and cardiovascular diseases (Stevenson and Hurst, 2007). The information obtained is essential as a preliminary step to proceed with a significant use of both these plants as source of health related products serving as functional foods or pharmaceuticals to prevent or treat certain diseases.

Experimental

Chemical and Reagents. Chemicals such as dinitrophenyl hydrazine (DNPH), phosphate buffer of different molarity, adenosine monophosphate (AMP), sodium hydroxide, benzene, and 5,5'-dithio-bis(2-nitrobenzoic acid) were purchased from Sigma-Aldrich, Chemie GmbH (Steinheim, Germany). Acetic acid was procured from BDH Chemical Ltd., (England). Except otherwise stated, all other chemicals and reagents are of analytical grade while the water was glass distilled. A Jenway UV-visible spectrophotometer (Model 6305; Jenway, Barlo world Scientific, Dunmow, United Kingdom) was used to measure absorbance throughout the experiment. All the chemicals used were of analytical grade, while the water was glass distilled.

Sample collection. Sample of the vegetables *Gongronema latifolium* known as Utazi and *Gnetum africanum* known as Afang were purchased from a local market in Cross river state Nigeria.

Aqueous extract preparation. The *Gongronema latifolium* (Utazi) leaves and *Gnetum africanum* (Afang) leaves were thoroughly washed in distilled water to remove any dirt, chopped into small pieces by table knife, air dried and milled into fine powder. The aqueous extracts were prepared by soaking 5g of the grinded samples in 50ml of distilled water for 24hrs at 37°C. The mixture was later filtered through Whatman No. 2 filter paper and centrifuged at 4000 rpm to obtain a clear supernatant which was then

stored in the refrigerator for subsequent analysis (Oboh and Rocha, 2007).

Determination of total phenol content. The total phenol content was determined by mixing 0.2 ml of the sample extract with 2.5 ml 10% Folin-Ciocalteu reagent (v/v) and 2.0 ml of 7.5% sodium carbonate was subsequently added. The reaction mixture was incubated at 45°C for 40 min and the absorbance was measured at 765 nm using a spectrophotometer. Gallic acid was used as standard while the total phenol content was subsequently calculated as gallic acid equivalent (Singleton *et al.*, 1999).

Determination of total flavonoid content. The total flavonoid content was determined by mixing 0.5 ml of appropriately diluted sample with 0.5 ml methanol, 50 µl 10% AlCl₃, 50 µl 1M Potassium acetate and 1.4 ml distilled water, and allowed to incubate at room temperature for 30 min. The absorbance of the reaction mixture was subsequently measured at 415 nm; Quercetin is used as standard flavonoid. The total flavonoid content was subsequently calculated as quercetin equivalent. The non-flavonoid polyphenols were taken as the difference between the total phenol and total flavonoid content (Meda *et al.*, 2005).

Determination of reducing power (FRAP). The reducing property of the extracts was determined by assessing the ability of the extracts to reduce the FeCl₃ solution as described by Oyaizu ((1986). A 2.5-ml aliquot was mixed with 2.5 ml of 200 mmol/L sodium phosphate buffer (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min, and then 2.5 ml of 10% TCA was added. This mixture was centrifuge at 650 rpm for 10 min. five millilitres of the supernatant was mixed with an equal volume of water and 1 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm. The ferric-reducing antioxidant

property was subsequently calculated using ascorbic acid as standard.

Fenton reaction (Hydroxyl radical scavenging ability OH*). The extract 0 - 100 µL was added to a reaction mixture containing 120 µL of 20 mM deoxyribose, 400 µL of 0.1 M phosphate buffer, 40 µL of 500 µM of FeSO₄, and the volume were made up to 800 µL with distilled water. The reaction mixture was incubated at 37°C for 30 minutes and the reaction was then stopped by the addition of 0.5 mL of 2.8% trichloroacetic acid. This was followed by addition of 0.4 mL of 0.6% thiobarbituric acid (TBA) solution. The tubes were subsequently incubated in boiling water for 20 minutes. The absorbance was measured at 532. The OH* scavenging ability was subsequently calculated (Halliwell and Gutteridge, 1981).

DPPH free radical scavenging ability. The free radical scavenging ability of the extracts against DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical was evaluated as described by Gyamfi *et al.*, (1999). Briefly, appropriate dilution of the extracts (0 – 500 µl) was mixed with 1 ml, 0.4 mM methanolic solution containing DPPH radicals, the mixture was left in the dark for 30 min and the absorbance was taken at 516 nm. The DPPH free radical scavenging ability was subsequently calculated.

Fe²⁺ Chelation assay. The Fe²⁺ chelating ability of the extract was determined using a modified method of Minotti and Aust (1987), with a slight modification by Puntel *et al.*, (2005). Freshly prepared 500 µM FeSO₄ (150 µL) was added to a reaction mixture containing 168 µL 0.1 M Tris-HCl (pH 7.4), 218 µL saline and the extracts (0 – 25 µL). The reaction mixture was incubated for 5 min, before the addition of 13 µL 0.25% 1, 10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm. The Fe (II) chelating ability was subsequently calculated.

Data analysis. The results of the replicate readings were pooled and expressed as mean \pm standard deviation. Student *t* test was performed and significance was accepted at $P \leq 0.05$ (Zar, 1984). IC_{50} (concentration of extract that will cause 50% inhibitory activity) was determined.

RESULTS

The results in Table 1 represent the phenolics and biological constituents of *Gongronema latifolium* and *Gnetum africanum*. The results show that *Gnetum africanum* had higher total phenol (2.15 ± 0.02 mg/GAE/g) than *Gongronema latifolium* (1.86 ± 0.02 mg/GAE/g). Also the total flavonoid content of *Gnetum africanum* (1.00 ± 0.02 mg/gQE) as shown in the table is higher than that of

Gongronema latifolium (0.81 ± 0.02 mg/gQE). Also, the reducing power of *Gnetum africanum* (4.23 ± 0.05 mg/g) is higher than that of *Gongronema latifolium* (2.15 ± 0.03 mg/g). Table 2 and Figures 1 - 3 also revealed that *Gongronema latifolium* (4.22 ± 0.09 mg/ml) has better 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging ability than *Gnetum africanum* (4.66 ± 0.01 mg/ml). It also shows that *Gongronema latifolium* (3.24 ± 0.05 mg/ml) has a higher ability to chelate Fe^{3+} to Fe^{2+} in a concentration dependent manner than *Gnetum africanum* (3.75 ± 0.05 mg/ml) while there was no significant change in their OH-scavenging ability [*Gongronema latifolium* (1.08 ± 0.01 mg/ml) *Gnetum africanum* (0.92 ± 0.05 mg/ml)].

Table 1: Total phenol, total flavonoid, reducing power of aqueous extract of *Gongronema latifolium* and *Gnetum africanum* extract.

Sample	Total phenol(mgGAE/g)	Total flavonoid(mgQE/g)	Reducing power(mg/g)
Utazi (GL)	1.86 ± 0.02^a	0.81 ± 0.01^a	2.15 ± 0.03^a
Afang (GA)	2.15 ± 0.02^b	1.00 ± 0.02^b	4.23 ± 0.05^b

Data represent means of triplicate determinations. Values with the same letter along the same row are significantly different ($p < 0.05$).

Table 2: IC_{50} of the Antioxidants activities of aqueous extract of *Gongronema latifolium* and *Gnetum africanum* extract.

Sample	Iron chelating ability(mg/ml)	DPPH scavenging ability(mg/ml)	OH-fenton reaction(mg/ml)
Utazi (GL)	3.24 ± 0.05^a	4.22 ± 0.09^a	0.92 ± 0.05^a
Afang (GA)	3.75 ± 0.05^b	4.66 ± 0.01^b	1.08 ± 0.01^a

Data represent means of triplicate determinations. Values with the same letter along the same row are significantly different ($p < 0.05$).

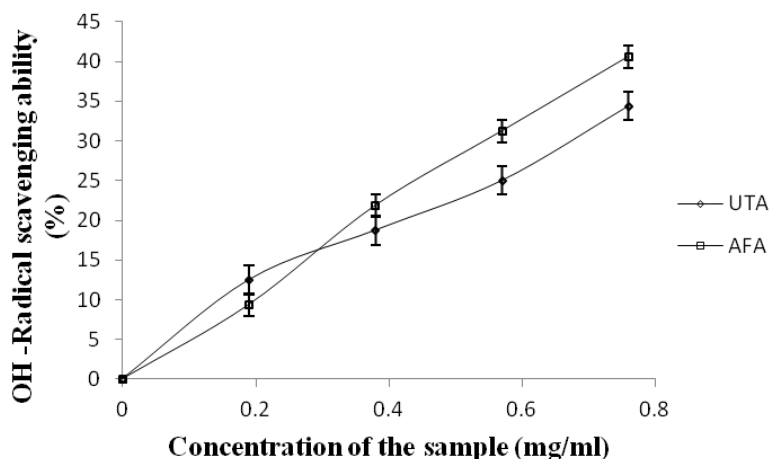


Figure 1: percentage OH-radical scavenging ability of *Gongronema latifolium* and *Gnetum africanum*. SD represent means of replicate readings

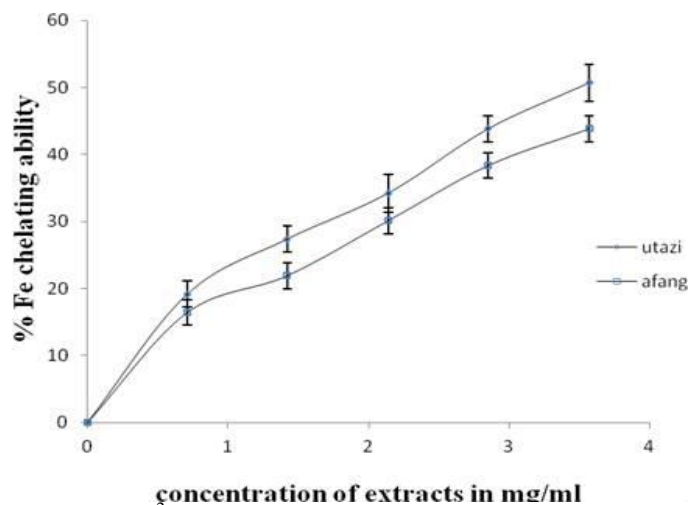


Figure 2: Fe-chelating ability curve. Fe^{2+} chelating ability of the aqueous extracts from *Gongronema latifolium* and *Gnetum africanum*. SD represent means of replicate readings.

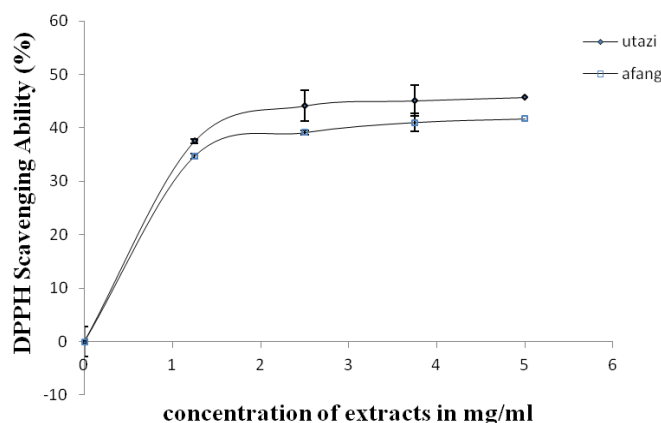


Figure 3: DPPH scavenging ability curve in a concentration dependent manner. SD represent means of replicate readings using linear regression analysis.

DISCUSSION

The positive health effects of phenolic phytochemical are linked to their ability to counter the negative effects of reactive oxygen species generated during cellular energy metabolism (Urquiaga and Leighton, 2000). Flavonoids are also known as a class of widely distributed phytochemical with antioxidant activities (Halliwell, 2007). The potent antioxidant activity of flavonoids reveals their ability to scavenge hydroxyl radicals, superoxide anions and lipid peroxy radicals; this may be the most important function of flavonoids. Its detoxification and antioxidant activities have been established (Alan and Miller, 1996). The results of the

total phenol and flavonoid contents of the aqueous extract as presented in Table 1 as gallic acid (GAE) and quercetin (QE) equivalent respectively. The total flavonoid content recorded in this study showed to be higher compared to what was recorded for *Aframomum* species by Adefegha and Oboh (2012). Flavonoids are natural biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergen, virus and carcinogens (Egharavba and Kunle, 2010). It is known to inhibit platelets aggregation, and could exert a membrane stabilizing action that may protect liver from injury. Its detoxification and antioxidant

activities have been established (Alan and Miller, 1996).

The aqueous extract exhibited strong scavenging abilities against DPPH radical by mopping reactive oxygen species and this can be attributed to the presence of phenolic compounds presents in the plants extract. More so, the hydroxyl radical scavenging ability of the aqueous extract through decomposition of deoxyribose could be attributed to the presence of polyphenols which are capable of donating hydrogen atoms to free radicals, thus inhibiting oxidation process (Zhang *et al.*, 2000). The aqueous extract chelate Fe^{2+} solution. This ability might be due to the presence of some phytochemicals such as polyphenol with potent Fe^{2+} chelating ability. The phytochemicals form complexes with the metal, thereby aiding its excretion from the body. The ferric reducing antioxidant properties as shown in this study have found out that a good antioxidant must be a strong reducing agent. Hence, the ferric reducing antioxidant property of the plants extract suggests it has potent antioxidant constituents. The damage caused by Fe^{2+} through lipid peroxidation of cell membranes, reduces antioxidant enzyme and antioxidant substrates to induce oxidative stress which contributes to the causation of injuries in different body tissues (Szymonik-Lesiuk *et al.*, 2003). Jia and his group (2001) investigated that oxidative damage can occur in DNA during the peroxidative breakdown of membrane polyunsaturated fatty acids. DNA damage affects homeostasis of various cells leading to cell damage and death (Khanna and Jackson, 2001). The results reveal that the aqueous extract scavenge free radicals in a concentration-dependent manner. The antioxidant result revealed that *Gongronema latifolium* (4.22 ± 0.88 mg/ml) had a better 1,1-diphenyl-2-picrylhydrazyl ability than *Gnetum africanum* (4.66 ± 1.05 mg/ml). *Gongronema latifolium* (3.24 ± 0.04 mg/ml) had higher

ability to chelate Fe^{3+} to Fe^{2+} than *Gnetum africanum* (3.75 ± 0.05 mg/ml) in concentration-dependent manner. Fe-chelating ability may also be one of the possible mechanisms through which antioxidants phytochemicals in aqueous extracts prevent lipid peroxidation in tissues, and it may be by forming a complex with Fe, thus preventing the initiation of lipid peroxidation (Obboh *et al.*, 2007). While there was no change in the hydroxyl (OH^*) scavenging activity which also agrees with the report of Nwanna *et al.*, 2013. The results from this study showed an important antioxidant mechanism demonstrated by the plant and could play some part in the prevention of oxidative-stress induced degeneration.

Conclusion. Both aqueous extracts of *Gongronema latifolium* (Utazi) and *Gnetum africanum* (Afang) are rich in phenolic compounds with high antioxidant activity, but Utazi had the highest antioxidant activity. Both of the vegetables showed potential as a functional food/or nutraceuticals in the management of degenerative conditions as well as preventing oxidative stress-induced conditions.

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