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Proximate and antioxidant activity analyses of six indigenous Nigerian vegetables

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ABSTRACT

Six commonly consumed indigenous vegetables in Nigeria, *Ocimum gratissimum*, *Talinum triangulare*, *Telfairia occidentalis*, *Amaranthus hybridus*, *Vernonia amygdalina*, and *Basella alba* were analysed for their proximate composition and antioxidant properties. The antioxidant properties were measured qualitatively and quantitatively by DPPH. The ash contents of the vegetables ranged from 3.99±0.01 to 11.5±1.40%, while the crude fibre content was between 5.35±0.04 and 10.20±0.1% based on dry weight. *B. alba* had the highest moisture content (92.55±0.07%), but the lowest ash and crude fibre contents.All the vegetable extracts exhibited antioxidant activities in terms of free radical scavenging abilities and the activities were concentration dependent. *O. gratissimum* and *V. amygdalina* exhibited antioxidant activities that were comparable to the reference antioxidant compound, ascorbic acid. The vegetables contained different quantities of phenolic compounds, which seemed significantly contributing to differences in their antioxidant activities. The IC_{50} value of *A. hybridus, O. gratissimum, V. amygdalina*, *B. alba* and *T. occidentalis* were 0.20, 1.23, 10.52, 44.65 and 76.90 µg/mL respectively. All the vegetables displayed high antioxidant ability except *T. trianguleae* with IC_{50} 502.81 µg/mL. Our results showed that all the vegetables are suitable sources of antioxidant compounds and could be health promoting.

Key Words: Nigerian vegetables, nutrient content, phenolic content, antioxidant activity

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1. INTRODUCTION

Oxygen is utilized by living organisms for the metabolism and utilization of dietary nutrients to produce energy. However, due to the highly reactive nature of oxygen, the metabolism often results in the formation of reactive oxygen species (ROS) such as superoxide anions, hydroxyl radicals and hydrogen peroxides (Kris-Etherton *et al* 2004). Excessive generation and accumulation of these free radicals as a result of environmental stress such as heat exposure or ionizing radiation results in oxidative stress causing damage to biomolecules and cell membranes and resulting in inflammatory diseases, diabetes, cancer, cardiac dysfunction, ageing, cataracts, immune system decline and other degenerative diseases (Wang *et al,* 2004; Percival, 1998). Overall, free radicals have been implicated in the pathogenesis of several diseases (Percival, 1998). However, the formation of free radicals in human systems can be controlled using some compounds obtained in the diets known as antioxidants. These compounds inhibit or delay the oxidation process by blocking the initiation or the propagation of free radical chains. When the availability of antioxidants is limited however, the damage can be quite devastating.

Interest in natural antioxidants is growing due to their perceived safety and healthfulness. This has prompted increased research into plants with natural antioxidant potential (Karakaya *et al,* 2001; El and Karakaya, 2004; Tepe *et al*, 2006). In addition to being good sources of nutrients, vegetables are also good sources of antioxidants (Robinsin, 1990). Nigeria, a tropical country in the Western region of Africa, is endowed with a large variety of vegetables that are consumed whole or in part, either raw or cooked as salad (Uzo, 1989). Vegetables are also made into refreshing drinks and are consumed as medicinal herbs (Uwaegbule, 1989). A lot of people consume vegetables without adequate nutritional information on them or the quantity required for beneficial nutrition. Nutritional scientific information and documentation on the health-promoting potential of Nigerian vegetables are scanty; hence, this study was conducted to determine the nutritional composition and antioxidant properties of six Nigerian indigenous vegetables.

The six vegetables investigated are very commonly consumed in various parts of Nigeria. *Ocimum gratissimum* Linn*,* also known as Clove basil or African basil (locally known as *Efirin, Daidoya* and *Nchuanwu* respectively among the Yorubas, Hausas and Igbos of Nigeria), *Talinum triangulare* (Jacq) Willd, otherwise known as Waterleaf or *Gbure* in Yoruba*, Vernonia amygdalina* Del or Bitter leaf (*Ewuro, Onugbu* or *Shuwaka* in Yoruba, Igbo and Hausa respectively)*, Basella alba* Lin or Red vine spinach or climbing spinach (*Amunututu* in Yoruba, *Gborongi* in Igbo)*, Telferaira occidentalis* Hook or Fluted pumpkin leaves (*Ugu* or *Ugwu* in Igbo and Yoruba and *Kabewa* in Hausa) and *Amaranthus hybridus* Linn, commonly known as African spinach or green amaranth (*Tete* in Yoruba, *Inine* in Igbo and *Allayahu* in Hausa) are all used in the preparation of various soups and dishes. Apart from being ingredients for delicacies, these vegetables have also been reported (scientifically and folklorically) to have some health benefits which need to be verified (Rubatzky and Yamaguchi, 1997; Prahbu *et al,* 2009; Nwokolo, 2016). Comprehensive scientific information on Nigerian vegetables and their nutritional benefits will serve as a guide to consumers to select vegetables based on nutritional needs and health benefits.

2. MATERIALS AND METHODS

2.1 Plant Identification and Preparation

The plants: *Ocimum gratissimum* Linn*, Talinum triangulare* (Jacq) Willd*, Vernonia amygdalina* Del*, Basella alba* Lin*, Telferaira occidentalis* Hook *and Amaranthus hybridus* Linn were purchased directly from gardeners who produced them and sell to market women at Tanke, Oke-odo in Ilorin, North-Central Nigeria between January and February, 2013. Plant samples were identified at the herbarium of the Plant Biology Department of the University of Ilorin and specimen copies were deposited.

The vegetables were rinsed with tap water and later with distilled water. Leaves and succulent stems were plucked, air dried and pulverized. Each sample was divided into two parts; one part was used for proximate analysis while the remaining part was soaked in ethanol to obtain the crude extract, which was used for antioxidant analysis. Fresh samples were used for moisture content determination.

2.2 Proximate Analysis

2.2.1 Moisture and Ash contents

Moisture content was determined using 10 g of the fresh vegetables in a crucible placed in an oven set at 105^oC while the ash content determination was achieved by incinerating 2 g of the air-dried sample in a porcelain crucible for 5 h in a muffle furnace at 550^oC. Analysis was done using standard methods described by AOAC (2000).

2.2.2 Crude Fibre

A 2 g sample of the dried vegetable was accurately weighed into a round bottom flask and digested with 25 ml of 1.25% H_2SO_4 for 30 minutes. After filtration, the residue was further digested with 25 ml of 1.25% NaOH solution for another 30 minutes. The residue obtained from the filteration of the digest was then dried, weighed and incinerated in a muffle furnace at 550^oC for 5 hours. The fibre content was determined from the weight of residual ash. Each analysis was done in triplicates and the mean calculated (AOAC, 2000).

2.2.3 DPPH Anti-oxidant analysis

Dried, pulverized samples were weighed and macerated twice in cold ethanol. It was extracted for six days in the first instance and then for another three days with fresh solvent. All extracts were pooled and concentrated *in vacuo*. The crude extracts obtained were refrigerated until needed for assays.

The radical scavenging abilities of the vegetables were qualitatively and quantitatively determined using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (Aldrich, USA).

2.2.4 Qualitative Assay

Dilute solutions of the vegetable extracts were prepared with ethanol and spotted on pre-coated silica gel TLC plates (G_{254}, Merck). The plates were developed using different solvent systems of varying polarities (1:2 Methanol/Ethyl acetate; 3:1 Ethyl acetate/n-hexane and 3:1 N-hexane/ethyl acetate mixtures) to resolve polar and non-polar components of the extracts. The plates were allowed to dry at room temperature and the separation was visualized using 0.02% ethanolic DPPH as a spray reagent.

Bleaching of DPPH by the resolved spots was observed for 10 minutes and thereafter colour changes were observed for the spots (yellow on purple background) (Sadhu *et al.,*2003).

2.2.5 Quantitative Assay

Quantitative assay was done to determine the proportion of antioxidant compounds in the vegetable extracts. 1 mg/mL ethanolic solution of each extract was prepared and diluted serially to obtain 10, 20, 40, 50, 60, 80, 100 µg/mL solutions. Blois method (Blois, 1958), as modified by Saha *et al.,* (2004) was employed in the determination of the free radical scavenging activity of the extracts using 1, 1-diphenyl-2-picrylhydrazyl (DPPH). Control experiment was conducted alongside and absorbance was measured at 517 nm. The percentage inhibition was calculated using the equation below and plotted against log of concentration of the extract. Generated IC_{50} was calculated from the graph. Ascorbic acid was used as a standard inhibitor. All the tests were performed in triplicates and the graph was plotted with the mean values.

$$DPPH\ radical\ scavenging = 1 - \frac{A_s}{A_c} \times 100 \qquad (1)$$

Where, A_C is the absorbance of control, and A_S is the absorbance of sample solution.

2.2.6 Total Phenolic Compounds Determination

Total soluble phenolic compounds in the vegetable extracts were determined using the Folin-Ciocalteu reagent, with the method of Slinkard and Singleton (1977) with little modification. A 1 mg sample of the extract was dissolved in 1 ml of distilled water and a portion of this was further diluted with distilled water, Folin-Ciocalteu's reagent (FCR) was added and the mixture was kept in the dark for 30 min after which 2% Na_2CO_3 was added to the mixture. The absorbance of the sample was measured at 760 nm after the mixture had been shaken for 2 h at room temperature. All tests were performed in triplicates and the mean calculated. A calibration curve was prepared using gallic acid (10 – 100 µg/ml) and the concentrations of the total phenolic compounds were determined from this calibration curve and expressed as gallic acid equivalent on dry matter basis.

2.2.7 Statistical Analysis

Experimental results are presented as mean ± SD of three replicate measurements. Statistical analysis was performed using the student t-test and ANOVA. The values for $P < 0.05$ were regarded as significant. The correlation coefficient (r) and regression analysis between the two variables in Table 4 were calculated by MS Excel software. The IC_{50} were calculated using Curve Expert statistical program.

3. RESULTS AND DISCUSSION

3.1 Proximate Analysis

Fresh plants generally have moisture contents ranging from about 72 % in potatoes to about 95 % in cucumber (USDA, 2009). The proximate analysis of the vegetables investigated show high moisture content in all samples (Table 1), thus the vegetables can be consumed to address dehydration problems in man. *B. alba* and *T. triangulare* are particularly high in moisture with values 92.55±0.07 and 92.02±0.25% respectively. High moisture content makes large quantity of water soluble minerals and vitamins readily available to consumers, but it also hastens both hydrolytic and microbial spoilage. Vegetables are classed among the perishable foods and are therefore usually consumed fresh for optimal nutrients and taste.

Dietary crude fibres reduce serum cholesterol level, hypertension, diabetes, breast cancer and constipation (Ishida *et al.,* 2000; Ramula and Rao, 2003). All the vegetables under investigation contain indigestible fibres in varying high amounts (Table 1). The quantities of crude fibre in *T. triangulare* and *V. amygdalina* are remarkably high (10.20±0.10 and 10.18±0.02% respectively), closely followed by *O. gratissimum* and *T. occidentalis* with 9.33±0.15 and 9.0±1.75% crude fibre respectively. These indigenous vegetables are therefore good and cheap sources of fibre which help to prevent diabetes and constipation. The generally high mineral content of the vegetables are evident from the results presented in Table 1 which agree with the earlier work of Ifon and Bassir (2000) on the nutritive value of some Nigerian leafy vegetables. *A. hybridus* and *T. triangulare* are exceptionally rich in minerals as shown by their high ash contents (11.5±1.40 and 9.41±0.38% respectively) while *B. alba* has a lower ash content compared to others (3.99%). All the vegetables under study except *B. alba* can therefore serve as high mineral foods or supplements especially in individuals who may be deficient in one or more mineral elements (Idris *et al*, 2011). Apart from adding nutrients to the diet, vegetables are also sources of herbal medication for the rural populace due to the presence of phytochemicals in them. In Nigeria, for example, *O. gratissimum* and *V. amygdalina* leaf extracts are reportedly used to treat diarrhoea, and the cold leaf infusions are used in the treatment of stomach upset and haemorrhoids (Kabir *et al,* 2005). Vegetables are used in folk medicine for the treatment of various diseases and illnesses such as upper respiratory tract infections, diarrhoea, headache, diseases of the eye and skin, pneumonia, cough, fever and conjunctivitis (Adebolu and Salau, 2005). The benefits derived from vegetables consumption are therefore numerous, including nutrients supply, diseases control and antioxidant activities.

Table 1. Proximate analysis of the Vegetables

Vegetable	Moisture content (%)	Ash content (%), (dry matter basis)	Crude fibre (%), (dry matter basis)
Ocimum gratissimum	80.83 ± 0.30	4.167 ± 0.29	9.33 ± 0.15
Talinum triangulare	92.02 ± 0.25	9.41 ± 0.38	10.20 ± 0.10
Telfairia occidentalis	86.73 ± 0.12	6.54 ± 0.84	9.0 ± 1.75
Amaranthus hybridus	84.73± 0.87	11.5 ± 1.40	7.25 ± 1.06
Vernonia amygdalina	76.2 ± 0.40	7.26 ± 0.03	10.18 ± 0.02
Basella alba	92.55 ± 0.07	3.99 ± 0.01	5.35 ± 0.04

3.2 Antioxidant Activity

3.2.1 Qualitative assay

The colour changes of the extracts (yellow on purple background) on TLC plates were because of the bleaching of DPPH on the resolved spots, which is indicative of the presence of antioxidants.

3.2.2 Quantitative assay

DPPH is a stable free radical and its reduction by the extracts indicates the ability of the extracts to function as antioxidant. The DPPH radical contain odd number of electrons, which absorbs around 517 nm with visible deep purple colour. When an antioxidant donates an electron, the DPPH colour is discharged and this loss is measured by change in absorbance that can be used to calculate the antioxidant capacity quantitatively. Extracts of the vegetables were found to reduce the DPPH radical and the antioxidant activities of the extracts were concentration dependent i.e. high concentration results in high percentage of DPPH inhibition with low IC_{50} value. The scavenging effects of the extracts as compared with ascorbic acid are shown in Table 2. *O. gratissimum* and *V. amygdalina* were found to display inhibition activities comparable to the reference antioxidant compound. It was observed that at low concentrations, *O. gratissimum* showed higher percentage of DPPH inhibition than ascorbic acid at the same concentration. For instance, concentrations of 10 and 20 µg/mL of *O. gratissimum* had percentage inhibitions of 71.3492 and 75.4365% respectively compared to ascorbic acid with 62.7381 and 74.2064% inhibition. At 100 µg/mL, the percentage inhibition of *O. gratissimum* was 91.3095% and *V. amygdalina* 87.1408% while ascorbic acid had 98.7698%. These results support the use of these vegetables in ethno-medicinal applications, since drugs used for treating diseases caused by free radical accumulation in human body utilizes antioxidant mechanism as one of the routes of action (Chowdhury *et al*, 2008). Several anti-inflammatory, digestive, antinecrotic, neuroprotective and hepatoprotective drugs have recently been shown to have antioxidant or radical scavenging mechanism as part of their mode of action for therapeutic purposes (Lin and Huang, 2000; Perry *et al*, 1999). *T. occidentalis* and *B. alba* have percentage DPPH inhibition between 45% and 55% with IC_{50} of 76.90 and 44.65 µg/mL respectively which classified them into vegetables with medium antioxidant properties (Perry *et al*, 1999). *A. hybridus* and *O. gratissimum* have IC_{50} of 0.20 and 1.23 µg/mL, which ranked them as very strong natural anti-oxidants.

Saponins, tannins and flavonoids are some of the phytochemicals known to be responsible for the antioxidant properties of medicinal plants and these phytochemicals have been previously reported to be present in these vegetables (Dosumu *et al*, 2013). The presence of these phytochemicals can therefore account for the free radical scavenging properties of these vegetables.

Table 2. Percentage Inhibition of DPPH by the Vegetable extracts and Ascorbic acid:

	Percentage DPPH Inhibition of the Vegetables						
Extract Conc (µg/ml)	*O. gratissimum*	*T. triangulea*	*T. occidentalis*	*A. hybridus*	*V. amygdalina*	*B. alba*	Ascorbic acid
10	71.3492	56.6667	45.4286	53.7302	53.8071	45.3884	62.7381
20	75.4365	50.3968	45.6349	54.2064	53.8904	47.0571	74.2064
40	88.6111	52.3810	48.4127	55.6349	68.4037	47.5687	96.0714
50	93.4127	58.7302	48.8571	55.7937	78.1384	50.5104	98.4524
60	95.3175	57.5794	48.9611	54.6429	79.1092	50.0792	98.6111
80	90.6349	49.9206	52.5397	54.5635	82.9214	52.6891	98.6508
100	91.3095	49.4444	49.0873	56.8651	87.1408	54.4431	98.7698
IC_{50}	1.23	502.81	76.90	0.20	10.52	44.65	4.16

3.3 Total phenolic Compounds

The results of the total phenolic compounds determination revealed that all the vegetables contain phenolic compounds that are responsible for the antioxidant activities recorded. Phenolic compounds are responsible for antioxidant activities and the number and position of the hydroxyl groups of the compounds determine the degree of activity. Many structure-activity-relationship studies have reported the effect of the number and position of phenolic hydroxyls in the radical scavenging potential of phenolic compounds (Seyoum *et al.*, 2006). For instance, when one of the *ortho*- or *para*-free hydroxyl in a phenolic compound is protected by glycosylation or methylation, its radical scavenging activity is drastically reduced (Jassbi *et al.*, 2004).

V. amygdalina and *O. gratissimum* have high percentage of DPPH inhibition which is complemented by the high total phenolic content in 1 mg of crude extract (Tables 3 and 4). The IC_{50} of the vegetables corroborated the total phenolic content of the extracts; for instance, *A. hybridus*, *V. amygdalina* and *O. gratissimum* have total phenolic content of 0.158, 0.130 0.101 µg/mL respectively in 1 mg of extract and the IC_{50} of the vegetables are 0.20, 10.52 and 1.23 µg/mL respectively. Thus, *A. hybridus, V. amygdalina* and *O. gratissimum* contain high concentrations of phenolic compounds with many hydroxyl groups.

Table 3. Total Phenolic content in Vegetables

Vegetable	***O. gratissimum***	***T. triangulea***	***T. occidentalis***	***A. hybridus***	***V. amygdalina***	***B. alba***
Total Phenolic content (50% dilution)	42.66	7.97	23.91	96.09	69.84	7.03

IC_{50} is a measure of the amount of antioxidant material required to scavenge 50% of free radical (DPPH) in the assay system with values inversely proportional to the antioxidant activity. Possession of antioxidant activities by food materials assists in reducing ROS concentration in the human body by hydrogen donation (Khanam *et al*, 2004; Jayaprakash *et al*, 2001). The high percentage of DPPH inhibition by the extracts implies capabilities for good hydrogen donation. Phenolic compounds are known to have direct antioxidant activities due to the presence of hydroxyl groups which function as hydrogen donors (Dreosti, 2000; Duh *et al*, 1999).

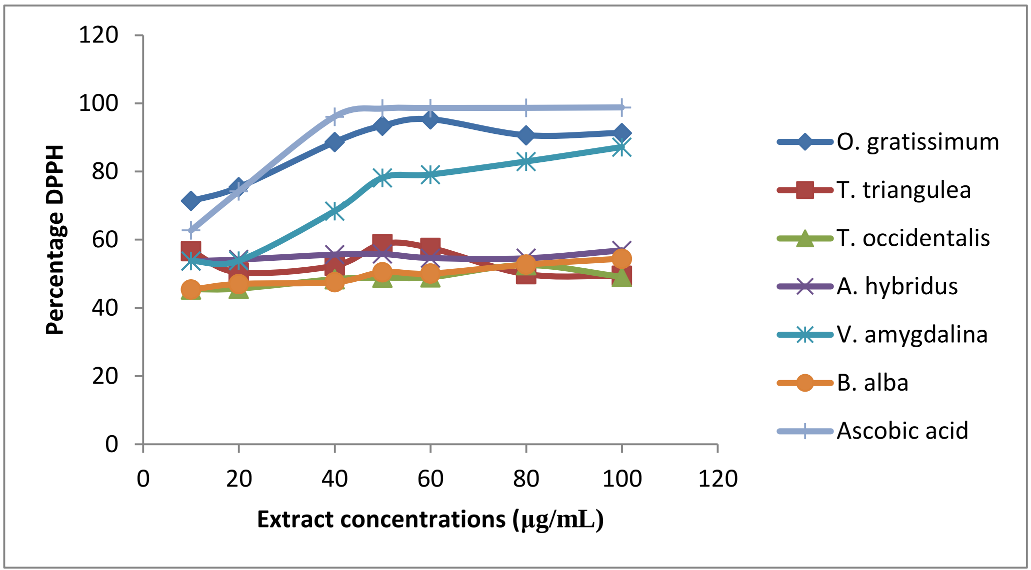

Figure 1. Free radical Scavenging activities of the vegetable extracts and Ascorbic acid by 1,1-diphenyl-2-picryl hydrazyl radicals

A positive correlation exists between the antioxidants activities and the total phenolic compounds for all the vegetables at 95% confidence level except *A. hybridus.* This suggests that the antioxidant activities are traceable to phenolic compounds for all the vegetables. *A. hybridus* has the highest anti-oxidant activity and highest percentage of total phenolic compound but other anti-oxidant compounds must have contributed to the activity.

Table 4. Total phenolic content and DPPH radical scavenging potential of the vegetable extracts

Vegetable	*O. gratissimum*	*T. triangulea*	*T. occidentalis*	*A. hybridus*	*V. amygdalina*	*B. alba*
Total Phenolic content (50% dilution)	42.66	7.97	23.91	96.09	69.84	7.03
IC_{50} DPPH	1.23	502.81	76.90	0.20	10.52	44.65

4. CONCLUSION

This study has shown that the six Nigerian indigenous vegetables possess both nutritional and health-benefitting potential as revealed by the proximate composition and antioxidant properties. Further work on other antioxidant assays particularly at lower concentrations of the extracts is suggested. The health-benefitting potential of these vegetables should be established for possible industrial utilization as source of natural antioxidants. We are presently working on the effect of processing on the antioxidants as well as the isolation of the phytochemicals in the vegetables.

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