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PHYTOCHEMICAL COMPOSITION, ANTIOXIDANT PROPERTIES AND ANTIBACTERIAL ACTIVITIES OF FIVE WEST-AFRICAN GREEN LEAFY VEGETABLES

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ABSTRACT

Green leafy vegetables have been reported as sources of nutrients and folklore remedy for the treatment of infections in West Africa. The present study was designed to investigate the antioxidant, antibacterial and phytochemical components of the methanol extracts from five selected green leafy vegetables (*Telfaria occidentalis, Amaranthus viridis, Amaranthus hybridus, Lactuca taraxicofolia* and *Solanium aethiopium*). The *in vitro* antioxidant activities of the extracts were measured using 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity and the antibacterial activities were evaluated using the well agar diffusion assay. The antioxidant activity of the methanolic extracts of the selected vegetables revealed that the EC_{50} ranged from 1.8 to 9.0µg/ml. The EC_{50} values for free radical scavenging activity of *T. Occidentalis, L. taraxicofolia* and *A. viridis* extracts were significantly superior over the standard, gallic acid. The extracts also exhibited inhibitory effects against *Pseudomonas aeruginosa, Bacillus cereus, Enterobacter cloacae*, *Bacillus subtilis* and *Staphylococcus aureus* at the concentration of 100µg/ml. The present study scientifically validated the traditional use of the vegetables as possible therapeutic agents.

Keywords: West Africa, vegetables, antioxidant properties, antibacterial activity, phytochemicals.

INTRODUCTION

Africa is endowed with a variety of traditional vegetables and different types are consumed by the various ethnic groups for different reasons (Salawu *et al*., 2009). In some West-African countries, vegetables are considered as the cheapest and most available sources of important proteins, carbohydrates, vitamins, minerals and essential amino acids (Francis *et al*., 2012). Previous studies have shown that the consumption of vegetables is closely related with the decrease risks of diseases that resulted from oxidative stress, including cancer, diabetes and various infectious diseases (Doll, 1990; Liu, 2004). These findings have encouraged people to source for locally available vegetables for the treatment of various diseases (Akindahunsi and Mulinacci, 2009). Therefore, five vegetables (Table 1) were selected for this study because of their popular use as food additives and remedy for the treatment of infections in West Africa.

T. Occidentalis (fluted pumpkin) is one of the popular and widely grown vegetable crops in West Africa (Akoroda, 1990). Previous study revealed that long term feeding of *T. Occidentalis* supplemented diet caused a significant increase in weight of animals which may be due to its nutrients (Oboh *et al*., 2006). A study has shown that the ethanol root extracts of *T. occidentalis* possess antimicrobial potential (Odoemena and Essien, 1995; Okokon *et al*., 2007). *S. aethiopicum* is commonly known as 'African eggplant' and grown in West Africa for its immature fruit (garden egg) and leaves. *S. aethiopicum* is highly valued constituents of the Nigerian foods and indigenous medicines and commonly consumed almost on a daily basis by both rural and urban families (Akoroda, 1990). *L. taraxacifolia* has been domesticated as a leafy vegetable in West Africa (Burkill, 1985). *L. taraxacifolia* is used as a remedy for prevention and treatment of measles, diabetes mellitus. It is reported to possess hypolipidaemic effect (Ayensu, 1978; Adebisi, 2004; Obi *et al*., 2006). The leaves of *L. taraxacifolia* plant are fed to nursing cows to stimulate lactation and also to sheep and goat to induce multiple births (Wichtl, 1994).

A. viridis L. is also a widespread weed and occasionally cultivated in Nigeria, Gabon and DR Congo. *A. viridis* L is a traditional food which has potential to improve nutrition, boost food security, foster rural development and support sustainable land care (NRC, 2006). In Côte d'Ivoire, the leaf sap is used as an eye wash to treat eye infections and for treating convulsions and epilepsy in children. Furthermore, the plant possesses anti-proliferative, anti-fungal and anti-viral activities (Obi *et al*., 2006). *A. hybridus* is commonly called smooth amaranth, smooth pigweed and red amarantha in Nigeria. Leaves and young seedlings can be cooked as a spinach, added to soups or eaten raw (Tindall, 1983).

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So far, a great number of these vegetables have not been tested for their chemical composition, antimicrobial and antioxidant activities (Oboh *et al*., 2006). The aim of this study is to determine the phytochemical components, antioxidants and antibacterial potentials of five locally sourced green leafy vegetables. Our ultimate target is to give an insight on the health benefits of these vegetables in local dishes in order to sensitize the people on the need for their consumption.

MATERIALS AND METHODS

Selection of species

The botanical identity of the plant materials (Table 1) was confirmed by the Taxonomist at the Forest Research Institute of Nigeria (FRIN) Jericho Ibadan, Nigeria and the voucher specimens were stored in the herbarium.

Preparation of crude extract

The plant materials (leaves of *A. viridis, A. hybridus, L. taraxicofolia, S. aethiopicum, T. occidentalis*) were air-dried at room temperature for 3 weeks and then ground into powdered form. The powdered vegetable (100g) was macerated in 500ml of methanol for 72hours at room temperature. The extracts were then filtered through a filter paper (Whatman GF/C, England), concentrated using a rotary evaporator at 36^0C and stored at 4^0C until needed.

Phytochemical screening

The Folin-Ciocalteu assay using gallic acid as standard was used for the qualitative test of total phenolics from methanolic extracts of the selected vegetables (Makkar, 2000). Total phenolic concentrations were expressed as gallic acid equivalents (GAE) per gram dry matter. The test as described by Tadhani and Subhash (2006) was used to test for the presence of saponin in the vegetables. The presence of alkaloids was based on the quantitative method of Makkar and Goodchild (1996). The flavonoid content was determined as described by Hagerman (2002), with some modifications. 50µl of each MeOH extract were diluted with 950µl glacial acetic acid, followed by the addition of 2.5 ml of 4% HCl in methanol (v/v) and 2.5 ml vanillin reagent (4% vanillin in glacial acetic acid, w/v), after which the reaction mixture was incubated for 20 min at room temperature. After incubation, absorbance at 500nm was measured using a UV–Vis Spectrophotometer against water blank. Flavonoid content was expressed as catechin equivalents (CTE) per gram dry matter.

Condensed tannin content was evaluated using the butanol–HCl assay as described by Makkar (2000) and Ndhlala *et al*. (2007) and the percentage dry matter was calculated as equivalent amount of leucocyanidin (LCE) using the equation below:

$$\text{Condensed tannin}(\%) = \frac{(A_{550} \times 78.26 \times \text{dilution factor of extract})}{\%\ \text{dry matter}} \times 100$$

where A550 = absorbance of sample at 550nm. The formula assumes the effective E1%, 1cm, 550nm of leucocyanidin to be 460.

Table 1. Ethnobotanical data and percent (w/w) extraction yields of methanol extracts from selected Nigerian green leafy vegetables.

Plant species (family)	Voucher number	Traditional use	Extraction yield (%)
Telfairia occidentalis Hook.f. (Cucurbitaceae)	FHI. 107340	The leaves and vines are consumed as vegetables and the young seeds are eaten as food (Akoroda, 1990; Badifu and Ogunsua, 1991).	12.1
Lactuca taraxicofolia (Willd.) Schum. (Asteraceae)	FHI. 107399	The leaves are eaten fresh as a salad or cooked in soups and sauces (Burkill,1985).	10.7
Amaranthus viridis L. (Amaranthaceae)	FHI. 107395	The leaves are used as vegetables or as ingredients in sauces (Grubben and Denton, 2004).	14.2
Solanum aethiopicum L. (Solanaceae	FHI. 107394	The leaves are eaten raw and also when boiled or fried as ingredient of stews, soups and vegetable sauces.	11.8
Amaranthus hybridus L. (Amaranthaceae)	FHI. 107401	The leaves combined with condiments are used to prepare soup (Mepha *et al*., 2007). In Congo, the leaves are eaten as spinach or green vegetables (Dhellot *et al*., 2006). These leaves boiled and mixed with groundnut sauce are eaten as salad in Mozambique and in West Africa (Oliveria and DeCarvalho, 1975).	15.0

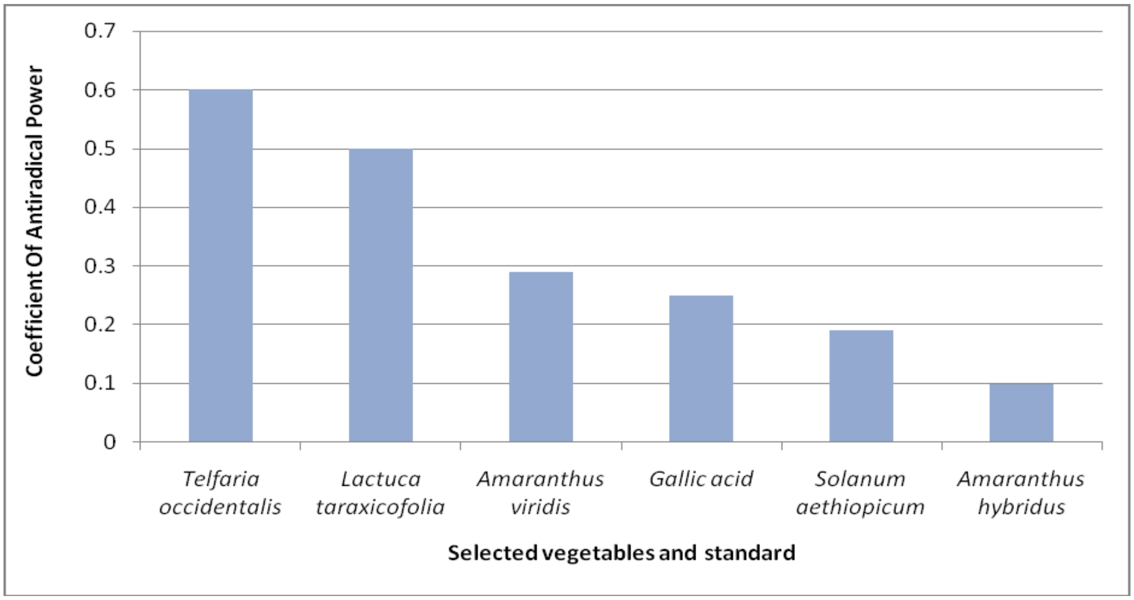

Fig. 1. Antiradical power of the selected vegetables and standard, gallic acid.

Radical scavenging activity of plant extract and gallic acid against DPPH radicals

Radical scavenging activity of the methanolic plant extracts and gallic acid against DPPH were determined using a modification of Hatano *et al*. (1988). Serial dilutions were made to obtain a concentration range of 10 to 0.0393µg/ml for the plant extracts and gallic acid. The reaction mixtures were made by adding 100 µl of the test sample to 900µl of 6.5 x 10^{-5} M DPPH solution in methanol. Then the absorbance was recorded at 515nm after 1minute and 5 minute interval up to 40minutes. The experiments were carried out in triplicate. However, the percentages of DPPH reduction of the test samples were calculated using the following formula:

$$\% \text{ Radical Scavenging Activity (RSA)} = \frac{Ab\ \ As}{Ab} \times 100$$

Where Ab is absorbance of blank (DPPH solution) (t=0min)

As is absorbance of test sample ($t \neq 0$min)

The reaction kinetics for each concentration of the plant extract was plotted. From these graphs, the percentage of DPPH remaining at the steady state was determined. The antiradical activity (ARP) was then calculated from the equation: ARP = 1/EC50.

Antimicrobial assay

Bacterial cultures

The following bacteria were used: *Bacillus cereus, Bacillus subtilis, Enterobacter cloacae, Staphylococcus aureus* and *Pseudomonas aeruginosa*. All the cultures were isolated in our laboratory from fresh food samples and maintained on nutrient agars (Oxoid, England) at 4^0C (Ruijssenaars and Hartsmans, 2000). Selected isolates were plated on respective agar plates for subsequent work and maintained as pure cultures according to Bergey's Manual of Systemic Bacteriology (Sneath *et al*., 1984).

Agar well diffusion assay

The antibacterial activities of vegetable extracts were determined using an agar well diffusion assay (Lyudmila *et al*., 2003). The bacterial strains were cultured in a Mueller-Hinton broth (Oxoid, England) for 24h and diluted with sterilised distilled-deionised water. Then, 10ml of each culture (0.5 McFarland standards) was spread onto the surface of Mueller–Hinton agar (Oxoid, England) to create a bacterial lawn. The sterile cork borer (6mm) was used to make wells in the agar medium. These wells were then filled with 50 and 100mg/ml of the extracts prepared in 2% of DMSO. The plates were incubated at 37^0 C for 24 hrs. The antibacterial activity was compared with chloramphenicol (1mg/ml). Tests were carried out in triplicates.

Statistical analysis

All the data were expressed as mean ± standard deviation (S.D). Results were analyzed using one-way ANOVA followed by Student's t-test. Differences with p values less than 0.05 were considered to be significant.

RESULTS

Phytochemical screening

S. aethiopium and *A. hybridus* showed high contents of gallotannin, while *S. aethiopicum* and *L, taraxicofolia* presented high content of flavonoids (Table 2). In the condensed tannin content, higher levels of phytochemicals were contained in the *T. occidentalis*. In addition, all the extract indicated the presence of alkaloids.

Antibacterial assays

All the extracts of the vegetables showed broad spectrum antibacterial activity, with zone of inhibition ranging from 10-25mm (Table 2). Besides the *T. occidentalis* extract, which had a zone of inhibition value of 25mm against *P. aeruginosa*, all the extracts showed low antimicrobial activity. With the exception of the extract of the *T. occidentalis* and *A. hybridus*, almost all the plants showed a low measure of the diameter of the zone of inhibition in (mm), against the gram-negative bacteria strains tested.

Scavenging effect of extracts and gallic acid on DPPH radicals

Table 4 shows the EC_{50} of the extracts in comparism with gallic acid (standard) relative to their ARP values. Figure 1 shows activity-relativity of the extracts in respect to the ratios of their ARP values, using gallic acid as a standard. Figure 1 indicates the antiradical power of the selected vegetable in relation to standard compound (gallic acids). *T. occidentalis* shows the highest activity-relativity, hence possessing the highest antioxidant activity. The order of increasing activity is *A. hydridus>S.aethiopicum* >gallic acid > *A.viridis> L.taraxicofolia>T. occidentalis.*

DISCUSSION

In some African countries keen interest has been committed to the commonly available green leafy vegetables which possess a remarkable potential to help people overcome the lethal diseases of modern society (Aletor and Adeogun, 1995; Aletor *et al*., 2002; Schonfeldt and Pretorius, 2011). In the present study, we have attempted to evaluate the antibacterial and antioxidants properties of five green leafy vegetables, namely *T. occidentalis, L. taraxicofolia, A. viridis, S. aethiopium and A. hybridus*. A preliminary phytochemical analysis was also carried out to determine the presence of different classes of secondary metabolites. Determination of antibacterial activity of the selected vegetable extracts tested against a panel of isolated pathogenic bacteria indicated that the concentration of the extracts directly influenced the antibacterial potentials of the vegetables (Table 3). Extracts with more than 14mm zone of inhibition were considered as having good antimicrobial activity (Aligiannis *et al*., 2001).

Table 2. Total phenolics, condensed tannin and flavonoid content of the selected vegetable extracts.

Plant secondary metabolites	*S. aethiopium*	*T. occidentalis*	*L. taraxicofolia*	*A. viridis*	*A. hybridus*
Total phenolics (mg GAE/g dry matter)	40.60±7.88	49.32± 4.07	28.38 ± 1.07	49.3±9.07	39.32± 6.07
Condensed tannin (% LCE/g dry matter)	0.46 ± 0.02	1.32 ± 0.12	0.96 ± 0.04	0.87± 0.05	0.90 ± 0.03
Gallotannin (µg GAE/g dry matter)	29.32±0.07	19.01±0.07	16.32 ± 0.07	23.2±0.07	31.32±4.12
Flavonoid (mg CTE/g dry matter)	0.96 ± 0.03	0.71 ± 0.07	0.97 ± 0.10	0.76± 0.05	0.34 ± 0.03

GAE, gallic acid equivalents; LCE, leucocyanidin equivalents; CTE, catechin equivalents. Data represented as means ± SD (n = 3).

Table 3. Inhibitory activities of plant extracts against five isolated bacteria (*B. cereus, B. subtilis, E. cloacae, S. aureus* and *P. aeruginosa*).

Bacterial strains	Diameter of zone of inhibition (mm)					
	Concentration (mg/ml)	*S. aethiopium*	*T. occidentalis*	*L. taraxicofolia*	*A. viridis*	*A. hybridus*
B. cereus	100	11±1.3	17±2.3	18±2.0	14±0.9	16±2.1
	50	12±1.1	13±0.5	12±0.9	12±1.0	14±1.2
B. subtilis	100	14±1.2	17±2.0	13±1.1	15±2.0	16±2.6
	50	12±1.2	14±1.1	NI	11±0.6	14±0.8
E. cloacae	100	14±0.9	20±2.8	NI	14±0.3	16±2.9
	50	NI	14±1.3	NI	11±0.9	13±1.2
S. aureus	100	14±1.0	20±3.2	13±0.9	11±0.8	15±0.9
	50	12±0.9	14±1.2	11±0.3	10±0.8	12±0.8
P. aeruginosa	100	11±0.8	42±5.9	NI	12±1.2	15±2.1
	50	NI	20±3.2	NI	NI	12±0.9

NI-No inhibition; Data represented as means ± SD (n = 3).

Among the studied vegetables, DPPH radical scavenging activity was found to be highly significant in *T. occidentalis* ($p<0.01$) while the remaining four vegetables showed good activity when compared with gallic acid (standard) (Table 4). The EC_{50} of *T. occidentalis* extract was about 3 times greater than standard antioxidant (gallic acid). The trend of antioxidant activity amongst the extracts in comparism with gallic acid is *T. occidentalis*<*L. taraxicofolia*<*A. viridis*<gallic acid < *S. aethiopicum*<*A.viridis* (Table 4). This revealed that *T. occidentalis, L. taraxicofolia* and *A. viridis* possess a higher antioxidant activity than gallic acid (positive control). In recent times restriction has been placed on synthetic antioxidant like butylated hydroxyl anisol, butylated hydroxyl toluene, tertiary butylated hydroquinone and gallic acid esters; which have been suspected to prompt negative health effects (Uusiku *et al*., 2010). Therefore, there is an urgent need to substitute these synthetic products with naturally occurring antioxidants. The selected vegetables may be considered as a good alternative for the synthetic antioxidants based on the high antioxidant activities exhibited by the selected vegetables.

Table 4. EC_{50} of the vegetable extracts and gallic acid (standard).

Vegetable extracts	EC_{50} (µg/ml)
T. occidentalis	1.8±0.2
L. taraxicofolia	2.0±0.3
A. viridis	3.4±0.25
S. aethiopicum	5.2±0.5
A. hybridus	9.0±2.1
Gallic acid	4.0±0.4

Data represented as means ± SD (n = 3)

Previous investigations have shown that the presence of phytochemicals in the selected vegetables showed an underlying contribution in respect to their medical and pharmaceutical relevance (Aletor and Adeogun, 1995; Aleto *et al*., 2002; Uusiku *et al*., 2010). Phenolic compounds including tannins, flavonoids, saponins and alkaloids have been implicated in pharmacological activities such as antioxidants, antimicrobial and anti-inflammatory activities (Wichtl, 1994). For instance, the use of *A. viridis* L. as an astringent (Francis *et al*., 2012) and the anti-venom properties of *L. taraxicofolia* were attributed to the presence of tannins.

Flavonoids, phenolics and tannins which are known antioxidants are found to be distinctively present amongst all the phytochemicals tested in all the selected vegetables (Table 2). The presence of these compounds in the extracts of these vegetables may also be the main cause of their high radical-scavenging activity (Table 4) and popularly reported high health beneficial properties (Oboh *et al*., 2006; Uusiku *et al*., 2010). Therefore, the use of the selected vegetable as a food supplement by the majority of people in West African may help in contributing to the total antioxidant defense system of the human body and this may account for the protection against diseases (Francis *et al*., 2012).

Consequently, the presence of phytochemicals such as tannins, phenolics as well as flavonoid in the selected vegetables (Table 2) may have crucial roles in the observed antioxidant and antibacterial potential of the leaves. Therefore, further work is being channelled towards the isolation and identification of the active ingredients in these vegetables.

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