Evaluation of Selected Phytochemicals and Bioactive Components in Fluted Pumpkin (*Telfairia occidentalis*) and Amaranth (*Amaranthus caudatus*) Leaves

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ABSTRACT

Aims: This study was carried out to assess the contents of total phenolics, flavonoids, condensed tannins, saponins and alkaloids in *Telfairia occidentalis* and *Amaranthus caudatus* leaves.
Study design: The experiment employed a 2×2×5 factorial design.
Place and Duration of Study: Food Chemistry/Biochemistry Laboratory, Department of Food Science and Technology, Federal University of Technology, Akure, Nigeria, between November 2010 and June 2011.
Methodology: The leaves were sun dried and milled into flour using a laboratory blender. The flour samples were separately soaked in ethanol and distilled water, filtered and rotary evaporated to obtain the respective aqueous (AECT) and ethanolic (EECT) concentrates. The concentrates were used for the determination of the above-mentioned phytochemicals and also for the assessment of free radical scavenging capacity using α,α-diphenyl-β-picrylhydrazyl (DPPH).
Results: The results obtained clearly showed that using ethanol as a solvent to extract the bioactive components was more efficient than using distilled water, especially for total phenolic and flavonoid contents of the leaf extracts. The contents of total phenolics, flavonoids, saponins and alkaloids were higher in *Amaranthus caudatus* leaf extract than in *Telfairia occidentalis* leaf extract; whereas the content of condensed tannins was higher in *T. occidentalis* leaf extract than in *A. caudatus* leaf extract. The free radical scavenging capacity of *Telfairia occidentalis* leaf extract was higher than that of *Amaranthus caudatus* leaf extract at both 0.3 and 0.6µl concentration of the extract. At 0.3µl concentration of the extract, the free radical scavenging capacity of *T. occidentalis* leaf extract ranged from 81% to 89% while that of *A. caudatus* leaf extract ranged from 67% to 74%.
Conclusion: The leaves contain substantial concentrations of the assayed phytochemicals. *T. occidentalis* leaves had higher free radical scavenging capacity than *A. caudatus* leaves.

Keywords: Telfairia occidentalis, Amaranthus caudatus, leaf extracts, phytochemicals, DPPH

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1. INTRODUCTION

Many studies aim at determining the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of both topical and systemic microbial infections as possible alternatives to chemical synthetic drugs that induce antimicrobial resistance (Akinpelu and Onakoya, 2006; Chopra et.al., 2007). Although advances have been made in pharmacology and synthetic organic chemistry, this reliance on natural products, particularly on plants, remains largely unchanged (Trevor, 2001). A wide variety of indigenous and minor plants have been utilized for daily consumption since ancient times. They are not only important ingredients of unique gastronomic dishes but also traditional functional foods usually consumed to maintain wellness (Kazuhiko et al., 2002).

With the increase in the awareness of the importance of vegetables as sources of vitamins and minerals in human diets, the cultivation of leafy vegetables has been on the increase (Ojeifo and Lucas, 1986; Okunlola and Enujiugha, 2010). Most of the vegetables consumed are from the market gardens grown in Fadama, flood plains, river banks and on flat land close to source of water supply. The purpose of market gardens is to produce economic crops for sale thus guaranteeing the farmer a steady income especially when operated on a full time basis. Vegetables are also good sources of regular food, being relatively cheap and rich in necessary vitamins.

Fluted pumpkin (*Telfaria occidentalis*) is one of the popular and widely grown vegetable crops in Nigeria particularly in the eastern (Anambra, Imo, Abia and Ebonyi States) and mid-western areas (Edo, and Delta States) and to an appreciable degree in the south western states (Ondo, Ogun, Ekiti, Oyo and Lagos) (Okoli and Mgbeogu, 1982). The nutritional composition of the leaves per 100g edible portion is as follows: water (86g), energy (47calories), protein (2.9g), fat (1.8g), carbohydrates (7g), fibre (1.7g). Oboh *et al.* (2006) reported that both the ethanolic and aqueous extracts of *Telfairia occidentalis* leaves contain saponin, alkaloid and tannins, with the aqueous extract having significantly higher ($p<0.05$) total phenol (12.2%), reducing power (1.9 OD_{70}) and free radical scavenging ability (92%) than the ethanolic extract [total phenol (5.5%), reducing power (1.5 OD_{70}), free radical scavenging ability (25%). Akoroda (1988) observed that *Telfaria occidentalis* is a common homestead garden crop in southern Nigeria, mostly cultivated by women. The crop is grown close to trees, walls, fence sand structures on which the shoots are allowed to climb (Okoli and Mgbeogu, 1982).The leaves of *Telfairia occidentalis* have long been recognized as veritable suppliers of iron and other important micro-minerals.

Amaranthus shows a wide variety of morphological diversity among and even within certain species. Although the family (Amaranthaceae) is distinctive, the genus *Amaranthus* has few distinguishing characters among the 70 species included (Juan et al., 2007). The leaves of *Amaranthus caudatus* contain water-soluble red-violet betacyanin pigments. In aqueous plant extracts they consist on average of 81% amaranthine and 19% isoamaranthine; in dried extracts of 67% amaranthine and 33% isoamaranthine. Amaranth leaves are a very good source of vitamins, including vitamin A, vitamin K, vitamin B_6, vitamin C, riboflavin, and folate, as well as dietary minerals including calcium, iron, magnesium, phosphorus, potassium, zinc, copper, and manganese. The compounds in amaranth can enhance human growth and development, improve general health, and strengthen immune responses to combat diseases. Veeru et al. (2009) reported that 0.14 mg/l of aqueous extract of *Amaranthus caudatus* was needed to bring about 50% inhibition (IC_{50}) of DPPH free radical.

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Antioxidants are protective molecules, which trap and neutralize free radicals and consequently protect the body from damage due to oxidative stress (Enujiugha, 2010). Antioxidants neutralize free radicals by accepting or donating an electron to eliminate the unpaired condition. Thus they may well be defined as free radical stabilizers and quenchers. The antioxidants themselves do not become free radicals by donating electrons because they are stable in either form. Evaluation of the antioxidant activities of natural substances has been of interest in recent years (Enujiugha, 2010). Antioxidants scavenge free radicals and reactive oxygen species and can be extremely important in inhibiting oxidative mechanisms that lead to degenerative diseases (Cardador–Martinez et al., 2002). It is widely accepted that significant antioxidant activity of food is related to high total phenolic content. Ethanol, methanol and acetone are commonly used solvents to extract antioxidants from plant foods (Sun et al., 2005; Enujiugha et al., 2012).

Bioactive components play a vital role in the treatment of various diseases; therefore it is important to identify, isolate, purify, characterize and quantify these bioactive components using various analytical methods and also to determine their antioxidant and free radical scavenging capacity. This study was aimed at determining and comparing the phytochemical constituents of the water and ethanol extracts of *Amaranthus caudatus* and *Telfairia occidentalis* leaves and assessing their respective capacities to scavenge free radicals using α,α-diphenyl-β-picrylhydrazyl (DPPH).

2. MATERIALS AND METHODS

2.1 Source of materials

Telfairia occidentalis and *Amaranthus caudatus* leaves were purchased from oja oba market Akure, Ondo state. All chemicals and reagents used were of analytical grade.

2.2 Sample preparation and solvent extraction

The leaves were sun dried for 48hrs and milled into flours using a laboratory blender. Leaf flours (50 g) were soaked in ethanol or distilled water (500 ml) for 24 h, filtered (Whatman no. 4 filter paper) and concentrated under reduced pressure with a rotary vacuum-evaporator at 40°C to obtain aqueous (AECT) and ethanolic (EECT) concentrated leaf extracts. The filtrate was then concentrated using rotary evaporator. The concentrate was then used for total phenol, flavonoid, tannin, saponin and alkaloid estimations and also for the assessment of antioxidative capacity employing DPPH.

2.3 Determination of total phenolic content

The total phenolic content (TPC) was determined by a Folin–Ciocalteu assay described by Singleton and Lamuela–Raventos (1999) using tannic acid as standard. The mixture of the sample solution (0.2ml), distilled water (0.2ml), 0.5ml of Folin-Ciocalteu reagent solution and 2.5ml of 7.5% Na_2CO_3 was vortexed in a test tube and incubated for 40 minutes at 45^{0}C. The absorbance was measured at 700nm against a blank prepared without the sample using a UV-160A spectrophotometer (Shimadzu, Kyoto, Japan). The total phenolic content was expressed as gallic acid equivalent (mg of GAE/ml sample) through the calibration curve of gallic acid. Linearity range of the calibration curve was 50 to100µg/ml (r=0.99)

2.4 Determination of total flavonoid content

The flavonoid content was determined using a spectrophotometric method described by Meda et al (2005) using caffeic acid as standard. The mixture of 0.5ml of the sample solution, 0.5ml of methanol, 0.05ml of aluminium chloride, 0.05ml of potassium acetate and 1.4ml of distilled water was allowed to stand for 30 minutes at room temperature. The absorbance was then measured at 415nm against the blank prepared without the sample

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using a spectrophotometer. The result were calculated and expressed as caffeic acid equivalent (mg of CAE/g sample) using the calibration curve of caffeic acid.

2.5 Determination of condensed tannin content

The analysis of tannin content was carried out according to the method described by Broadhurst and Jones (1978). A measured quantity (0.2ml) of the sample was added to 10ml of 70% acetone. And a volume (0.5ml) of the mixture was then added to 2.5ml of sodium carbonate and 0.5ml of Folin–Ciocalteu reagent was added to it in a test tube. The mixture was allowed to stay for1hour at room temperature and the absorbance was measured at 700nm against the blank prepared without the sample.

2.6 Determination of alkaloid content

This was done by the alkaline precipitation gravimetric method described by Harbone (1973). A known volume (0.2ml) of sample was dispersed into 50ml of 10% acetic acid solution in ethanol to form a ratio of 1:10 (10%) in a beaker. Few drops of ammonia solution were added to it and it was allowed to stay for 4 minutes. It was then placed on the hot plate for it to concentrate to half of its volume. Few drops of ammonia solution were added to it again after which it was then filtered using a weighed filter paper. The filter paper with the residue was dried in the oven at 80°C for some minutes. The weight of the filter paper was determined using a measuring cylinder. Alkaloid content was calculated and expressed as a percentage of the weight of the sample analysed.

2.7 Determination of saponin content

The direct spectrophotometric method of Brunner (1984) was used to determine the saponin content. The mixture of 0.2ml of the sample and 10ml of isopropyl alcohol was allowed to stand for 24 hours to ensure uniform mixing 0.2ml of 40% magnesium carbonate was dispersed into the mixture and the solution was properly shaken together after which it was filtered using no 42 Whatman filter paper. The mixture of the filtrate (0.5ml), distilled water (25ml) and 1ml of ferric chloride solution in a beaker was allowed to stand for 30minutes for colour development after which the absorbance was measured at 380nm.

2.8 Determination of DPPH radical scavenging capacity

The free radical scavenging ability of the leaf extract against DPPH free radical was evaluated as described by Gyamfi et al. (1999). Briefly, appropriate dilution of the leaf extract was prepared by adding 9ml of distilled water to 1ml of the concentrated sample. Each 0.6ml of this mixture was dispensed into a test tube and 0.6ml of DPPH solution was added to it and the mixture was left in the dark for 30mins after which the absorbance was taken at 517nm. Further dilution was done by adding 0.3ml of distilled water to 0.3ml of the first mixture and 0.6ml of DPPH solution was added to it. This mixture was also left in the dark for 30 mins after which the absorbance was taken at 517nm using a UV-spectrophotometer. The DPPH free radical scavenging ability of the sample was subsequently calculated in percentage.

2.9 Statistical analysis

The data were analysed by ANOVA using SPSS version 16 for Windows. This was used to test any significant differences between solvent used and also between the different leaves extracts. The significant levels were defined using $p \leq 0.05$

3. RESULTS AND DISCUSSION

3.1 Total phenolic content

Total phenolic content (TPC) of the extracts from *Amaranthus caudatus* and *Telfairia occidentalis* leaves and the effect of the three solvents are presented in Table 1. Natural

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phenolics exert their beneficial health effects mainly through their antioxidant activity (Wang et.al 1996). These compounds are capable of decreasing oxygen concentration, intercepting singlet oxygen, decomposing primary product of oxidation to non radical species, and breaking chains to prevent continued hydrogen abstraction from substances (Shahidi and Naczk, 2004). Phenolic compounds contribute to the overall antioxidant activities of plant foods.

Table 1. Contents of total phenolics, flavonoids and condensed tannins in the leafy vegetable extracts

Leafy vegetable	Aqueous extract	Ethanol extract
Total phenolic content (mg gallic acid equiv./g)		
Telfairia occidentalis	0.000±0.000c	0.005±0.000ab
Amaranthus caudatus	0.002±0.001b	0.007±0.003a
Total flavonoid content (mg caffeic acid equiv./g)		
Telfairia occidentalis	0.212±0.004c	0.776±0.000b
Amaranthus caudatus	0.176±0.004d	0.840±0.004a
Condensed tannin content (mg tannic acid equiv./g)		
Telfairia occidentalis	0.047±0.003b	0.050±0.000a
Amaranthus caudatus	0.045±0.000c	0.035±0.000d

Values with same following letters within a row are not significantly different (p≤0.05)

Amaranthus caudatus and *Telfairia occidentalis* leaf extracts from different solvents differ significantly (p≥0.05) in their total phenolic content, as the TPC of the *Amaranthus caudatus* leaf extract was higher than that of the *Telfairia occidentalis* leaf extract. The total phenolic content of *T. occidentalis* leaf extract treated with ethanol was 0.005mgGAE/ml while TPC was 0.000mg GAE/ml in the extract treated with distilled water. For *Amaranthus caudatus* extract, the TPC of the extract treated with ethanol ranged from 0.004 to 0.010 mgGAE/ml compared with that of the extract treated with distilled water which ranged from 0.001 to 0.003 mgGAE/ml. This implies that the recommended solvent for extracting total phenolic content from these leaves is ethanol since it was more efficient. The TPC of the extracts with different treatments are presented in the Table 1.

3.2 Total flavonoid content

In order to estimate the potential role of flavonoids on the antioxidant activity of *Telfairia occidentalis* and *Amaranthus caudatus* leaves, the total flavonoid contents of the extracts were analysed. Also the effect of different solvents on the extract was examined and the results are presented in Table 1.

Flavonoids are widespread plant secondary metabolites, including flavones and flavonols. Epidermiological studies suggest that the consumption of flavonoid–rich foods protect against human diseases associated with oxidative stress. In vitro, flavonoids have shown free radical scavenging activity and protection against oxidative stress. As components of green leafy vegetables, they are regularly contained in human food.

Telfairia occidentalis and *Amaranthus caudatus* leaves extracts differ significantly (p≥ 0.05) in their flavonoid contents as TFC of *Amaranthus caudatus* leaf extract was higher than that of *T. occidentalis* leaf extract. The flavonoid contents of *T. occidentalis* and *A. caudatus* leaves extracts treated with ethanol were higher than those of the extracts treated with distilled water, implying that the more efficient solvent for extracting flavonoid from these leaves is ethanol. The total flavonoid content (TFC) of the *Telfairia occidentalis* leaf extract treated with ethanol was 0.776mgCAE/ml while the extract treated with distilled water ranged from 0.208 to 0.216mgCAE/ml. The TFC of *Amaranthus caudatus* leaf extract treated with

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ethanol was high, ranging from 0.836 to 0.844mgCAE/ml, compared to extract treated with distilled water which ranged from 0.172 to 0.180mgCAE/ml as shown in Table 1.

3.3 Condensed tannin content

The results for the condensed tannin contents of both aqueous and ethanolic extracts of the two leafy vegetables are presented in Table 1. Tannins are produced via condensation of simple phenolics and have a variety of molecular structure. They are generally divided into hydrolysable and condensed proanthocyanidins (polymer of flavan-3-ols) (Haslam, 1989). Tannins are biological active compounds and may have beneficial or adverse nutritional effect. It has been suggested that phenolic substances occur primarily in seeds of certain leafy vegetable. Tannins play an important role in the defence system of seeds that are exposed to oxidative damage by many environmental factors (Trosynska et al 2002).

The tannin content of *T. occidentalis* leaf extract was found to be almost the same with that of *A. caudatus* leaf extract ranging from 0.044 to 0.05mgTAE/ml while that of *A. caudatus* leaf extract ranged from 0.035 to 0.045mgTAE/ml. It is worthwhile to note that the tannin content of *T. occidentalis* leaf extract treated with ethanol was 0.05mgTAE/ml compared to the extract treated with distilled water which ranged from 0.044 to 0.05mgTAE/ml; while for *A. caudatus* leaf extract, the tannin content of the extract treated with ethanol was 0.035mgTAE/ml which was low compared to that of the extract treated with distilled water which was 0.045mgTAE/ml as shown in Table 1.

3.4 Alkaloid content

The alkaloid contents of the leaf extracts of the two assayed green leafy vegetables are presented in Table 2. Alkaloids are a group of naturally occurring chemical compounds which mostly contain basic nitrogen atoms. This group also includes some related compounds with neutral and even weakly acidic properties. Also some synthetic compounds of similar structure are attributed to alkaloids. In addition to carbon, hydrogen and nitrogen, alkaloids may also contain oxygen, sulphur and more rarely other elements such as chlorine, bromine and phosphorus. Alkaloids are produced by a large variety of organisms, including bacteria, fungi, plants, and animals and are part of the group of natural products (also called secondary metabolites). Many alkaloids can be purified from crude extracts by acid-base extraction and many are toxic to other organisms. They often have pharmacological effects and are used as medications, as recreational drugs, or in entheogenic rituals. Examples are the local anaesthetic and stimulant, cocaine; the stimulant, caffeine; nicotine; the analgesic, morphine; the antibacterial, berberine; the anticancer compound, vincrinstine; the antihypertension agent, resepine; the cholinomimeric, galatamine; the spasmolysis agent, atropine; the vasodilator, vincamine; the anti-arhythmia compound, quinidine; the anti-asthma therapeutic, ephedrine; and the antimalarial drug, quinine. Although alkaloids act on a diversity of metabolic systems in humans and other animals, they almost uniformly invoke a bitter taste.

Table 2. Alkaloid and saponin contents of the green leafy vegetable extracts

Leafy vegetable	Aqueous extract	Ethanol extract
Alkaloid content (mg/g)		
Telfairia occidentalis	0.350±0.010a	0.450±0.010c
Amaranthus caudatus	2.503±0.006a	0.500±0.010b
Saponin content (mg/g)		
Telfairia occidentalis	88.717±0.225c	11.933±0.251d
Amaranthus caudatus	122.80±0.252a	135.00±0.252b

Values with same following letters within a row are not significantly different (p≤0.05)

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The data generated in the present study showed that the alkaloid content of *Amaranthus caudatus* leaf extract was higher than that of *Telfairia occidentalis* leaf extract as it ranged from 49% to 251% compared to that of *Telfairia occidentalis* leaf extract which ranged from 34% to 46%. Also, the alkaloid content of *A. caudatus* extract treated with distilled water was higher than that of ethanol extract as it ranged from 250% to 251% compared to that of ethanol extract which ranged from 49% to 51%. On the other hand, for *Telfairia occidentalis*, the alkaloid content of extract treated with distilled water was low ranging from 34% to 36%, whereas the alkaloid content of ethanol extract ranged from 44% to 46% as shown in Table 2.

3.5 Saponin content

The saponin contents of both aqueous and eyhanolic extracts of *Telfairia occidentalis* and *Amaranthus* caudatus leaves are presented in Table 2. Saponins are a class of chemical compounds, one of many secondary metabolites found in natural sources, with saponins found in particular abundance in various plant species. Specifically, they are amphipathic glycosides grouped phenomenologically by the soap-like foaming they produce when shaken in aqueous solutions, and structurally by their composition of one or more hydrophilic glycoside moieties combined with a lipophilic triterpene derivative. A ready and therapeutically relevant example is the cardio-active agent digoxin, from common foxglove. In plants, saponins may serve as anti-feedants, and to protect the plant against microbes and fungi. Some plant saponins (e.g. from oat and spinach) may enhance nutrient absorption and aid in animal digestion. However, saponins are often bitter to taste, and so can reduce plant palatability (e.g., in livestock feeds), or even imbue them with life-threatening animal toxicity. Previous research has clearly demonstrated that some saponins are toxic to cold-blooded organisms and insects at particular concentrations.

The results from the present work showed that saponin contents of both the distilled water extract and ethanol extract of *Amaranthus caudatus* leaves were higher than the extracts from *Telfairia occidentalis* leaves. The saponin content of distilled water extract was high ranging from 88.5 to 89 mg/g while that of ethanol extract ranged from 11.7 to 12.2mg/g for *Telfairia occidentalis* leaves. The saponin content of distilled water extract ranged from 122.5 to 123.1mg/g while that of ethanol extract ranged from 134.7 to 135.3 mg/g for *Amaranthus caudatus* leaves. The result obtained also showed that the saponin content of the *T. occidentalis* extract treated with distilled water was higher than that of ethanol extract; while it is vice versa for *Amaranthus caudatus* leaf extract as shown in Table 2.

3.6 DPPH radical scavenging activity

It is generally recognised that free radicals produced in the body are partly associated with the aetiology of cancer and other chronic diseases. Dietary antioxidants, capable of scavenging free radicals are able to reduce risks of these diseases. Therefore it is important to determine the radical scavenging effect of antioxidants in *Telfairia occidentalis* and *Amaranthus caudatus* leaves. DPPH is a free radical which is stable at room temperature, and produces a violet solution in ethanol. Reduction of DPPH by antioxidant results in a loss of absorbance. Thus the degree of discoloration of the solution indicates the scavenging efficiency of the added substances. The use of DPPH provides an easy and rapid way to evaluate antioxidant activity.

The results obtained in this study revealed that ethanol extract of *Telfairia occidentalis* leaves had lower free radical scavenging capacity than the distilled water extract at 0.3µL concentration of the extract; DPPH scavenging capacity of aqueous extract ranged from 88 to 90% whereas that of ethanol extract ranged from 74% to 90%, but the reverse was the case at 0.6µL concentration of the extract. The free radical scavenging capacity of the ethanol extract was found to be lower than that of the distilled water extract for *Amaranthus caudatus* leaves at 0.6 µL while the free radical scavenging capacity of distilled water extract

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was lower than that of ethanol extract at 0.3µL. The free radical scavenging capacity of the ethanol extract ranged from 68% to 76% while that of distilled water extract ranged from 77% to 83% at 0.6 µL concentration of the extract. At both 0.3 and 0.6 µL concentrations of the extract, *Telfairia occidentalis* leaf extract had higher free radical scavenging capacity as shown in Figures 1 and 2.

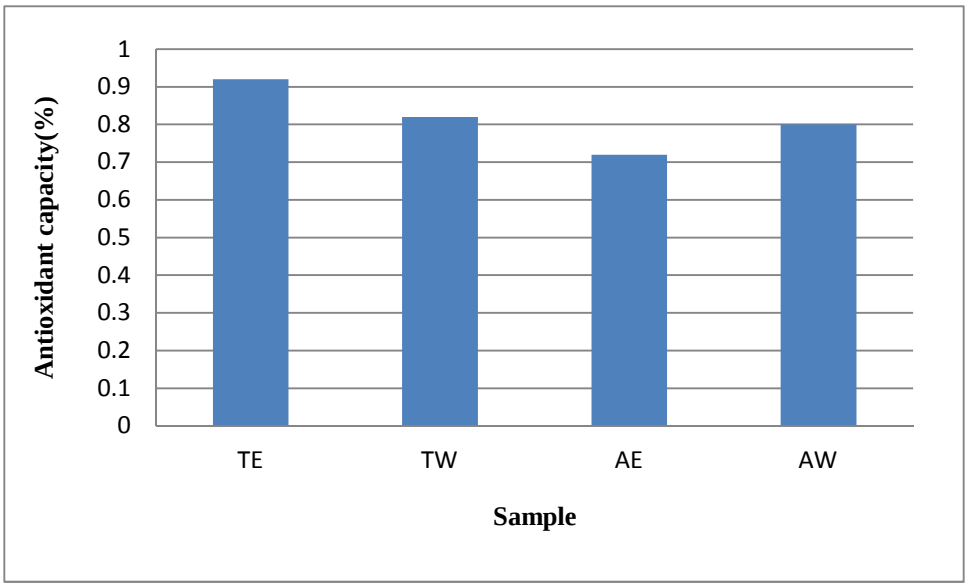

Fig. 1: DPPH Free Radical Scavenging Capacity of the vegetable extracts (0.6µL)
TE and TW = respective ethanol and aqueous extracts from Telfairia occidentalis leaves
AE and AW = respective ethanol and aqueous extracts from Amaranthus caudatus leaves

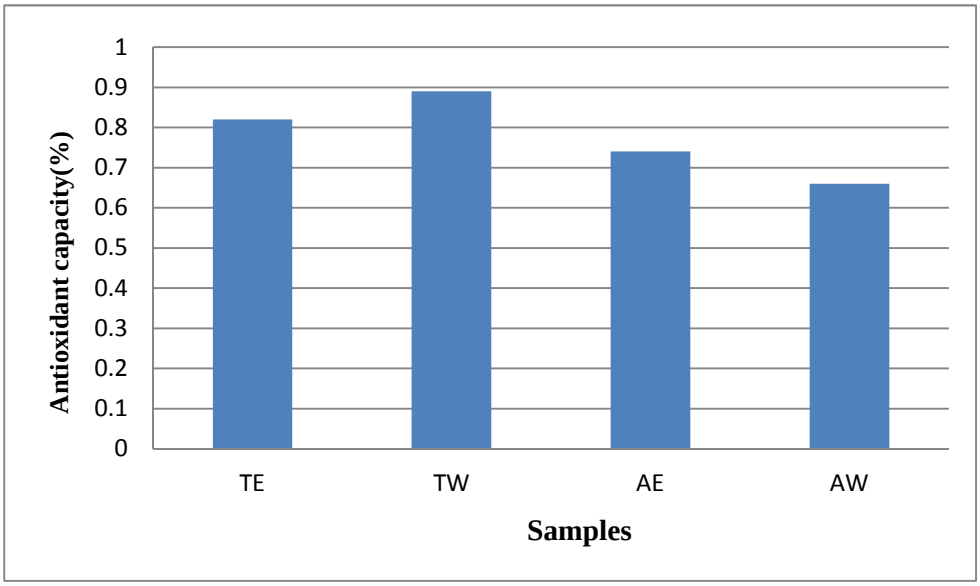

Fig. 2: DPPH Free Radical Scavenging Capacity of the vegetable extracts (0.3µL)
TE and TW = respective ethanol and aqueous extracts from Telfairia occidentalis leaves
AE and AW = respective ethanol and aqueous extracts from Amaranthus caudatus leaves

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4. CONCLUSION

The results of the present study showed that *Telfairia occidentalis* and *Amaranthus caudatus*, representing two of the commonly consumed green leafy vegetables in southern parts of Nigeria, contain substantial amounts of the selected bioactive components, which are helpful in the prevention of some cardiovascular and age-related diseases. However these bioactive components do not have a standard yet for a particular food. The results clearly showed that using ethanol as a solvent to extract the bioactive components was more efficient than using distilled water. *Telfairia occidentalis* leaf extract had higher free radical scavenging capacity than *Amaranthus caudatus* leaf extract.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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