

PHYTOCHEMICAL ANALYSIS, PROXIMATE AND MINERAL COMPOSITION AND *IN VITRO* ANTIOXIDANT ACTIVITIES IN *Telfairia occidentalis* AQUEOUS LEAF EXTRACT

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ABSTRACT

The present study sought to undertake the phytochemical analysis (using standard methods of analysis), proximate and mineral composition as well as *in vitro* antioxidant properties (involving inhibition of DPPH and reducing power ability) of aqueous leaf extract of *Telfairia occidentalis* consumed in Ekpoma, Edo state, Nigeria. The preliminary phytochemical analysis showed that the leaf of *T. occidentalis* contains flavonoids, tannins, saponins, alkaloids and phenolics. Quantitative analysis showed that phenol, flavonoids, tannins, alkaloids and saponins in percentage were 0.19 ± 0.12 , 7.12 ± 0.50 , 0.12 ± 0.08 , 1.03 ± 0.24 and 7.01 ± 0.30 respectively. Result on proximate analysis showed 89.01% dry matter, 10.99% moisture content, 21.14% crude protein, 6.46% lipid, 11.56% crude fiber, 8.31% Ash content, and 53.10 % carbohydrate. Result on Mineral composition in mg/100g dry matter showed Calcium (61.03 ± 0.04), Sodium (51.49 ± 1.32), Iron (25.75 ± 1.65), Zinc (13.15 ± 0.31), Potassium (801.21 ± 0.45), Magnesium (85.11 ± 1.22), Phosphorus (18.09 ± 0.09), Manganese (21.27 ± 0.32) and Copper (0.93 ± 0.03). *In vitro* antioxidant scavenging activity using 2, 2-diphenyl 1-picrylhydrazyl (DPPH) and Ascorbic as standard and reducing power ability of the plant extract was found to be concentration dependent with maximum inhibition and reducing power ability at 0.4mg/ml which were 69% (lower than 89% for ascorbic acid) and 0.199 respectively. Our findings provide evidence that aqueous extract of *Telfairia occidentalis* is a potential source of natural antioxidants. The result of these findings also revealed that *Telfairia occidentalis* leaves are good source of carbohydrates and energy. The leaves are also a good source of minerals.

KEYWORDS: *Telfairia occidentalis*; antioxidant activity; DPPH, free radicals, minerals, Phytochemicals

INTRODUCTION

Many oxidative stress related diseases are as a result of accumulation of free radicals in the body and a lot of researches are going on worldwide directed towards finding natural antioxidants of plant origin. Plants are endowed with free radical scavenging molecules, such as vitamins, terpenoids, phenolic acids, lignins, stilbenes, tannins, flavonoids, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are rich in antioxidant activity (Zheng and Wang, 2001; Cai *et al.*, 2003). Studies have shown that many of these antioxidant compounds possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, and antiviral activities (Sala *et al.*, 2002, Rice-Evans *et al.*, 1995). The ingestion of natural antioxidants has been associated with reduced risks of cancer, cardiovascular disease, diabetes, and other diseases associated with ageing, (Ashokkumar *et al.*, 2008; Veerapur *et al.*, 2009], and in recent years, there has been a worldwide trend towards the use of the natural phytochemical present in berry crops, teas, herbs, oilseeds, beans, fruits and vegetables (Kitts *et al.*, 2000; Muselík *et al.*, 2007; Wang and Jiao, 2000). In Nigeria, as in most other tropical countries of Africa where the daily diet is dominated by starchy staple foods, vegetables are the cheapest and most readily available sources of important proteins, vitamins, minerals and essential amino acids (Thompson and Kelly, 1990). Vegetables are the fresh and edible portions of herbaceous plants, which can be eaten raw or cooked (Dhellit *et al.*, 2006).

Telfairia occidentalis is a vigorous perennial vine, growing to 10m or more in length. The stems have branching tendrils and the leaves are divided into 3–5 leaflet. The fruits are pale green, 3 – 10 kg in weight, strongly ribbed at maturity and up to 25cm in diameter. The seeds are 3– 5cm in diameter (F.A.O., 1988). *T. occidentalis* popularly known as fluted pumpkin is cultivated mainly in Southern Nigeria mainly for the leaves and seeds. *T. occidentalis* leaf are often used as vegetable in the preparation of soups, while the seeds are eaten raw or roasted and also ground into powder and used as soup thickening. In Nigeria, the consumption of the leaf of *Telfairia occidentalis* as a leafy vegetable in the diet or as an infusion in medicinal preparation is being promoted in view of the various medicinal properties such as antianemic, antidiabetic, antioxidant, antimicrobial activities and as a purgative leafy vegetable (Obboh *et al.*, 2006). According to Obboh, (2005) *Telfairia occidentalis* prevents against garlic – induced oxidative stress. The plant also prevents the occurrence of abdominal pain, small intestine obstruction, dermatitis, asthma and increase of bleeding which are caused by garlic

Several works reporting compositional evaluation and functional properties of various types of edible wild plants in use in developing countries abound in the scientific literature (Ekop, 2007). However, much still needs to be done on the chemical composition of edible leafy vegetables grown in Nigeria. This study therefore investigated the phytochemical compositions, mineral and proximate compositions, the in vitro antioxidant and free radical scavenging

potential of *T. occidentalis* and if it could be used as supplement to other scarce or non available source of nutrients.

MATERIALS AND METHODS

Collection and Extraction

Fresh leaves of *Telfairia occidentalis* were purchased from a local market in Ekpoma, Edo state, Nigeria and identified. The Fresh leaves were thoroughly rinsed and air-dried at room temperature (24°C) and then pulverized, crushed into fine powder using a manual blender and weighed. Aqueous extracts of the plants were prepared by soaking 1000g of the dry powdered plant materials in 5 litres of double distilled water and then kept at room temperature for 48hours (for thorough extraction). At the end of the 48hours, the extracts were filtered first through a Whatmann filter paper No. 42 (125mm) and then through cotton wool. The filtrate was concentrated using a rotary evaporator with the water bath set 40°C to one-tenth its original volume and then finally with freeze drier. The dried residue (crude extract) was then stored at 4°C. Aliquot portions of the crude plant extract residue were weighed and dissolved in distilled water for use on each day of the experiments.

Qualitative Analysis of Phytochemicals

Phytochemical screening was performed using standard procedures (Sofowora, 1993; Trease and Evans, 1980; Ayoola *et al.*, 2008)

Quantitative Analysis of Phytochemicals

Determination of Alkaloids (Harborne, 1973)

A 5g sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol added. The beaker was covered

and allowed to stand for 4 hours. It was then filtered and the extract concentrated on a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide (2M) and then filtered. The residue (if available) is the alkaloid which is then dried and weighed.

Determination of Tannin (Van – burden and Robinson, 1981)

The sample (5g) was weighed into a 50 ml conical flask. 50 ml of distilled water was added and shaken for 1 hour in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtrate was pipette out into a test tube and mixed with 2 ml of 0.1M FeCl₃ in 0.1N HCl and 0.008M potassium ferrocyanide. The absorbance was measured at 395 nm within 10 minutes.

Determination of Saponin

The method used was as described by Obadoni and Ochuko (2001). A 20g sample was placed into a conical flask followed by the addition of 100 ml of 20% aqueous ethanol. This was then heated over a hot water bath for 4 hours with continuous stirring at about 55°C and thereafter filtered and the residue re-extracted with another 200 ml of 20% ethanol. The extract was reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml

of n-butanol was added. The n-butanol extracts was washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation, the sample was dried in the oven to a constant weight. Saponin content was calculated as percentage.

Determination of Flavonoids (Boham and Kocipai-abyazan, 1994)

The samples (10g) was extracted repeatedly with 100 ml of 80% aqueous methanol at room temperature. The entire solution was filtered through Whatman filter paper No 42 (125mm). The filtrate was transferred into a crucible and evaporated to dryness over a water bath and weighed to constant weight.

Determination of Total Phenols by Spectrophotometric Method

Fat free sample was boiled with 50 ml of diethyl ether for the extraction of the phenolic component for 15 minutes. The extract (5ml) was pipetted into a 50 ml flask, followed by the addition of 10 ml of distilled water. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amylalcohol were also added. The samples were made up to mark and the colour developed was measured after 30 minutes at 505nm at room temperature.

Determination of in vitro Antioxidant Properties of Extract

Determination of Reducing Power

The reducing power of the leaf extract was evaluated according to the method described by Aiyegoro and Okoh (2010). A mixture containing 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 ml of $K_3Fe(CN)_6$ (1% w/v) was added to 1.0 ml of the extract and standard (0.2–0.8 mg/ml) prepared in distilled water. The

resulting mixture was incubated for 20 min at 50°C, followed by the addition of 2.5 ml of TCA (10% w/v), which was then centrifuged at 3000 rpm for 10 min. Then 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (0.1% w/v). The absorbance was be measured at 700 nm against a blank sample. Increased absorbance of the reaction mixture indicates higher reducing power of the plant extract.

DPPH Radical Scavenging Ability

The method of Liyana-Pathiana and Shahidi (2005) was used for the determination of scavenging activity of DPPH free radical in the extract solution. DPPH (1 ml, 0.135 mM) prepared in methanol was mixed with 1.0 ml of aqueous extract ranging in concentration from 0.2 to 0.8 mg/ml. The reaction mixture was then vortexed thoroughly and left in the dark at room temperature for 30 min. The absorbance was measured at 517 nm using spectrophotometer. The scavenging ability of the plant extract was calculated using the equation:

$$\text{DPPH scavenging activity (\%)} = \frac{[(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})]}{(\text{Abs}_{\text{control}})} \times 100,$$

Where:

$\text{Abs}_{\text{control}}$ is the absorbance of DPPH + methanol and $\text{Abs}_{\text{sample}}$ is the absorbance of DPPH radical + sample (sample or standard).

Proximate Analysis

Dried and powdered plant samples were used for proximate analysis. The ash, crude fat, crude protein (nitrogen x 6.25) and crude fibre were determined using the standard methods of the Association of Official Analytical

Chemists (AOAC, 2000). Carbohydrate content was estimated based on the net difference between the other nutrients and the total percentage composition.

Mineral Analysis

An acid digest of the powdered plant was prepared by oxidizing 0.2g of the plant sample with conc. HCl/conc. nitric acid. The digests were diluted with distilled water and heated again (until colourless solution is obtained) to a certain volume and thereafter filtered with Whatmann filter paper No. 1 (110mm). The filtrate was then made up to 100ml with distilled water. Aliquots was thereafter used for mineral analysis using the Atomic Absorption Spectrophotometer (AAS). The blank and working standards were first run followed by the samples. Each sample was analysed twice, and the data reported as a mean of the analysed samples in mg/100g.

Phosphorus was determined by spectrophotometry. Here ammonium molybdate (40ml) plus 0.2g ascorbic acid was prepared. Sample aliquot (10ml) was added to ammonium molybdate and ascorbic acid (4ml) and then left to stand for few minutes (10minutes) for full color development and thereafter make up to 100ml with distilled water. After calibrating with different concentrations of phosphate standard solutions ranging from 0.5 to 2.5 mg/L, the absorbance of the sample was measured with spectrophotometer (HACH, DR 2800) at 660nm.

RESULTS

The phytochemical analysis conducted on *T. occidentalis* dried and powdered leaves (in Table 1 and 2)

revealed the presence of flavonoids, alkaloids, tannins, saponins and phenolics with composition as follows: flavonoids ($7.12 \pm 0.50\%$), tannins ($0.12 \pm 0.08\%$), saponins ($7.01 \pm 0.30\%$); alkaloids ($1.03 \pm 0.24\%$).

Percentage proximate composition of the leaves as shown in Table 3 are as follows: Dry matter (89.01 ± 0.13), moisture content (10.99 ± 1.34), crude protein (21.14 ± 0.32), crude fibre (11.56 ± 0.68), ash content (8.31 ± 0.21), crude lipid (6.46 ± 0.07), and carbohydrate (53.10 ± 0.68).

The mineral composition of *T. occidentalis* leaves in mg/100g are as shown in Table 4. Potassium had the highest concentration ($801.21 \pm 0.45 \text{mg}/100\text{g}$) and copper the least ($0.93 \pm 0.03 \text{mg}/100\text{g}$). The magnesium, iron, zinc, calcium, sodium, phosphorus and manganese content of the *T. occidentalis* leaves was 85.11 ± 1.22 , 25.75 ± 1.65 , 13.15 ± 0.31 , 64.03 ± 0.04 , 51.49 ± 1.32 , 18.09 ± 0.09 , and 21.27 ± 0.32 respectively.

Antioxidant ability, indicated by the ability of aqueous extract of *T. occidentalis* leaf to reduce iron (III) to iron (II) was examined (Table 5). Results showed that the higher the concentration, the higher the absorbance. Increased absorbance of the reaction mixture indicates higher reducing power of the plant extract i.e reducing power of the extract increased with concentration.

DPPH radical Scavenging activity of the aqueous extract of *T. occidentalis* leaves is shown in Table 5. The free radical scavenging activities was compared with activities of ascorbic acid, a known antioxidant. The activity was determined as a function of their %

inhibition (%I). The results showed that aqueous extract of *Telfairia occidentalis* leaf has the capability to scavenge the DPPH-radicals.

Table 1: Qualitative phytoconstituents of *Telfairia occidentalis* aqueous leaf extract

Plant phytochemicals	Positive or negative
Flavonoids	+
Alkaloids	+
Tannins	+
Saponins	+
Phenolics	+
Glycosides	-
Steroids	-
Triterpenes	-
Phylobatannins	-

Where, + = positive, - = negative

Table 2: Major phytoconstituents of *Telfairia occidentalis* powdered leaves

Sample	% Phenol	% Flavonoid	% Tannins	% Saponins	% Alkaloids
<i>Telfaria occidentalis</i>	0.19±0.12	7.12±0.50	0.12±0.08	7.01±0.30	1.03±0.24

Results are mean of triplicate determinations ± standard deviation.

Table 3: Proximate composition (g/100 g dry matter) of *Telfairia occidentalis* leaves

Parameters	% Composition
Dry matter	89.01 ± 0.13
Moisture content	10.99 ± 1.34
Crude protein	21.14 ± 0.32
Crude fibre	11.56 ± 0.68
Ash content	8.31 ± 0.21
Crude Lipid	6.46 ± 0.07
Carbohydrate	53.10 ± 0.68

Results are mean of triplicate determinations ± standard deviation.

Table 4: Mineral composition of *Telfairia occidentalis* leaves (mg/100 g dry weight)

Minerals	Concentration (mg/100g dry matter)
Calcium	61.03 ± 0.04
Sodium	51.49 ± 1.32
Iron	25.75 ± 1.65
Zinc	13.15 ± 0.31
Potassium	801.21 ± 0.45
Magnesium	85.11 ± 1.22
Phosphorus	18.09 ± 0.09
Manganese	21.27 ± 0.32
Copper	0.93 ± 0.03

Results are mean of duplicate determinations on a dry weight basis ± standard deviation.

Table 5: Radical scavenging activities and Reducing power activities of aqueous extract of *Telfairia occidentalis* and Ascorbic acid standard at different concentrations

Conc. (mg/ml)	% DPPH	Reducing power
0.05	44 (60)	0.058
0.1	55 (74)	0.07
0.2	63 (82)	0.121
0.4	69 (89)	0.199

Ascorbic acid values in parenthesis

DISCUSSION

The phytochemical analysis conducted on *T. occidentalis* dried and powdered leaves (Tables 1 and 2) revealed the presence of flavonoids, alkaloids, tannins, saponins and phenolics. These phytochemical compounds are known to support bioactive activities in medicinal plants and are thus responsible for the antioxidant activities of this plant. Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and have remarkable activity in cancer prevention (Ruch *et al.*, 1989, Motar *et al.*, 1985). Thus *T. occidentalis* containing tannins may serve as a

potential source of bioactive compound in cancer prevention and treatment. Flavonoids are antiinflammatory, anti-tumor, anti-viral, antiplateletes (Corkan *et al.*, 1998, Pourmorad *et al.*, 2006)). Flavonoids also are potent water soluble antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong anticancer activity (Salah *et al.*, 1995, Del-Rio *et al.*, 1997, Okwu, 2004). It has been recognized that flavonoids, which contain hydroxyl groups are responsible for the radical scavenging effects of most plants. They show antioxidant activity and their effects on human nutrition and health is considerable. The mechanisms of action

of flavonoids are through scavenging or chelating process (Pourmorad *et al.*, 2006; Omale and Okafor, 2008). Alkaloids are beneficial chemicals to plants serving as repellent to predators and parasites. This probably endows these group of agents its antimicrobial activity. Alkaloids have been found to have microbiocidal effect and the major anti-diarrheal effect is probably due to their effects on small intestine and antihypertensive effect (Trease and Evans, 1985). Some alkaloids are useful against HIV infection as well as intestinal infection associated with AIDS (McDevitt *et al.*, 1998).

Also, the plant extract was revealed to contain saponins, known to produce inhibitory effect on inflammation (Just *et al.*, 1998). Saponins are known bioactive substances that can reduce the uptake of cholesterol and glucose in the gut through intra-luminal physiochemical interaction (Price *et al.*, 1987). Saponins as a class of natural products are also involved in complexation with cholesterol to form pores in cell membrane bilayers (Francis *et al.*, 2002) as such may be used as anticholesterol agents or cholesterol lowering agent. The presence of these phenolic compounds in this plant contributes to their antioxidative properties and thus the usefulness of these plants in herbal medicament. Phenols have been found to be useful in the preparation of some antimicrobial compounds such as dettol and cresol. Phenols act as free radical chain reaction terminators thereby acting as antioxidant (Shahidi and Wanasundra, 1992). Phenols also have a potential of combating oxidative stress syndrome, causative of some neurodegenerative diseases and

cardiovascular diseases. These properties bestow high medicinal activities on *T. occidentalis*.

The nutrient composition revealed that *T. occidentalis* leaves contained protein, fiber, ash, fats/oil as well as carbohydrate as shown in table 4.3. Plant proteins are source of food nutrient especially for the less privileged population in developing countries including Nigeria. Fibre cleanses the digestive tract by removing potential carcinogens from the body and prevents the absorption of excess cholesterol. Fibre also adds bulk to the diet and may therefore guard against metabolic conditions such as hypercholesterolemia and diabetes mellitus. Thus, the benefit of consumption of *T. occidentalis* leaves cannot be over-emphasized. The high ash content is a reflection of the mineral content of this food material. The results therefore suggest a high deposit of mineral elements in the leaves (Antia *et al.*, 2006). Carbohydrates are essential for the maintenance of life in both plants and animals and also provide raw materials for many industries (Ebun-Oluwa and Alade, 2007). The plant is a good source of carbohydrate when consumed because it meets the recommended dietary allowance (RDA) values (FND, 2002). The plant is considered a good source of protein because it provides more than 12% of caloric value from protein (Pearson, 1976). Dietary fat increases the palatability of food by absorbing and retaining flavours (Antia *et al.*, 2006). A diet providing 1 - 2% of its caloric of energy as fat is said to be sufficient for humans as excess fat consumption is implicated in certain cardiovascular disorders (Antia *et al.*, 2006).The

moisture content of the dried *T. occidentalis* leaves was 10.99% which is relatively low, and would therefore hinder the growth of spoilage microorganisms and thereby enhance the shelf life.

The mineral composition of *T. occidentalis* leaves in mg/100g is as shown in Table 4 with potassium having the highest concentration and copper the least. Other mineral revealed includes calcium, sodium, iron, magnesium, zinc, phosphorus and manganese. Sodium and potassium are important intracellular and extracellular cations respectively. Sodium is involved in the regulation of plasma volume, acid-base balance, nerve and muscle contraction (Akpanyung, 2005). Sodium remains one of the major electrolytes in the blood. Without sodium the body cannot be hydrated, it would dry up.

A balanced proportion of calcium and phosphorus is needed in the body. Phosphorus is an essential component of bone mineral. Deficiency of phosphorus-calcium balance results in osteoporosis, arthritis, pyorrhea, rickets and tooth decay. Calcium is necessary for the coagulation of blood, the proper functioning of the heart and nervous system and the normal contraction of muscles. Its most important function is to aid in the formation of bones and teeth. Copper helps in the absorption of iron, it is therefore often seen with Iron naturally. Copper is important for cellular defense and protection of the mucous membranes, it is anti-anemic and essential for the formation of iron and haemoglobin (Claude and Paule, 1979). Magnesium is a component of chlorophyll and it is an important mineral element in connection

with ischemic heart disease and calcium metabolism in bones (Ishida *et al.*, 2000). Iron is an essential element for haemoglobin formation, normal functioning of the central nervous system and oxidation of carbohydrate, protein and fats (Adeyeye and Otokili, 1999). Iron plays crucial roles in haemopoiesis, control of infection and cell mediated immunity (Bhaskaran, 2001). The deficiency of iron has been described as the most prevalent nutritional deficiency and iron deficiency anemia is estimated to affect more than one billion people worldwide (Trowbridge and Martorell, 2002). The consequences of iron deficiency include reduced work capacity, impairments in behaviour and intellectual performance and decrease resistance to infection (Dioxin *et al.*, 2004). Zinc is involved in normal function of immune system and is a component of over 50 enzymes in the body (Okaka *et al.*, 2006). An estimated 20% of the world population is reported to be at risk of inadequate zinc intake (Hotz and Brown, 2004). Studies on Nigerian shows that zinc deficiency affects 20% of children less than five years, 28.1% of mothers and 43.9% of pregnant women (Dioxin *et al.*, 2004).

Majority of antioxidant enzymes or defense systems of the body and processes involved in lipid metabolism in general make use of mineral elements (Gorman, 1992), and an imbalance in these elements usually leads to nutritional disorders and complications of nutritionally related diseases for example diabetes. The presence of these mineral elements in *T. occidentalis* could, therefore, be relevant in exerting antihyperglycemic activity and the

amelioration of the attendant macrovascular complications.

Various studies have indicated that the electron donation capacity of bioactive compounds is associated with their antioxidant activity (Siddhuraju *et al.*, 2002; Arabshahi-Deloue, 2007). Antioxidant ability, indicated by the ability of aqueous extract of *T. occidentalis* leaf to reduce iron (III) to iron (II) was examined (Table 5). Results showed that the higher the concentration, the higher the absorbance. Increased absorbance of the reaction mixture indicates higher reducing power of the plant extract i.e reducing power of the extract increased with concentration.

DPPH radical Scavenging activity of the aqueous extract of *T. occidentalis* leaves is shown in Table 5. The free radical scavenging activities was compared with activities of ascorbic acid, a known antioxidant. The activity was determined as a function of their % inhibition (%I). The results showed that aqueous extract of *T. occidentalis* leaf has the capability to scavenge the DPPH-radicals. DPPH is a stable free radical generally used to determine the ability of compounds to scavenge free radicals. DPPH stable free radical method is an easy, rapid and sensitive way to evaluate the antioxidant activity of a specific compound or plant extracts (Koleva *et al.*, 2002). Free radical oxidative stress has been implicated in the pathogenesis of a variety of human diseases like: arteriosclerosis, diabetes mellitus, hypertension, inflammation, cancer and AIDS. The use of DPPH scavenging assays in assessing the cell membrane integrity/cell membrane stabilizing capacities of plant constituents has given

explanations as to the possible ways by which phytomedicine could help to reduce diseases caused by infections, inflammation and oxygen radical generation affecting the cell membrane (Shahidi and Wanasundara, 1992). The model of scavenging DPPH free radicals used in the rapid screening method commonly employed for evaluating antioxidant activities is based on their ability to donate hydrogen ion (Kumazawa *et al.*, 2002). The stable radical 2-diphenyl-1-picrylhydrazyl radical (DPPH), which is known to generate free radicals was used as the radical sources. From our analysis, a larger percentage of the sample exhibited the ability to scavenge the free radical used in a concentration dependent manner, as their %I decreased with decrease in concentration. This is agreement with the work of Onocha *et al.*, (2010). The reduction in absorbance of DPPH caused by the aqueous extract of *T. occidentalis* leaves was measured and the percentage inhibition showed a decreasing trend with decrease in concentration of extract of *T. occidentalis* leaf. The scavenging activity of the extract could be linked to the presence of secondary metabolites such as flavonoids. This study justifies the use of *T. occidentalis* leaves in treatment of certain diseases such as diabetes, hypercholesterolemia, liver problems e.t.c, in which the participation of reactive oxygen species have been implicated. This could be as a result of the phytochemicals, nutritional, minerals, antioxidant and free radical scavenging ability of *T. occidentalis* leaf. Thus *T. occidentalis* leaf if consumed in sufficient amount could contribute greatly towards

meeting human nutritional requirement for normal growth and adequate protection against diseases arising from malnutrition.

REFERENCES

- Adeyeye, E. and Otokili M. K. O. 1999. Proximate composition and some nutritionally valuable minerals of two varieties of *Capsicum annum*. *Discov. Innov.* 11: 75-81.
- Aiyegoro, O. A. and Okoh, A. I. 2010. Preliminary phytochemical screening and *in vitro* antioxidant activities of the aqueous extract of *Helichrysum longifolium* DC. *BMC Complementary and Alternative Medicine* 10:21-32
- Akpanyung, E. O. 2005. Proximate and mineral composition of bouillon cubes produced in Nigeria. *Pakistan Journal of Nutrition* 4(5), 327-329.
- Antia, B. S., Akpan, E. J., Okon, P. A. and Umoren, I. U. 2006. Nutritive and Anti-Nutritive Evaluation of Sweet Potatoes (*Ipomoea batatas*) Leaves. *Pak. J. Nutr.* 5:166-168.
- AOAC (Association of Official Analytical Chemists). 1980. *Official method of analysis*. 15th Edition, Washington D.C. p143
- AOAC (Association of Official Analytical Chemists). 2000. *Official method of analysis*. 15th Edition, Washington D.C. p212
- Arabshahi-Deloue, S. and Urooj, A. 2007. Antioxidant properties of various solvent extracts of mulberry (*Morus indica* L.) leaves. *Food Chem.* 102: 1233–1240.
- Ashokkumar, D., Mazumder, U. K., Gupta, M., Senthilkumar, G. P. and Selvan, V. T. 2008. Evaluation of antioxidant and free radical scavenging Activities of *Oxystelma esculentum* in various *in vitro* Models. *J. Comp. Integ. Med.* 5(1):9
- Ayoola, G. A., Coker, H. A. B., Adesegun, S. A., Adepoju-Bello, A. A., Obaweya, K., Ezennia, E. C. and Atangbayila, T. O. 2008. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malarial therapy in South-Western Nigeria. *Trop. J. Pharm. Res.* 7: 1019-1024.
- Bhaskaran, P. 2001. Immunobiology of mild nutrient deficiency. *Br. J. Nutr.*, 85, S75-S80.
- Boham, B. A. and Kocipai-Abuyazan. 1994. Flavonoids and condensed tannins from the leaves of *Vaccinium raticulatum* and *Vaccinium calycinium*. *Pacific Sci.*, 48:458-463
- Cai, Y. Z., Sun, M., and Corke, H. 2003. Antioxidant activity of betalains from plants of the Amaranthaceae. *J. Agric. Food Chem.* 51(8):2288-2294.
- Claude, B. and Paule, S. 1979. Text book of Manual of Natural Living. 1st ed. Biddles Ltd. Guildford, Surrey, 98-101.
- Corkan, A., Fuh, R. A., Li, J. and Linssey, J. S. 1998. Phytochem. Auto CAD. A Computer Aided Design and Research IN: *Phytochemistry and Phytobiology* 68:141- 145.
- Del-Rio, A., Obdulio, B. G., Castillo, J., Martin, F. G., and Orluno, A. 1997. Uses and properties of citrus flavonoids. *J. Agric. Food Chem.* 45:4505-4515.
- Dioxon, B. M., and Haris, E. M. 1997. Nigeria food consumption and nutrition survey, 2001-2003. FNB.

- Food and Nutrition Board, Institute of medicine: Dietary reference intakes for calcium, phosphorus, magnesium.* Washington DC, National Academy Press.
- Dhellot, J. R., Matouba, E., Maloumbi, M. G., Nzikou, J. M., Safou-Ngoma, D. G., Linder, M., Desobry, S. and Parmentier, M. 2006. Extraction, chemical composition and nutritional characterization of vegetable oils: Case of *Amaranthus hybridus* (Var 1 and 2) of Congo Brazzaville. *African Journal of Biotechnology* **5**(11), 1095-1101.
- Ebun-Oluwa P.O., and Alade A.S. 2007. Nutritional potential of Belandieri Nettle spurge *Jatropha cathatica* seed. *Pak. J. Nutr.* 6:345:348.
- Ekop, A.S. 2007. Determination of chemical composition of *Gnetum Africana* (AFANG) seeds. *Pakistan Journal of Nutrition* **6**(1),40-43.
- FAO. 1998. Traditional Food Plants. FAO Food and Nutrition. Paper 42 pp. 489.
- FND. Food and nutrition board, Institute of medicine. National Academy of Sciences. 2002. Dietary reference Intake for Energy, Carbohydrate, Fibre, Fat, Fatty Acids, Cholesterol, protein and Amino acid (micro-nutrients). www.nap.edu
- Francis, C., George, G., Zohar, K., Harinder, P. S., Makhar, L. M., and Klaus, B. 2002. The biological action of saponins in animal system: a review. *British J. Nutrition* **88**(6):587-6051
- Gbolahan, D. 2001. Lesson Note on medical importance of trace elements. Centre for Natural Health Studies.
- Harborne J. 1973. *Phytochemical Methods: A Guide to Modern Technique of Plant Analysis*, Chapman and Hall, Thompson Science, London, pp. 107.
- Hotz, C. and Brown, K. C. 2009. International Zinc Nutrition Consultative Group (IZINCG) Technical document.
- Ishida, H., Suzuno, H., Sugiyama, N., Innami, S., and Todokoro, T. 2000. National evaluation of chemical component of leaves stalks and stem of sweet potatoes. *Ipomea batatas* *poir. Food Chem.* **68**: 359-367.
- Just, M. J., Recio, M. C., Giner, R. M., Cuellar, M. J., Manez, S., Bilia, A. R., and Rios, J. L. 1998. Anti-Inflammatory activity of unusual lupine saponins from. *Bupleurum fruticosens* **64**:404-407.
- Kayode, A. A. A. and Kayode, O. T. 2011. Some Medicinal Values of *Telfairia occidentalis*: A Review. *Am. J. Biochem. Mol. Biol.*, **1**:30-38.
- Koleva, I. I., Van Beek, T.A., Linssen, J. P. H. and de Groot, L. N. 2002. Evstatieva. Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. *Phytochem. Anal.*, **13**:8-17
- Kumazawa, S. Taniguchi, M. Suzuki, Y., Shimura, M, Kwon, M, and Nakayama, T. 2002. Antioxidant activity of polyphenols in Carob pods. *J. Agric. Food. Chem.* **50**:373-377.
- Kitts, D. D., Yuan, Y. V., Wijewickreme, A. N. and Hu, C. 2000. Antioxidant properties of a North American ginseng extract. *Mol. Cell Biochem.* **203**:1-10.
- Liyana-Pathiana, C. M. and Shahidi, F. 2005. Antioxidant activity of

- commercial soft and hard wheat (*Triticum aestivium* L) as affected by gastric pH conditions. *J Agric Food Chem.* 53:2433–40.
- McDevitt, J. T., Schneider, D. M., Katiyar, S. K., and Edlind, T. D. 1998. Berberina: a candidate for the treatment of diarrhea in AIDS patients abstr. 175. In Program and Abstract of the 36th Interscience Conference on Antimicrobial Agents And Chemotherapy. Am. Soc. Microbiol. Washington D.C.
- Motar. M. L. R., Thomas, G. and Barbosa Fillo, J. M. 1985. Effects of *Anacardium occidentale* stem bark extract on *in vivo* inflammatory models. *J. Ethnopharm.* 95(2-3):139-142.
- Muselík, J., García-Alonso, M., Martín-López, M. P., Želmička, M. and Rivas-Gonzalo, J. C. 2007. Measurement of Antioxidant Activity of wine catechins, procyanidins, antocyanins and piranoantocyanins. *Int. J. Mol. Sci.* 8:797-809.
- Obadoni, B. O. and Ochuko, P. O. 2001. Phytochemical studies and comparative efficacy of the crude extract of some homeostatic plants in Edo and Delta states of Nigeria. *Global J. Pure Appl. Sci.* 8:203-208
- Oboh, G., Nwanna E.E. and Elusiyan C.A. 2006. Antioxidant and antimicrobial properties of *Telfairia occidentalis* (fluted pumpkin) leaf extracts. *J. Pharmacol. Toxicol.* 1(2):167-175.
- Okaka, J. C., Akobundu, E. N. T. and Okaka, A. C. N. 2006. Food and Human Nutrition, an Integrated Approach. O. J. C. Academic Pub. Enugu, Nigeria.
- Okwu, D. E. 2004. Phytochemicals and Vitamin content of indigenous spices of South eastern Nigeria. *J. Sustain. Agric. Environ.* 6(1): 30-37.
- Omale, J. and Okafor, P. N. 2008. Comparative antioxidant capacity, membrane stabilization, polyphenol composition and cytotoxicity of the leaf and stem of *Cissus multistriata*. *Afr. J. Biotechnol.* 7(17): 3129-3133.
- Onocha, P. A., Oloyede, G. K. and Afolabi, Q. O. 2010. Phytochemical investigation, cytotoxicity and free radical scavenging activities of non-polar fractions of *Acalypha hispida* (leaves and twigs). *Excli J.* 10:1-8
- Pearson, D. H. 1976. Chemical Analysis of Foods. Churchhill London. pp. 335-336.
- Pourmorad, F., Hosseinimehr, S. J. and Shanabi Majd. N. 2006. Antioxidant activity, Phenol and flavonoid contents of some selected Iranian medicinal plants. *Afr. J. Biotechnol.*, 5(11):1142-1145.
- Price, K. R., Johnson, L. I. And Feriwick, H. 1987. Chemical and biological significance of saponin in food science. *Nutrition*, 26:127-135.
- Rice-Evans, C. A., Miller, N. J., Bolwell, P. G., Bramley, P. M., and Pridham, J. B. 1995. The relative activities of plant-derived polyphenolic flavonoid. *Free radical Res.* 22:375-383.
- Ruch, R. J., Cheng, S. J. and Klaunig, J. E. 1989. Prevention of cytotoxicity and inhibition of Intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogens* 10:1003-1008.
- Salah, N., Miller N.J. Pagange G. Tijburg L, Bolwell G.P., Rice E, Evans C. 1995. Polyphenolic flavonoids as

- scavenger of aqueous phase radicals as chain breaking antioxidant.
- Sala A., Recio M.D., Giner R.M., Manez S., Tournier H., Schinella G., and Rios J.L. 2002. Antiinflammatory and antioxidant properties of *Helichrysum italicum*. *J. Pharm Pharmacol.* 54(3):365-371.
- Shahidi F., and Wanasundara P.K.J.P.D. 1992. Phenolic antioxidants. Critical Reviews. In: Food Science and Nutrition. 32:67-103
- Siddhuraju P, Mohan P.S., Becker K. 2002. Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): a preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. *Food Chem.* **79**, 61-7.
- Sofowora, L.A. 1993. Medicinal plants and Traditional Medicine in Africa. Spectrum Books Ltd, Ibadan, pp. 55-71.
- Thompson, H.C. and Kelly, W.C. 1990. *Vegetable Crops*. 5th Ed. New Delhi: Mac Graw Hill Publishing Company Ltd, pp. 120-125.
- Trease G.E., and Evans W.C. 1985. Pharmacognosy 11th Ed., Tindall Ltd, London, pp. 60-75.
- Trowbridge, F., and Martorell, M. 2002. Forging effective strategies to combat iron deficiency. Summary and recommendations. *J. Nutri.*, 85, 875-880.
- Wang S.Y. and Jiao H. 2000. Correlation of antioxidant capacities to oxygen radical scavenging enzyme activities in blackberry. *J. Agric. Food Chem.* 48:5672-5676.
- Van-Burden, T.P. and Robinson, W.C. 1981. Formation. of complexes between proteins and tannic acid. *J. Agric. Food Chem.* 1:77
- Veerapur V.P., Prabhakar K.R., Parihar V.P., Kandadi M.R., Ramakrishana S., Mishra B., Satish Rao B.S., Srinivasan K.K., Priyadarsini K.I., Unnikrishnan M.K. 2009. *Ficus racemosa* stem bark extract: A potent antioxidant and a probable natural radioprotector. *Evid. Based. Complement Alternat. Med.* 6(3):317-324.
- Zheng W., and Wang S.Y. 2001. Antioxidant activity and phenolic compounds in selected herbs. *J. Agric. Food Chem.* 49(11):5165-5170.