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# Antioxidant and Antimicrobial Properties of *Telfairia occidentalis* (Fluted pumpkin) Leaf Extracts*

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**Abstract:** Dietary antioxidants are beneficial because of their protective roles against oxidative stress involved in the pathogenesis of multiple diseases such as cancer, anaemia, diabetics and cardiovascular diseases. This study sought to investigate the antioxidant and antimicrobial properties of ethanolic and aqueous extracts of *Telfairia occidentalis,* a popularly consumed green leafy vegetable in Nigeria. *Telfairia occidentalis* is popularly used in soup and folk medicine preparation in the management of various diseases such as diabetics, anaemia and gastrointestinal disorder. The result of this study revealed that both the ethanolic and aqueous extracts of *Telfairia occidentalis* leaves contain saponin, alkaloid and tannins, however, the aqueous extract had a significantly higher ($p<0.05$) total phenol (12.2%), reducing power (1.9 $OD_{700}$) and free radical scavenging ability (92%) than the ethanolic extracts [total phenol (5.5%), reducing power (1.5 $OD_{700}$), free radical scavenging ability (25%)]. Conversely, the ethanolic extract (0.5 mg $mL^{-1}$) had a higher inhibitory effect on some of the commonly encountered *Enterobacterioceae in* Nigeria, namely *Escherichia coli* (4.0 mm), *Pseudomonads aeruginosa* (8.0 mm) and *Proteus* sp. (4.0 mm), except *Salmonella typhii* (2.0 mm) where the aqueous extracts had a higher inhibition of the growth. However, both extracts did not inhibit the growth of the fungi tested, which are *Aspergillus flavus, Aspergillus fumigatum, Penicillum italicm, Geotrichum albidum.* It could be inferred from the study that extracts from *Telfairia occidentalis* leaf could scavenge and prevent free radical production and same time have antimicrobial property; although the aqueous extract had a higher antioxidant property, while the ethanolic extract had a higher antimicrobial property.

**Key words:** *Telfairia occidentalis*, antioxidant, antimicrobial, *Enterobacterioceae*

## Introduction

Oxidation which is essential for the production of energy to fuel biological processes usually produce free-radicals and other reactive oxygen species that can damage tissue and cause cell death (Yang *et al*., 2002). Although almost all organisms posses antioxidant defence and repair systems that have evolved to protect them against oxidative damage these systems are insufficient to prevent the damage entirely. However, antioxidant supplements or foods containing antioxidants may be used to help human body reduce oxidative damage (Yang *et al*., 2002). In recent years, there has been a particular interest in the antioxidant and health benefit of phytochemicals in foods and vegetables. This was as a result of their potential effects on human health (Wei and Shiow, 2001). These antioxidants are a wide range of substances or molecules that neutralise harmful compounds called free radicals that

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damage living cells. They are powerful substances that slow down the aging process, fight diseases and even prevent some cancers (Wei and Shiow, 2001). Antioxidants can take the form of enzymes in the body, vitamin supplement and nutritional additives. Although there are several enzyme systems, within the body to scavenge free radicals, the principal micronutrients (vitamins) antioxidants are vitamin E, β-carotene, flavonoids and vitamin C. The body cannot manufacture these micronutrients, so they must be supplied in the diet. Many vegetables contain health protective constituents; they are essential to preventing diseases and maintaining a state of well-being (Apple *et al*., 1997).

In Nigeria, *Telfairia occidentalis* (Fluted pumpkin) leaf is consumed in different parts of the country because of the numerous nutritional and medicinal attributes ascribed to it. It has different traditional names; among Igbos, it is known as Ugu, iroko or aporoko in Yoruba, ubong in Efik, umee in Urhobo and umeke in Edo (Akoroda, 1990). Oyolu (1978) reported that the leaves together with the edible shoots contain 85% moisture; 11% crude protein, 25% carbohydrates, 3% oils, 11% ash and as much as 700 ppm of iron, while Longe *et al*. (1983) reported that the minerals namely: calcium, potasium, magnesium, iron, sodium and phorphorus are concentrated in the testa, pulp and husk. The crop is primarily grown as a leafy vegetable and is used for human consumption and animal fodder, the high level of iron in *Telfairia occidentalis* leaves extract seems to provide a basis for the folklore that it can be administered as a blood tonic to convalescent persons. However, our preliminary investigations revealed that dietary intake of the leaf could prevent garlic-induced hemolytic anaemia in rats (Oboh, 2004).

The aqueous extracts of *Telfairia occidentalis* had been reported to reduce blood glucose level and also have antidiabetic effects in glucose induced hyperglycaemic and streptozotocin (STZ) induced diabetic mice (Aderibigbe *et al*., 1999), while it did not alter the glucose levels in normoglyceamic mice. Type 2 diabetes is associated with increased oxidative stress, which probably results both from excess generation of reactive oxygen species and decreased antioxidant defenses (Baynes, 1991; Tribe and Poston, 1996). In recent years, it has been known that, the most important factor to increase the free radicals production in diabetes is the hyperglycemic status, which can induce damage through overproduction of superoxide radical in the mitochondrion (Brownlee, 2001). Superoxide is converted to hydroperoxyls, which can diffuse through membranes and initiate lipoperoxidation. The oxidation of unsaturated lipids has implications not only for atherosclerosis, but also for stability and integrity of the red cell membranes (Steinberg *et al*., 1989). Increased levels of lipoperoxidation as evidenced by breakdown products like malondialdehyde, have been found in erythrocytes and plasma of type 2 diabetic patients. Supplementation with antioxidants is therefore, an attractive potential therapy.

Recently, Dina *et al*. (2001) reported that the aqueous extract of *Telfairia occidentalis* leaf could assist in the purging of the gastrointestinal tract as revealed by the purgative effect of the aqueous extracts of *Telfairia occidentalis* leaf on isolated guinea pig ileum and he concluded that there are some pharmacological effects underlying their mode of action. This study therefore sought to provide some biochemical basis for both the antidiabetic, antianaemic and the role of *Telfairia occidentalis* in maintaining/or cleansing of gastrointestinal tract, by determining the phytochemicals constituents of the aqueous and ethanolic extracts of *Telfairia occidentalis* leaves and to assess their antioxidant and antimicrobial property.

## Materials and Methods

### *Materials*

*Telfairia occidentalis* leaves were collected from a local market in Akure, Nigeria, while the authentication was done at the department of Crop Production, Federal University of Technology, Akure. The water used was glass-distilled, while the chemicals were analytical grades.

*Methods*

*Preparation of Aqueous Extracts*

Fresh leaves (317 g) of *Telfairia occidentalis* were homogenized with distilled water using a PowerGen 1800 D homogenizer and the mixture were filtered with Whatman No.1 filter paper. And the filterate was concentrated to 1/10 of its original volume at 40°C and freeze-dried.

*Preparation of Ethanolic Extracts*

The ethanolic extract was prepared following the same procedure as for the aqueous extract but using 80% ethanol instead of distilled water.

*Phytochemical Screening*

Both the aqueous and ethanolic extracts of the *Telfairia occidentalis* leaf was screened for the presence of alkaloids, tannins, saponnin, anthraquinone and cardiac glucosides using the method reported by Farnsworth (1988).

## Antioxidant Activity

*Total Phenol Content*

The total phenol content was determined by mixing 0.5 mL aliquot (0.05 g of the extract dissolved 20 mL of 70% Acetone) with equal volume of water, 0.5 mL Folin-Cioalteu's reagent and 2.5 mL of Sodium carbonate were subsequently added and the absorbance was measured after 40 min at 725 nm (Singleton *et al*., 1999).

*Reducing Property*

The reducing property of the vegetable was determined by assessing the ability of the vegetable extracts to reduce $FeCl_3$ solution as described by Pulido *et al*. (2000), briefly 2.5 mL aliquot (0.05 g of extract dissolved in 20 mL methanol) was mixed with 2.5 mL, 200 mM Sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% Potassium ferricyanide, the mixture was incubated at 50°C for 20 min, thereafter 2.5 mL, 10% Trichloroacetic acid was added and subsequently centrifuged at 650 rpm for 10 min, 5 mL of the supernatant was mixed with equal volume of water and 1 mL of 0.1% ferric chloride, the absorbance was later measured at 700 nm, a higher absorbance indicates a higher reducing power.

*Free Radical Scavenging Ability*

The free radical scavenging ability of the vegetables against DPPH (1, 1-diphenyl-2 picrylhydrazyl) free radical was also evaluated (Ursini *et al*., 1994), briefly, 1 mL aliquot (0.05 g of the extract was dissolved in 20 mL methanol) was mixed with 1 mL, 0.4 mM methanolic solution containing 1, 1-diphenyl-2 picrylhydrazyl (DPPH) radicals, the mixture was left in the dark for 30 min before measuring the absorbance at 516 nm.

*Antimicrobial Activity*

Antimicrobial activity of ethanolic and aqueous extracts of *Telfairia* occidentalis leaf was conducted using agar-diffusion methods (Ojala *et al*., 2000). The extracts were dissolved in sterile water to give a concentration range of 0.1-0.5 mg $mL^{-1}$. The extracts were tested using the hole-plate diffusion method. The bacterial plates were incubated at 23°C for 1 h to facilitate diffusion and then

incubated at 35°C for 24 h. The antifungal test plates were refrigerated at 8°C for 1 h and then incubated at 25°C for 72 h. The effect was evaluated by measuring the diameter of the inhibitory zones.

*Analysis of Data*

The result of the three replicates were pooled and expressed as mean±standard error (SE). Student t-test was used for the analysis of the data (Zar, 1984). Significance was accepted at $p \leq 0.05$.

## Results and Discussion

Epidemiological studies have shown that consumption of fruits and vegetables is associated with reduced risk of chronic diseases (Chu *et al*., 2002), in Nigeria the consumption of the leaf of *Telfairia occidentalis* as a leafy vegetable in diet or as an infusion in medicinal preparation is being promoted in view of the various medicinal properties such as antianeamic, antidiabetic and as a pugative leafy vegetable. In this study the phytochemical screening, antioxidant properties as typified by the total phenol content, reducing power and free radical scavenging ability and the antimicrobial activity as typified by its inhibition of the growth of some commonly encountered *Enterobacterioceae* and fungi of the aqueous and ethanolic extract of *Telfairia occidentalis* leaf are highlighted below.

The result of the phytochemical screening is shown in Table 1, the result revealed that both extracts (aqueous and ethanol) had tannin, alkaloid and saponins, this finding agrees with earlier report of Apple *et al*. (1997) in that vegetables are rich sources of phytochemicals, this phytochemicals have a protective and therapeutic effect essential to preventing diseases and maintaining a state of well being, by stimulating enzymes in the liver that render some carcinogens harmless and help the body stimulate others (Duke, 1992). Epidemiological studies have shown that regular consumption of fruits and vegetables has been correlated with a reduced risk of developing chronic diseases, such as cancer and cardiovascular disease. It is thought that the phytochemicals found in fruits and vegetables may be responsible in part for these health benefits. However, the actions of the antioxidant nutrients alone

Table1: Phytochemical constituent of *T. occidentalis* leaf extracts

| | Observation | |
|---|---|---|
| Plant Metabolite | Aqueous | Ethanol |
| Alkaloids | + | + |
| Saponins | + | + |
| Tannins | + | + |
| Anthraquinones | - | - |
| Cardiac glycosides | - | - |
| Phytobatannins | - | - |
| Present | + | |
| Absent | - | |

Table 2: Antioxidant properties on *Telfairia occidentalis* leaf extracts

| | Observation | |
|---|---|---|
| Antioxidant parameter | Aqueous | Ethanol |
| Total phenol (%) | $12.2 \pm 0.2^{a}$ | $5.5 \pm 0.1^{b}$ |
| Reducing power ($OD_{700}$) | $1.9 \pm 0.3^{a}$ | $1.5 \pm 0.1^{b}$ |
| Free radical scavenging ability (%) | $92.0 \pm 0.4^{a}$ | $50.0 \pm 0.2^{b}$ |

Values represent means of triplicate, values with the same superscript along the same row are not significantly different ($p>0.05$)

do not explain the observed health benefits of diets rich in fruits and vegetables for chronic diseases because taken alone, the individual antioxidants studied in clinical trials do not appear to have consistent preventive effects. However, the additive and synergistic effects of phytochemicals in fruits and vegetables are responsible for these potent antioxidant and anticancer activities and that the benefit of a diet rich in fruits and vegetables is attributed to the complex mixture of phytochemicals present in whole foods (Liu, 2005).

The total phenol content of the ethanolic and aqueous extracts are presented in Table 2. Phenols have antioxidant capacities that are much stronger than those of Vitamin C and E. Flavonols and flavonones are flavonoids of particular importance because they have been found to posses' antioxidant and free radical scavenging activity in vegetables (Amic *et al*., 2003). Some evidence as shown that flavonoids could protect membrane lipids from oxidation and a major source of flavonoids is vegetables and fruits (Amic *et al*., 2003). Polyphenols, particularly the flavonoids, are among the most potent plant antioxidants. Polyphenols can form complexes with reactive metals such as iron, zinc and copper-reducing their absorption. At first glance, this may seem to be a negative side effect (reducing nutrient absorption), but excess levels of such elements (metal cations) in the body can promote the generation of free radicals and contribute to the oxidative damage of cell membranes and cellular DNA. In addition to their chelating effect on metal cations, polyphenols also function as potent free radical scavengers within the body, where they can neutralize free radicals before they can cause cellular damage. Recent experimental studies in both animals and humans have shown that increasing polyphenol intake can protect LDL cholesterol from becoming oxidized (a key step in developing atherosclerosis), lower blood pressure in hypertensive subjects, reduce the tendency of the blood to clot and elevate total antioxidant capacity of the blood. The result of the study revealed that the aqueous extracts had a significantly higher ($p<0.05$) total phenol (12.2%) content than the ethanolic extracts (5.5%), this clearly indicates that the phenols present in *Telfairia occidentalis* leaves are more soluble in water than ethanol, consequently, the aqueous extracts could be a more potent antioxidant than the ethanolic extracts, this gives credit to the fact that aqueous extracts of the leaf is presently used in the management and prevention of anaemia and diabetes. This high phenol content in the aqueous extracts could have contributed to the prevention/ management of hemolytic anaemia (Oboh, 2004) and type 2 diabetes (Baynes, 1991) which are associated with free radical damage.

Antioxidants may be put into two separate groups: those that suppress the generation of reactive oxygen species and those that scavenge the reactive oxygen species generated. The result of the antioxidant activity of both the ethanolic and aqueous extracts as typified by the reducing power and free radical scavenging ability is shown in Table 2, the results indicate that both the aqueous and ethanol extract of *Telfairia occidentalis* leaf had high reducing power (1.5-1.9 $OD_{700}$). However the aqueous extract had a significantly higher ($p<0.05$) reducing power (1.9 $OD_{700}$) than the ethanolic extracts (1.5 $OD_{700}$), the higher phenolic content in the aqueous extract would have accounted for the higher ability of the aqueous extract to reduce Fe (III) to Fe (II) in the FRAP test for reducing ability (Amic *et al*., 2002). In addition, to the chelating properties of phenols they have been reported to have high reducing power (Blázovics *et al*., 2003), so it is expected that the extract with the higher phenol content will have higher reducing property.

The free radical scavenging ability of both the ethanolic and aqueous extract is also shown in Table 2, both extracts had a high free radical scavenging ability (50.0-92.0%), likewise the aqueous extract (92.0%) had a higher free radical scavenging ability than the ethanolic extract (50.0%), this findings is in line with earlier finding on the total phenol content of both extracts.

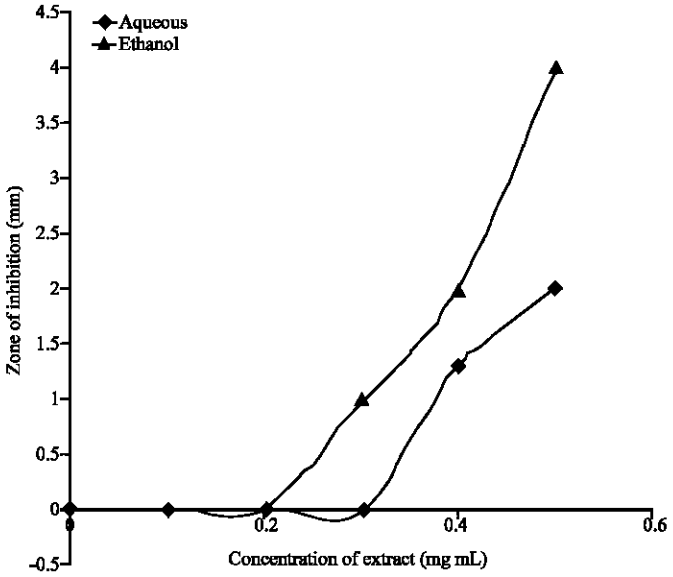


Fig. 1a: Inhibition of E. coli growth by *Telfairia occidentalis* leaf extract

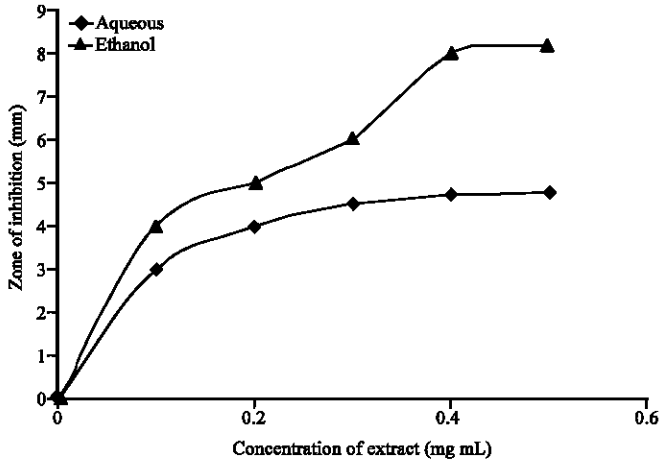


Fig. 1b: Inhibition of *Pseudomonads aeruginosa* growth by *Telfairia occidentalis* leaf extract

And the higher phenolic content in the aqueous extract would have accounted for the higher ability of the aqueous extract to scavenge DPPH free radicals (Amic *et al*., 2002). This finding clearly indicate that *Telfairia occidentalis* leaf antioxidant potentials will be more harness in its aqueous extraction than the ethanolic extraction and this is in accord with the form in which the plant is presently been used. Furthermore, the high reducing power and free radical scavenging ability of the extracts clearly indicate that both extracts of *Telfairia occidentalis* could suppress the generation of free radical and scavenge free radical.

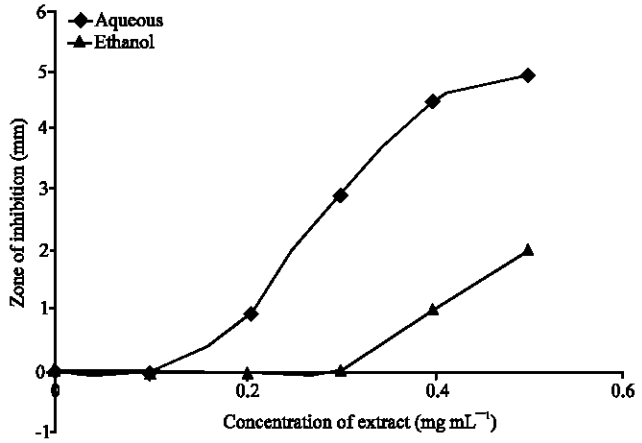


Fig. 1c: Inhibition of *Salmonella typhii* growth by *Telfairia occidentalis* leaf extract

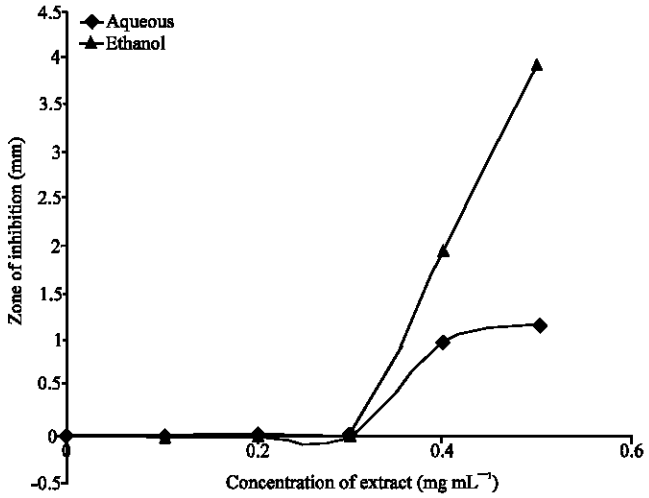


Fig. 1d: Inhibition of *Proteus sp* growth by *Telfairia occidentalis* leaf extract

The aqueous extract of *Telfairia occidentalis* leaf extracts had been reported to have purgative effect on the gastrointestinal tract and this action had been reported to be pharmacological (Dina *et al*., 2001). As a result, the inhibitory property of aqueous and ethanolic extracts of *Telfairia occidentalis* leaf against some commonly encountered *Enteriobacterioceae* namely *Pseudomonads aeruginosa*, *Proteus* sp. *Salmonella typhii* and *Escherichia coli*, as well as some fungi namely *Aspergillus flavus*, *Aspergillus fumigatum*, *Penicillum italicm* and *Geotrichum albidum* was also assessed. As shown in Fig. 1a-d, the result clearly revealed that the ethanolic extracts had a significantly higher ($p<0.05$) inhibitory effect on all the bacteria tested at all concentration (0.1-0.5 mg $mL^{-1}$) except in *Salmonella typhii* where the aqueous extract had a significantly higher

($p<0.05$) inhibitory effect than the aqueous extracts. Furthermore, of all the bacteria tested *Pseudomonads aeruginosa* had the highest susceptibility to inhibition by both extracts, while *Salmonella typhii* had the least susceptibility to inhibition by the ethanolic extracts, while *Proteus* sp. had the highest resistance to inhibition by the aqueous extract.

However, the modes of action of both extracts are dose-dependent and bacteriostatic. This finding agrees with earlier findings of Oboh and Ayoola (2003) on the mode of action of ethanolic extracts of *Alchornea laxiflora* leaves on some *Enterobacterioceae* as well as the findings of Oboh (2001) on the mode of action of onion and garlic volatile oil on some *Enteriobacterioceae*. Conversely, both extracts at the concentration tested in this study did not inhibit the growth of all the fungi tested. The basis for the antibacterial activity of both extracts cannot be categorically stated, however, it will not be far fetch from the array of group of secondary metabolites present such as saponins, tannins and alkaloids and all this secondary metabolites had been reported to have antimicrobial and therapeutic activity (Oboh *et al*. 1998; Oboh and Ayoola, 2003). In conclusion, *Telfairia occidentalis* leaf is a rich sources of phytochemicals and the intake of this plants chemicals has a protective potential against some tropical diseases such as anaemia, diabetes and *Enterobacterioceae* and the use of the leaf in folk medicine in Nigeria could be as a result of its ability to suppress the production of free radical and to scavenge already produced free radicals; and the antibacterial activity, although the aqueous extract had higher antioxidant property, while the ethanolic extract had higher antimicrobial activity.

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