Evaluation of Antioxidant Activity And Chemical Analysis Of The Leaf Of *Telfairia occidentalis*

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ABSTRACT

The methanol extract of *Telfairia occidentalis* leaf and n-hexane, ethyl acetate, butanol fractions were evaluated for their free radical scavenging activity, with DPPH assay. n-hexane fraction had the highest activity exhibiting an IC_{50} of 78.50µg/ml, comparable to that of the commercial antioxidant BHT. Chromatographic analysis showed the presence of three components in n-hexane fraction ($2H_1$, $2H_3$ and $2H_4$) with Rf values of 0.86, 0.71 and 0.55, respectively. Two components were seperated in ethyl acetate fraction ($2E_1$ and $2E_5$) with Rf values of 0.85 and 0.25, respectively, while the three components of butanol fraction ($2B_1$, $2B_2$, and $2B_3$) have Rf values of 0.90, 0.88 and 0.83 respectively. In addition, total phenolic content of *T. occidentialis* was determined as catechin equivalents. The n-hexane fraction which had the highest DPPH free radical scavenging activity also had the highest total flavonoid contents. The high flavonoid content was responsible for the antioxidant and free radical scavenging activities of *Telfairia occidentalis* leaf.

KEY WORDS: Antioxidants, DPPH, free radical scavenging activity, flavoniods.

INTRODUCTION

Reactive oxygen species (ROS), including free radicals are reported to cause damage of biological system, and to be involved in aging and in the pathogenesis of some diseases such as arthritis, atherosclerosis, diabetes and cancer (Ames, 1983; Feher et al., 1987; Aruoma, 1998). Almost all organisms possess antioxidants and repair systems that evolved to protect them against oxidative damage, these systems are insufficient to prevent them entirely. However, antioxidants may be used to help human body to reduce oxidative damage (Yang et al., 2002). Plants contain different natural products, which have a remarkable role in the traditional medicine in different countries. Nowadays the prevention of many diseases has been associated with the ingestion of different plants rich in natural antioxidants (Johnson, 2001; Virgilli et al., 2001; Adedapo et al., 2008). In recent years, there has been a particular interest in the antioxidant and health benefit of phytochemicals in food and vegetables. This was as a result of their potential effects on human health (Wei and Shiow, 2001). Many researchers especially in the filed of medical science have observed free radical scavenging ability and antioxidant property in *Telfairia*

occidentalis (Oboh and Akindahunsi, 2004; Oboh et al., 2007; Iweala and obidina, 2009, Kayode et al., 2009, kayode et al., 2010). The hypoglyceamic properties of the plant have also been reported (Aderibigbe et al., 1999; Eseyin et al., 2000; Eseyin et al., 2005; Eseyin et al, 2007; Eseyin et al., 2010).

The antioxidant effect is mainly due to compents, such as flavoniods. Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals, acting as oxygen scavengers (Shahidi and Wanasundara, 1992) and preventing lipid auto-oxidation (Brand-Williams et al., 1995; Bondet et al., 1997). Research work had justified the use of the leaf of *Telfairia occidentialis* in Nigeria in the treatment of certain disease in which the participation of reactive oxygen species (ROS) have been implicated. This could be as a result of an antioxidant and free radical scavenging ability (Kayode and Kayode, 2011). Much have been reported on the various medicinal properties of the leaf extract of *Telfairia occidentalis* but little or no report have been published on the active pharmacological constituents isolated from this very important plant. In this research work, attempt were made to isolate some constituents using column and thin layer chromatographic methods.

MATERIALS AND METHODS

Collection of plant materials Plant material

Telfairia occidentalis leaf used for this work was obtained from a local market in Uyo, Akwa Ibom State, Nigeria in March, 2010 and authenticated by Dr. Mrs. Margaret Bassey, a taxonomist in Botany Department, University of Uyo, Uyo, Akwa Ibom State, Nigeria. The leaves were cleaned and dried in the shade, then powered to 40 mesh and stored in an airtight container at 25^{o}c.

Extraction and isolation of the chemical constituents

Air dried powered leaf of *Telfairia occidentalis* (731g) was exhaustively extracted with a total volume of 2.5L of methanol in a soxhlet apparatus using continuous extraction. Evaporation and concentration of the solvent afforded the methanol extract (198.3g). This was further extracted successively with a total volume of 2.5L of the following solvent: n-hexane, ethyl acetate and butanol. Evaporation and concentration of the solvents afforded the n-hexane fraction (HF,93.89g), ethyl acetate fraction (EF, 22.43g) and butanol fraction (BF, 13.23g).

Phytochemical test was carried out on the crude extract and the fractions.

The fractions were fractionated on Silica gel (absorbent) (60-120µg mesh) as follows:

n-hexane fraction: Silica gel (148.3g) was used to fractionate 1.0gm of the HF using n-hexane, chloroform, methanol and water as solvent in that order.

Ethyl acetate fraction: Silica gel (93.6g) was used to separate 2.0g of the EAF using benzene, chloroform, ethyl acetate, methanol and water as solvent in that order.

Butanol fraction: Silica gel (114.2g) was used to separate 2.7g of the BF using benzene, ethyl acetate, methanol and water as solvent in that order.

New solvents were introduced gradually in these ratios 80:20; 50:50; 20:80 to prevent cracking. In each chromatographic analysis, 15ml was collected in each test tube and the solvent allowed to evaporate at room temperature. Thin layer (TLC)

chromatographic analysis was carried out on these eluents and the Rf of each eluent was determined. Eluent with identical Rf values were pooled together to obtain the following pooled fractions:

HF: $2H_1$, $2H_3$, $2H_4$ with Rf values 0.86, 0.71 and 0.55, respectively.

EAF: $2E_1$ and $2E_5$ with Rf values 0.85 and 0.25, respectively.

BF, $2B_1$, $2B_2$ and $2B_3$ with Rf values 0.90, 0.88 and 0.83, respectively. The TLC plates of these fractions were sprayed with the following colour reagents:

Conc. sulphuric acid: A light yellow or pale orange colouration confirmed the presence of steroids.

Ferric chloride: A blue colouration confirmed the presence of tannins.

Dragendorff's solution: A reddish-brown colouration confirmed the presence of alkaloids.

Determination of Total Phenolic Content (TPC) of Extract and Fractions

Total phenolics was quantified and expressed as gallic acid equivalent according to a method proposed by Singleton and Rossi (1999). 1ml of Folin-Ciocalteu's reagent, previously diluted (1:20), was added to 1ml of samples (250µg/ml) and mixed thoroughly. To the mixture, 4ml of sodium carbonate (75g/L) and 10ml of distilled water were added and mixed well. The mixture was allowed to stand for 2hr at room temperature. Contents were then centrifuged at 2000g for 5min and the absorbance of the supernatant was taken at 760nm. A standard curve was obtained using various concentrations of gallic acid. Results were expressed as percentage of Gallic Acid Equivalents (GAE) per 100gram of fresh mass.

Determination of Total Flavonoids Contents (TFC) of extract and Fractions

Total flavoniod contents was measured by aluminum chloride colourimetric assay based on the method modified by Marinova, Ribarova and Atanasova (2005). To 0.1ml of extracts in a 10ml volumetric flask, distilled water was added to make the volume to 5ml and 0.3ml 5% $NaNO_3$ was added to this. 3ml of 10% $AlCl_3$ was then added 5minutes later. After 6 minutes, 2ml of 1M NaOH was added and the absorbance measured at 510nm. Cathecol was used as a standard.

DPPH free radical scavenging assay

Free radical scavenging activities was determined using the DPPH free radical method. Various concentrations of the samples were added to 3ml of daily-prepared methanol DPPH solution (0.1nm). The mixture was shaken and left to stand at room temperature in the dark. After 30min, absorbance was measured at 517nm against a blank (containing all reagents except the test samples). Assays were carried out in triplicates. The concentrations of the samples that gave 50% inhibition of DPPH (IC_{50}) were obtained from the graph of 1% (inhibition percentage) versus concentration of the sample in µg/ml. The percentage inhibition of DPPH (1%) was calculated using the equation.

$1\% = (A_{blank} - A_{sample}/A_{blank}) \times 100$

Where A_{blank} is the absorbance of the blank solution

A_{sample} is the absorbance of the test sample.

RESULTS AND DISCUSSION

Results of Phytochemical Analysis of Crude Extract and n-Hexane, Ethyl acetate, Butanol Fractions and Chromatography analysis for steroids, tannins and alkaloids are shown in tables 1 and 2. While the results of total Phenolic Content (TPC) and Total Flavonoid Content (TFC), and DPPH free radical scavenging activity (IC50) for methanol extract and fractions are shown in tables 3 and 4

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Table 1: Results of Phytochemical analysis of ME, HF, EAF and BF

S/N	Phytochemical test	Crude extract	n-hexane fraction	Ethyl acetate fraction	Butanol fraction
1	Tannin Test with Fe_2Cl_3	Present	Present	Present	Present
2	Saponnin test	Present	Present	Present	Present
3	Alkaloid	Present	Present	Present	Present
4	Test for Steriods Salkowski Test Lieberman-Burchard test	 Present Present	 Present Present	 Present Terpenes Present	 Present Present
5	Flavonoids	Present	Present	Present	Present
6	Glycoside	Present	Present	Present	Present

DISCUSSION

Currently, there is an increasing demand to evaluate the antioxidant properties from plants (Pratt, 1992). In this research work, the methanolic crude extract, fractions and separated constituents were analysed for their antioxidant activity and the phytochemical(s) responsible for this effect. Researchers have observed free radical scavenging ability and antioxidant property in *Telfairia occidentialis (*Oboh and Akindahunsi, 2004; Oboh et al., 2006; Iweala and Obida, 2009; Kayode et al., 2010).

The results of free radical scavenging activity of *Telfairia occidentalis* are shown in table 5. The crude methanolic extract presented a significant free radical scavenging activity, with an IC_{50} of 31.25µg/ml. Comparison of the obtained IC_{50} data (table 5) indicated a potent activity for the HF (IC_{50} = 78.50µg/ml) and a moderate free radical scavenging effect for EAF (IC_{50}=86.30µg/ml) and BF (IC_{50}= 142.40µg/ml). The fractions were purified by column and thin layer chromatography on silica gel to afford the constituents. N-hexane fraction gave three components $2H_1$, $2H_3$ and $2H_4$ with IC_{50} value of 32.5 µg/ml, 70.40 µg/ml and 100.50 µg/ml respectively. Ethyl acetate fraction gave two components $2E_1$ and $2E_5$ with IC_{50} value of 36.50µg/ml and 82.20µg/ml respectively. Butanol fraction gave three components $2B_1$, $2B_2$ and $2B_3$. The DPPH free radical scavenging activity of the constituents from BF were very low.

Table 2: Chromatography analysis for steroids, tannins and alkaloids

Isolated Compounds	Conc. H_2SO_4 (Steroid)	Fe_2Cl_2 (Tannins)	Dragendorff's soln (Alkaloids)
$2H_1$	Present	Present	Present
$2H_3$	Present	Present	Present
$2H_4$	Present	Present	Present
$2E_1$	Present	Present	Present
$2E_5$	Present	Present	Present
$2B_1$	Absent	Present	Absent
$2B_2$	Present	Absent	Present
$2B_3$	Present	Absent	Present
$2B_5$	Present	Present	Present

Table 3: Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) of Extracts and Fractions

Samples	MF	HF	EAF	BF	$2H_1$	$2H_3$	$2H_4$	$2E_1$	$2E_5$	$2B_1$	$2B_2$	$2B_3$
TPC (%)	0.0950	0.0700	0.0720	0.0600	0.0132	0.0124	0.090	0.0720	0.0540	0.0190	0.0740	0.0520
TFC (%)	0.0175	0.0195	0.0135	0.0165	0.0195	0.0110	0.0060	0.0030	0.0015	Very low conc.		

Table 4: DPPH free radical scavenging activity (IC_{50}) for methanol extract and fractions.

Sample	IC50 (μg/ml) (95% confidence limit)
Methanol extract (ME)	31.25(29.50-33.86)
n-Hexane fraction (HF)	78.50(74.59-82.41)
Ethyl acetate fraction (EAF)	86.30(81.93-90.62)
Butanol fraction (BF)	142.40(135.28-149.52)
$2H_1$	32.50(30.87-34.13)
$2H_3$	70.40(66.88-73.92)
$2H_4$	100.50(95.47-105.53)
$2E_1$	82.20(78.09-86.31)
$2E_5$	36.50(34.67-38.33)

The result of total flavonoid content (TFC) is shown in Table 4. The crude methanolic extract had a high TFC (0.0175%). In the fractions: HF had a high TFC (0.019%) followed by BF (0.016%) and EAF (0.013%). In the separated constituents of HF, $2H_1$ had the highest TFC (0.019%) followed by $2H_3$ (0.011%) and $2H_4$ (0.006%). In EAF, $2E_1$ was higher (0.003%) than $2E_5$ (0.0015). Constituents from BF had very low TFC.

The result of total phenolic content (TPC) (table 3) indicated higest TPC in EAF (0.072%)

followed by HF (0.07%) and BF (0.060%). In the separated constituents of HF the TPC was $2H_4$ had the highest (0.090%) then $2H_1$ (0.013%) and $2H_3$ (0.012%). In EAF, $2E_1$ had a higher TPC (0.072%) than $2E_5$ (0.0540%). In BF, $2B_2$ had the highest TPC (0.074%) followed by $2B_3$ (0.052%) and $2B_1$ (0.019%).

Several studies have demonstrated that plants are natural antioxidant sources due mainly to the presence of flavonoids, which act by reducing free radicals (Wilson, 1988; Miller 1996; Pietta, 2000; Geber et al., 2002; Matteo and Esposito, 2003; Behera et al., 2006). The n-hexane fraction (HF) indicated a high DPPH free radical scavenging activity which was contributed by component $2H_1$. The TPC was highest in HF and in the separated constituent $2H_1$. Comparing the BF, though the TPC was high in the separated constituents, it had little or no DPPH free radical activity which inferres that TPC had no effect on the DPPH free radical scavenging activity. It can therefore be inferred from the work that flavonoid contents of the leaves of *Telfairia occidentalis* was responsible for its DPPH free radical scavenging activity.

Researchers have justified the use of the leaf of *Telfairia occidentalis* in Nigeria in the treatment of certain diseases like diabetes, cholesterolaemia, liver problems etc in which the participation of reactive oxygen species have been implicated. This could be as a result of the antioxidant and free radical scavenging ability (Kayode and Kayode, 2011) which might have been attributable to the high flavonoid content of *Telfairia occidentalis* leaf.

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