GC-MS and HPLC profiles of phenolic fractions of the leaf of *Telfairia occidentalis*

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Abstract: *Telfairia occidentalis* possesses high antioxidant activity. However, the antioxidant components of the plant have not yet been identified. This study was undertaken to identify the phenolics in the leaf of the plant. Extract and fractions of the leaf of the plant were analysed using the HPLC and GCMS. HPLC analysis revealed the presence of gallic acid (22.19µg/mg), catechin (29.17µg/mg), caffeic acid (9.17µg/mg), ferulic acid (0.94µg/mg), sinapic acid (1.91 µg/mg) and 4-hydroxy benzoic acid (43.86 µg/mg) in the aqueous extract. Phenolics fraction contained gallic acid (0.88 µg/mg), catechin (2.70µg/mg), caffeic acid (7.92µg/mg), ferulic acid (2.72µg/mg), benzoic acid (6.36µg/mg), p-coumaric acid (1.48µg/mg), quercetin (12.00µg/mg). Only caffeic acid (2.50µg/mg), ferulic acid (0.44µg/mg) and quercetin (8.50µg/mg) were detected in the flavonoid fraction. While GCMS analysis showed the presence of methylparaben; ethylparaben; benzoic acid; 4-hydroxy-2-methoxy-3,5,6-trimethyl-, methyl ester; 4-hydroxy-3-methoxy; phenol, 5-methoxy-2-(methoxymethyl)-; phenol, 5-methoxy-2, 3- dimethyl; and phenol, 2-(2-benzothiazolyl)-. This study is the first to reveal the identity of some phenolics components of the leaf of *Telfairia occidentalis*.

Keywords: Fluted pumpkin, phenolics, antioxidant components, HPLC, GCMS.

INTRODUCTION

Telfaria occidentalis Hook. F (Cucurbitaceae), popularly called fluted pumpkin, is mainly cultivated because of the nutritional value of the leaves which are used mainly as vegetables in West Africa (Sanni, 1982). The leaves of the plant have high nutritive value compared with other vegetables (Okoli and Mgbeogu, 1983). The leaves have high content of vitamins and minerals (Whitaker and Davis, 1962; Jeffrey, 1980; Iwu, 1983). Some of the documented beneficial activities of the plant are: antidiabetic (Aderibigbe *et al.* 1999; Eseyin *et al.*, 2007; Eseyin *et al*, 2010 a, b, c; 2014), hepatoprotective (Oboh, 2005; Nwanna and Oboh, 2007; Bolaji and Olabode, 2011), haematological (Aiyeloja and Bello, 2006; Nwauzoma and Dappa, 2013; Verma and Baksh, 2013), antiplasmodial (Okokon *et al*., 2007; Okokon *et al*., 2009; Chenniappan and Kadarkarai, 2010), testiculoprotective (Saalu *et al*., 2010; Akang *et al*., 2011; Ejike and Ezeanyika, 2013), anticancer (Ejike and Ezeanyika, 2011a,b; Okokon *et al*., 2012), anti-inflammatory (Oluwole *et al*., 2005; Okokon *et al*., 2012), anxiolytic and sedative properties (Ajao and Akindele, 2013). A highlight of the pharmacological activities of the plant was written by Eseyin *et al* (2014). Some of these activities have been attributed to the plant's antioxidant property.

The antioxidant activity of *Telfairia occidentalis* has been widely reported. In terms of total phenol, free radical scavenging ability and reducing power the aqueous extract is better than the ethanolic extract (Oboh *et al*., 2010). The plant has higher free than the bound polyphenols and the free soluble polyphenols had better antioxidant activity than the bound one (Nwanna and Oboh, 2007). The leaf has the ability to reduce iron from +3 to +2 state (Oboh *et al*., 2010). *Telfairia occidentalis* leaf is very rich in vitamin C, flavonoids and phenolics, and it is better in free radicals inhibitory activity than the stem bark of *Psidium guajava* (Malam *et al*., 2012). The antioxidant property of *T. occidentalis* has been attributed to its high level of polyphenols, particularly flavonoids. Unfortunately, the identity of the individual phenolic components in the leaf is not yet known. This work was carried out with the objective of identifying as many phenolic compounds as possible in the leaf of *T. occidentalis* using GCMS and HPLC analyses.

MATERIALS AND METHODS

Collection and drying of plant material

The leaves of *Telfairia occidentalis* were obtained from the Medicinal plant farm of the University of Uyo, Nigeria. Identification of the plant was done in the same faculty and Voucher number UUH28 (d) was assigned to the plant. The fresh plant material was washed with distilled water, air dried and pulverized with a grinding machine.

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Preparation of extracts and fractions

The dried plant material (40 g) was heated in 500ml HCl (2M) for 30 mins in boiling water bath. The extract (300ml) was cooled, filtered and divided into two equal portions. One portion (150ml) was extracted with ether (100mlx5) to obtain the phenolics fraction. The other portion (150ml) was extracted with ethyl acetate (100mlx5) to obtain the flavonoids fraction (Harborne, 1998). To obtain the aqueous extract, 20g of the powdered leaf was macerated in distilled water (200ml) at 50^{o}C for 12 hours. The extract and fractions were filtered with Whatman filter paper and concentrated in vacuum with the aid of a rotary evaporator.

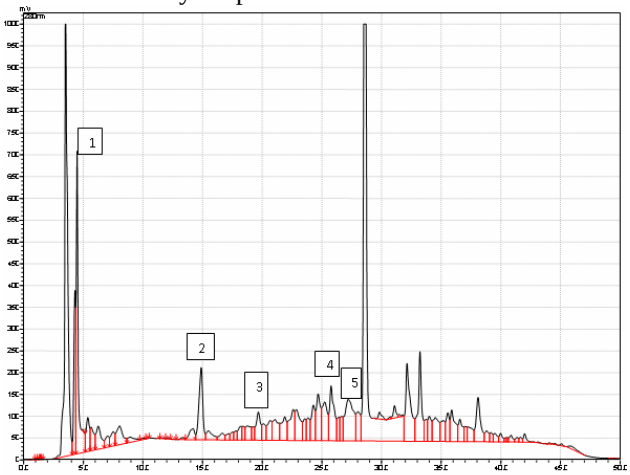

Fig. 1: HPLC chromatogram of aqueous extract of the leaf of *Telfairia occidentalis*. Peak: 1=Gallic acid, 2=Catechin, 3=Caffeic acid, 4=Ferulic acid, 5=Sinapic acid, 6=Benzoic acid.

GC-MS analysis of extracts and fractions

Phenolics and flavonoids fractions were analysed with GC-MS (Agilent 6890 gas chromatograph hyphenated to Agilent 5973 mass spectrometer and Agilent Chemstation software, Agilent Technologies, Palo Alto, CA, USA) with the following acquisition parameters: Oven Program – On 50°C for 3 min then 20°C/min to 280°C for 20 min, Run Time-34.5 min). Front SS Inlet- He; Mode-Splitless; Heater- On 250°C; Pressure- On 15.287 psi; Total Flow-On 54.8mL/min; Septum Purge Flow-On 3mL/min; Gas Saver- On 20mL/min After 3 min; Purge Flow to Split Vent- 50mL/min at 1 min. Back SS Inlet He, Mode-Splitless, Heater-Off, Pressure-Off, Total Flow-Off, Septum Purge Flow- Off, Gas Saver- On 15mL/min After 2 min, Purge Flow to Split Vent-15mL/min at 0.75 min. Thermal Aux 2 (MSD Transfer Line): Heater-On, Temperature Program On 280°C for 0 min, Run Time-34.5min. Column #1: Agilent 19091S-433: 93.92873, HP-5MS 5% Phenyl Methyl Silox, 325°C: 30 m x 250 µm x 0.25 µm. In: Front SS Inlet He. Out: Vacuum. (Initial)-50°C, Pressure -15.287 psi, Flow- .8mL/min, Average Velocity-48.896 cm/sec, Holdup Time-1.0226 min, Flow Program Off 1.8mL/min for 0min, Run Time- 34.5 min. *MS Acquisition Parameters*: Solvent Delay: 3.00 min, EMV Mode: Absolute, Resulting EM Voltage: 1118. Scan Parameters (Low Mass: 40.0, High Mass: 1000.0, Threshold: 100, Sample #: 2, Plot 2 low mass: 50.0, Plot 2 high mass: 550.0). MS Zones (MS Source: 230°C, maximum 350°C; MS Quad: 150°C, maximum 200°C). Emission: 34.610, Energy: 69.922, Repeller: 30.625, Ionfocus: 90.157, Entrance_Le: 28.500, Emvolts: 1188.235, Actual Emv: 1117.65, Gain Factor: 0.12. Identification of unknown compounds was done using the database of National Institute of Science and Technology.

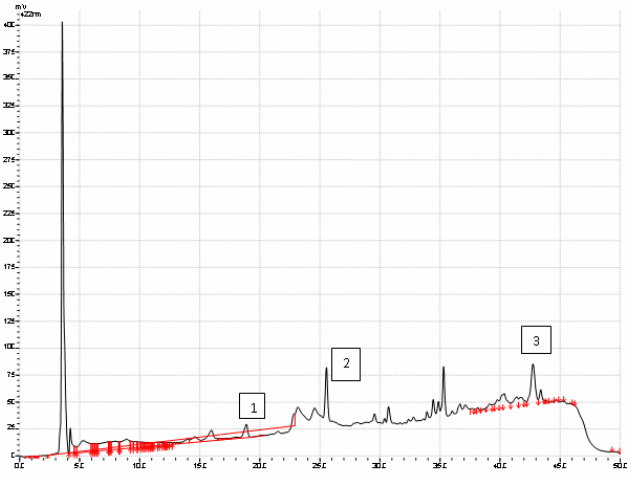

Fig. 2: HPLC chromatogram of flavonoids fraction of the leaf of *Telfairia occidentalis*. Peak 1= Caffeic acid, 2=Ferulic acid, 3=Quercetin

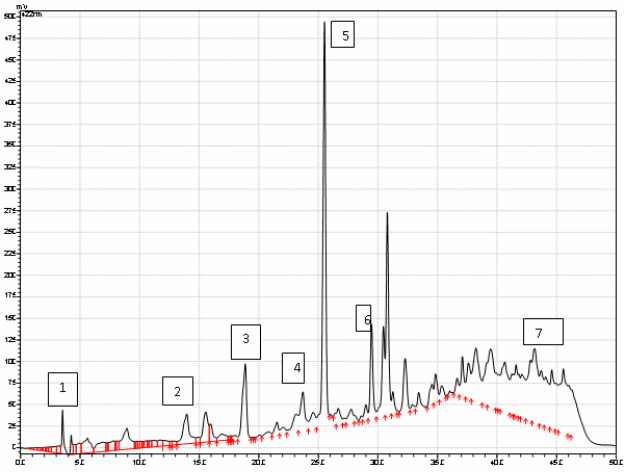

Fig. 3: HPLC chromatogram of phenolics fraction of the leaf of *Telfairia occidentalis*. Peak 1=Gallic acid, 2= Catechin, 3= Caffeic acid, 4=p-coumaric acid, 5=ferulic acid, 6=Benzoic acid, 7=Quercetin

HPLC Analysis

The reference compounds used for HPLC analysis which are quercetin, (-) catechin, gallic acid ferulic acid, *p*-coumaric acid, caffeic acid, vanillic acid, sinapic acid, tannic acid, chlorogenic acid, 4-hydroxybenzoic acid were all purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Table 1: Results of GC-MS analysis of flavonoids fraction

S/N	Rt (mins)	Name	M W	Formula	CAS #	R/A %
1	4.171	1,2-Butanediol	90	$C_4H_{10}O_2$	000584-03-2	2.56
2	5.468	Dicyclohexylmethanol	196	$C_{13}H_{24}O$	004453-82-1	3.20
3	5.978	Pentanoic acid, 4-oxo-, methyl ester	130	$C_6H_{10}O_3$	000624-45-3	3.40
4.	6.414	Butanedioic acid, dimethyl ester	146	$C_6H_{10}O_4$	000106-65-0	2.65
5	6.690	Pentanoic acid, 4-oxo-, ethyl ester	144	$C_7H_{12}O_3$	000539-88-8	2.63
6.	7.083	Butanedioic acid, ethyl methyl ester	160	$C_7H_{12}O_4$	000627-73-6	3.08
7	7.306	3-Acetoxy-3-hydroxypropionic acid, methyl ester	162	$C_6H_{10}O_5$	100019-01-0	5.50
9	9.730	Methylparaben	152	$C_8H_8O_3$	000099-76-3	3.0
10	10.144	Citric acid, trimethyl ester	234	$C_9H_{14}O_7$	001587-20-8	9.0
11	10.166	Ethylparaben	166	$C_9H_{10}O_3$	000120-47-8	0.80
12	10.569	Benzoic acid, 4-hydroxy-2-methoxy-3,5,6-trimethyl-, methyl ester	224	$C_{12}H_{16}O_4$	034874-83-4	0.70
13	10.548	Ethyl citrate	276	$C_{12}H_{20}O_7$	000077-93-0	10.30
14	11.802	Methyl 13-methyltetradecanoate	256	$C_{16}H_{32}O_2$	100033-63-4	1.40
15	12.334	Hexadecanoic acid, methyl ester	270	$C_{17}H_{34}O_2$	000112-39-0	12.60
16	12.536	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	000057-10-3	5.17
17	13.205	9-Octadecenoic acid (Z)-, methyl ester	296	$C_{19}H_{36}O_2$	000112-62-9	3.87
18	13.407	9,12-Octadecadienoic acid (Z,Z)-	280	$C_{18}H_{32}O_2$	000060-33-3	1.43
19.	15.034	Methyl 20-methyl-heneicosanoate	354	$C_{23}H_{46}O_2$	100033-64-4	1.08
20	15.522	Tricosanoic acid, methyl ester	368	$C_{24}H_{48}O_2$	002433-97-8	2.91
21	16.107	Tetracosanoic acid, methyl ester	382	$C_{25}H_{50}O_2$	002442-49-1	1.88
22	16.777	Pentacosanoic acid, methyl ester	396	$C_{26}H_{52}O_2$	055373-89-2	1.88
23	17.616	Hexacosanoic acid, methyl ester	410	$C_{27}H_{54}O_2$	005802-82-4	0.72
24	18.658	Cholest-5-en-24-one, 3-(acetyloxy)-, (3.beta.)-	442	$C_{29}H_{46}O_3$	020981-59-3	0.60
25	19.710	Stigmast-5-en-3-ol, oleate	679	$C_{47}H_{82}O_2$	100015-46-5	1.94

Table 2: Results of GC-MS analysis of Phenolics fraction

S/N	RT (mins)	Name	MW	Formula	CAS #	R/A %
1	4.224	Ethanedioic acid, dimethyl ester	118	$C_4H_6O_4$	000553-90-2	2.53
2	5.266	Propanedioic acid, dimethyl ester	132	$C_5H_8O_4$	000108-59-8	0.67
3	5.957	Pentanoic acid, 4-oxo-, methyl ester	130	$C_6H_{10}O_3$	000624-45-3	1.20
4	6.265	2-Butenedioic acid (E)-, dimethyl ester	144	$C_6H_8O_4$	000624-49-7	0.89
5	7.275	3-Acetoxy-3-hydroxypropionic acid, methyl ester	162	$C_6H_{10}O_5$	100019-01-0	1.64
6	7.264	2,4-Hexadienedioic acid, dimethyl ester, (Z,Z)-	170	$C_8H_{10}O_4$	000692-91-1	0.65
7	7.508	Octanedioic acid, dimethyl ester	202	$C_{10}H_{18}O_4$	001732-09-8	0.85
8	9.592	Methylparaben	152	$C_8H_8O_3$	000099-76-3	1.42
9	9.698	Citric acid, trimethyl ester	234	$C_9H_{14}O_7$	001587-20-8	1.28
10	9.751	Benzoic acid, 4-hydroxy-3-methoxy-, methyl ester	182	$C_9H_{10}O_4$	003943-74-6	0.75
11	10.123	Phenol, 5-methoxy-2-(methoxymethyl)-	168	$C_9H_{12}O_3$	062849-09-6	0.68
12	10.251	Phenol, 5-methoxy-2,3-dimethyl-	152	$C_9H_{12}O_2$	034883-01-7	0.49
14	10.463	Decanedioic acid, dimethyl ester	230	$C_{12}H_{22}O_4$	000106-79-6	0.53
15	10.835	Methyl tetradecanoate	242	$C_{15}H_{30}O_2$	000124-10-7	0.63
16	11.260	Undecanedioic acid, dimethyl ester	244	$C_{13}H_{24}O_4$	004567-98-0	0.55
17	11.388	Tetradecanoic acid	228	$C_{14}H_{28}O_2$	000544-63-8	0.79
18	11.462	Methyl 13-methyltetradecanoate	256	$C_{16}H_{32}O_2$	100033-63-4	1.17
19	11.802	Bicyclo[3.1.1]heptane, 2,6,6-trimethyl-	138	$C_{10}H_{18}$	000473-55-2	2.19
20	11.887	1-Hexadecyne	222	$C_{16}H_{30}$	000629-74-3	1.70
21	12.004	9-Octadecyne	250	$C_{18}H_{34}$	035365-59-4	2.62
22	12.100	1,3-Dimethyl-5-ethyladamantane	192	$C_{14}H_{24}$	001687-35-0	2.58
23	12.217	Hexadecanoic acid, methyl ester	270	$C_{17}H_{34}O_2$	000112-39-0	9.91
24	12.323	n-Hexadecanoic acid	256	$C_{16}H_{32}O_2$	000057-10-3	7.23
25	12.525	Oxalic acid, isohexyl nonyl ester	300	$C_{17}H_{32}O_4$	100030-93-2	0.95
26	12.876	9,17-Octadecadienal, (Z)-	264	$C_{18}H_{32}O$	056554-35-9	5.50
27	13.184	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-	278	$C_{18}H_{30}O_2$	000463-40-1	5.95
28	13.397	Methyl 8,11,14-heptadecatrienoate	278	$C_{18}H_{30}O_2$	100033-63-1	1.81
29	13.567	Eicosanoic acid, methyl ester	326	$C_{21}H_{42}O_2$	001120-28-1	0.88
30	14.183	Phenol, 2-(2-benzothiazolyl)-	227	$C_{13}H_9NOS$	003411-95-8	0.70
31	14.385	Heneicosanoic acid, methyl ester	340	$C_{22}H_{44}O_2$	006064-90-0	0.81
32	14.810	Methyl 20-methyl-heneicosanoate	354	$C_{23}H_{46}O_2$	100033-64-4	0.72
33	15.044	Tricosanoic acid, methyl ester	368	$C_{24}H_{48}O_2$	002433-97-8	1.91
34	15.544	Tetracosanoic acid, methyl ester	382	$C_{25}H_{50}O_2$	002442-49-1	1.07
35	16.118	Myristic acid, 2-(1-octadecenyloxy)ethyl ester, (Z)-	523	$C_{34}H_{66}O_3$	030760-01-1	0.54
36	16.915	Stigmastan-3,5-diene	396	$C_{29}H_{48}$	100021-41-4	0.59
37	18.243	7,9(11),22-Ergostatriene	380	$C_{28}H_{44}$	100025-30-9	0.46
38	18.499	Cholest-5-en-24-one, 3-(acetyloxy)-, (3.beta.)-	442	$C_{29}H_{46}O_3$	020981-59-3	2.20

Rt= Retention time, R/A= Relative abundance, MW= Molecular weight

HPLC profiles of the aqueous extract, phenolics fraction and flavonoids fraction were obtained using Shimadzu (Japan) HPLC which was equipped with Prominence Degasser (DGU-20A_5), Prominence pump (LC-20AD), Prominence CBM(CBM-20A), Prominence autosampler (SIL-20A_{HT}), column oven (CTO-10ASvp), fluorescence detector (RF-10AXL) and Hypersil Gold column (4.6x250 cm, 5 µm, Thermo scientific, USA). The plant materials (20mg/ml) and reference compounds (25, 50 and 100µg/ml) were filtered with syringe filter (0.45µm pore size). Injection volume was 20µl and oven temperature was 30°C. Flow rate was 1ml/minute and run time was 50 minutes. Solvent gradients were as described by Hao *et al* (2001). The proportion of solvent A [water–acetic acid (97:3, (v/v)] to solvent B (methanol) was varied and solvent program is as follows: Time (min) and Solvent B (%), 0 and 0, 10 and 10, 40 and 70, 44 and 0, 47 and 0, respectively. Detection was done at excitation wavelength of 260nm and emission wavelength of 422 nm. Each of the reference compounds and plant material was run in triplicate to obtain the retention time. References and plant materials were run under the same conditions. Chromatogram peaks of the plant materials were identified based on the correlation of their retention time with those of the reference compounds.

RESULTS

The results of GC-MS analyses of Flavonoids and Phenolics fractions are shown in tables 1 and 2, respectively.

The HPLC chromatograms of aqueous extract, flavonoids fraction and phenolics fraction are shown in fig. 1, 2 and 3, respectively. table 3 gives the concentration of phenolic compounds in the aqueous extract, phenolics and flavonoids fractions.

HPLC analysis revealed the presence of Gallic acid (22.19µg/mg), catechin (29.17µg/mg), caffeic acid (9.17 µg/mg), ferulic acid (0.94µg/mg), sinapic acid (1.91 µg/mg) and 4-hydroxy Benzoic acid (43.86µg/mg) in the aqueous extract. Phenolics fraction contained Gallic acid (0.88 µg/mg),catechin (2.70µg/mg), caffeic acid (7.92 µg/mg), ferulic acid (2.72µg/mg), benzoic acid (6.36 µg/mg), p-coumaric acid (1.48µg/mg), quercetin (12.00 µg/mg). Only caffeic acid (2.50µg/mg), ferulic acid (0.44 µg/mg) and quercetin (8.50µg/mg) were detected in the flavonoid fraction (fig. 2).

Phenolics compounds contained in the GCMS profile of the flavonoids fraction and their relative abundance (%) are methylparaben (3%), ethylparaben (0.8%) and benzoic acid, 4-hydroxy-2-methoxy-3,5,6-trimethyl-, methyl ester (0.7%) (table 1). The presence of methylparaben (1.42%); benzoic acid, 4-hydroxy-3-methoxy-(0.75%); phenol, 5-methoxy-2-(methoxymethyl)- (0.68%); phenol, 5-methoxy-2, 3- dimethyl (0.49%); and phenol, 2-(2-benzothiazolyl)-(0.70%) were detected in the phenolics fraction (table 3).

DISCUSSION

Natural products obtained from plants have strong antioxidant property, which is preferred to the often toxic commercially available synthetic antioxidants, mainly because of the presence of antioxidant constituents such as phenolics, vitamin C, carotenoids and lycopene.

Information available on Dr Duke's Phytochemical and Ethnobotanical Databases (2014) confirm the antioxidant activity of caffeic acid, ferulic-acid, quercetin, catechin, sinapic acid, myricetin and p-coumaric acid. The Database also confirms that these phenolic compounds are constituents of many plants. However, tannins, chlorogenic acid and vanillic acid were not detected in any of the extract and fractions. The aqueous extract contained the highest number of detected phenolic compounds followed by the phenolic fraction. The use of the water extract of the leaf is therefore encouraged.

Although eicosanoids are not phenolics compounds, they are a large class of bioactive metabolites of arachidonic acid which play vital functions in many physiological processes. The presence of eicosanoic acid, methyl ester; tricosanoic acid; tetracosanoic acid, methyl ester; Methyl 20-methyl-heneicosanoate, methyl ester; hexacosanoic acid, methyl ester; pentacosanoic acid, methyl ester; and heneicosanoic acid, methyl ester especially in the flavonoids fraction as revealed by GCMS is therefore worthy of note.

HPLC analysis methods for most kinds of the over eight thousand naturally occurring plant phenolic compounds have been developed (Cai *et al*., 2004). The number of phenolics identified from the HPLC analysis of *T. occidentalis* in this work was limited by the number of reference compounds (standards) used. This limitation also accounts for the unknown peaks in the HPLC chromatograms (fig. 1, 2 and 3). Similarly, GCMS is known to be effective in the identification of mainly volatile compounds. Consequently, the phenolics identified with GCMS in this work are only those that are volatile. It is therefore obvious that the list of phenolic compounds present in *Telfairia occidentalis* as obtained in this study is far from being exhaustive. It is expected that silylation of the extract before GCMS analysis would yield better results, as the compounds would be more volatile in the silylated form (Charalampos and Michael).

The presence of phenolics in the leaf of *Telfairia occidentalis* therefore provides the justification for the medicinal usefulness of the plant. The plant has the potential to protect the consumer from free radicals or oxidative stress-induced ailments.

Table 3: Concentration of phenolic compounds in the aqueous extract, Phenolics and flavonoids fractions of *Telfairia occidentalis* (µg/mg of plant material)

	Aqueous extract	Phenolics fraction	Flavonoids fraction
Gallic acid	22.19±2.03	0.88±0.07	ND
Catechin	29.17±2.23	2.70±0.81	ND
Caffeic acid	9.17±1.01	7.92±1.11	2.50±0.76
Ferulic acid	0.94±0.08	2.72±0.57	0.44±0.07
Sinapic acid	1.91±0.24	ND	ND
Benzoic acid	43.86±3.38	6.36±0.99	ND
p-Coumaric acid	ND	1.48±0.08	ND
Quercetin	ND	12.00±1.20	8.50±0.79
Tannins	ND	ND	ND
Chlorogenic acid	ND	ND	ND
Vanillic acid	ND	ND	ND

n=3, ND = Not detected

CONCLUSION

HPLC and GCMS analyses have revealed the presence of phenolic compounds in the leaf of Telfairia occidentalis. The results of this study give scientific basis for the antioxidant activity of the plant. This study is the first to give insight into the identity of some phenolic components of the leaf of *Telfairia occidentalis*.

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