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Article

Immunomodulatory, Anticancer and Anti-inflammatory Activities of *Telfairia occidentalis* Seed Extract and Fractions

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Abstract: The seed extract of *Telfairia occidentalis* used for nutritional purposes was investigated for anticancer activity against HeLa cells using SRB method and DNA interaction activity using gel electrophoresis. Antioxidative burst activity of the extract in whole blood, neutrophils and macrophages was also investigated using luminol/lucigenin-based chemiluminescence assay. While anti-inflammatory activity against egg albumin–induced paw oedema and xylene-induced ear oedema was also studied. The GC-MS analysis of hexane and dichloromethane fractions was carried out. The seed extract demonstrated significant anticancer activity with the hexane fraction exhibiting the most pronounced effect. The crude extract and the fractions did not interact with DNA when investigated using electrophoresis. The extract prominently inhibited oxidative burst activity in whole blood, isolated polymorphonuclear cells (PMNs) and mononuclear cells (MNCs) when two different phagocytosis activators (serum opsonizing zymosan-A and PMA) were used as well as significant anti-inflammatory activity against egg albumin and xylene-induced oedema. GC-MS analysis revealed some pharmacologically active components which are responsible for its activities. Thus, the seed extract possesses antioxidative burst, anticancer, and anti-inflammatory activities.

Keywords: *Telfairia occidentalis;* seed; antioxidative burst; anticancer; anti-inflammatory.

1. Introduction

T. occidentalis (Hook. F) Vahl. popularly known as fluted pumpkin is a member of Cucurbitaceae family. The plant is cultivated in Southern Nigeria mainly for the leaves and seeds which are eaten because of their high content of protein, vitamins and minerals (Johnson and Johnson, 1996). *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. Reports of hypoglycemic and antidiabetic activities (Aderibigbe *et al.,* 1999; Alada, 2000; Eseyin *et al.,* 2000 & 2005; Nwozo *et al.,* 2004), antioxidant and antimicrobial activities (Oboh *et al.,* 2006) of the leaf have been published. Several workers have reported on the nutritional composition, chemical characterization and functional properties of fluted pumpkin seed (Agatemor, 2006; Asiegbu, 1987; Badifu *et al*., 1995; Ezugwu and Nwodo, 2000; Fagbemi *et al*., 2005). The seed was reported by Okokon *et al.,* (2009) to possess antiplasmodial property. Most researches have focused on the leaf, and information on the medicinal properties of the seed is scanty. We, therefore, report in this study the anticancer, anti-inflammatory, and immunomodulatory activities as well as GC-MS analysis of hexane and dichloromethane fractions of the seed extract of *Telfairia occidentalis* from Nigeria.

2. Materials and Methods

2.1. Plant Collection

The plant material *Telfairia occidentalis* (seeds) were bought from local markets in Uruan area, Akwa Ibom State, Nigeria in April, 2011. The plant was identified and authenticated by Dr. Magaret Bassey of Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. A voucher specimen was deposited in the Faculty of Pharmacy Herbarium, University of Uyo, Uyo with voucher no. FPHUU 110.

2.2. Extraction

The seeds were washed and shade-dried for two weeks. The dried plants' materials were further chopped into small pieces and reduced to powder. The powdered material was macerated in 70% ethanol. The liquid filtrates were concentrated and evaporated to dryness in vacuo 40 °C using rotary evaporator. The crude ethanolic extract (100 g) was further partitioned successively into 1 L each of n-hexane, dichloromethane, ethyl acetate and butanol to give the corresponding fractions of these solvents.

2.3. Cellular Antioxidant Activity

The ethanolic crude extract was screened for cellular antioxidant activity in whole blood, neutrophils and macrophages using chemiluminescence assay. Briefly, luminol or lucigenin-enhanced chemiluminescence assay were performed as described by Helfand *et al.*, (1982) and Haklar *et al.*, (2001). Briefly, 25 µL diluted whole blood (1:50 dilution in sterile $HBSS^{++}$) or 25 µL of PMNCs (1×10^6) or MNCs (5×10^6) cells were incubated with 25 µL of serially diluted plant extract with concentration ranges between 6.25 and 100 µg/mL. Control wells received $HBSS^{++}$ and cells but no extract. Tests were performed in white 96 wells plates, which were incubated at 37 ^oC for 30 min in the thermostated chamber of the luminometer. Opsonized zymosan-A or PMA 25 µL, followed by 25 µL luminol (7×10^5 M) or lucigenin (0.5 mM) along with $HBSS^{++}$ was added to each well to obtain a 200 µL volume/well. The luminometer results were monitored as chemiluminescence RLU with peak and total integral values set with repeated scans at 30 s intervals and 1 s points measuring time.

2.4. Anticancer Activity

The growth inhibitory and cytotoxic activities of the ethanolic extracts and fractions were evaluated against HeLa cells (Cervix cancer cell) by using the sulforhodamine-B assay (Houghton *et al.*, 2007). The cells (10000 cells/100 µL) in 96-well plate were incubated for 24 h at 37 °C in a humidified 5% CO_2 incubator. The stock solutions of ethanolic extract, fractions were prepared in DMSO. Various dilutions of the ethanolic extracts and fractions (0.1, 1, 10, 100, and 250 µg/mL), were added (100 µL) in each well. After 48 h of incubation, 50 µL of cold TCA (50 %) was added gently and left for 30 min at room temperature, followed by washing with distilled water and drying overnight. To each well, 100 µL of SRB solution (0.4% wt/vol in 1% acetic acid) was added and after 10 min, the unbound stain was removed by washing with acetic acid (1%), and air-dried at room temperature. The protein bound stain was solubilized with tris base (pH 10.2), and was shaken for 5 min. Absorbance was measured at 515 nm using a microplate reader. The absorbance of the appropriate blanks, including test substance blank, and control (without drug), was used to calculate the growth inhibition, and cytotoxicity of the test compounds, which were represented as GI_{50}, TGI and LC_{50} (µg/mL) values.

2.5 Egg-albumin Induced Inflammation

Inflammation was induced in mice by the injection of egg albumin (0.1 mL, 1% in normal saline) into the sub planar tissue of the right hind paw (Akah and Nwambie, 1994; Okokon and Nwafor, 2010). The linear circumference of the injected paw was measured before and 0.5, 1, 2, 3, 4 and 5 hrs after the administration of the phlogistic agent. The seed extract (450, 900 and 1350 mg/kg

i.p) and ASA (100 mg/kg orally) were administered to 24 hrs fasted mice 1 hr before the induction of inflammation. Control group received 10 mL/kg of distilled water orally. Edema (inflammation) was assessed as the difference in paw circumference between the control and 0.5, 1, 2, 3, 4 and 5 hrs after the administration of the phlogistic agent (Hess and Milonig, 1972). The average (mean) edema was assessed by measuring with vernier calipers.

2.6. Xylene-Induced Ear Oedema

Inflammation was induced in mice by tropical administration of 2 drops of xylene at the inner surface of the right ear. The xylene was left to act for 15 min. *Telfairia occidentalis* seed extract (37, 74 and 111 mg/kg i.p), dexamethasone (4 mg/kg) and distilled water (0.2 mL/kg) were orally administered to various groups of mice 30 min before the induction of inflammation. The animals were sacrificed under light anaesthesia and the left ears cut off. The difference between the ear weights was taken as the oedema induced by the xylene (Mbagwu *et al*., 2007; Okokon and Nwafor, 2010; Tjolsen *et al.*, 1992).

2.7. GC-MS Analysis of Hexane and Dichloromethane Fraction

Quantitative and qualitative data were determined by GC and GC-MS, respectively. Each fraction was injected onto a Shimadzu GC-17A system, equipped with an AOC-20i autosampler and a split/splitless injector. The column used was an DB-5 (Optima-5), 30 m, 0.25 mm i.d., 0.25 µm df, coated with 5% diphenyl-95% polydimethylsiloxane, operated with the following oven temperature programme: 50 °C, held for 1 min, rising at 3 °C/min to 250 °C, held for 5 min, rising at 2 °C/min to 280 °C, held for 3 min; injection temperature and volume, 250 °C and 1.0 µL, respectively; injection mode, split; split ratio, 30:1; carrier gas, nitrogen at 30 cm/s linear velocity and inlet pressure 99.8 KPa; detector temperature, 280 °C; hydrogen, flow rate, 50 mL/min; air flow rate, 400 mL/min; make-up (H_2/air), flow rate, 50 mL/min; sampling rate, 40 ms. Data were acquired by means of GC solution software (Shimadzu).

Agilent 6890N GC was interfaced with a VG Analytical 70-250 s double-focusing mass spectrometer. Helium was used as the carrier gas. The MS operating conditions were: ionization voltage 70 eV, ion source 250 °C. The GC was fitted with a 30 m × 0.32 mm fused capillary silica column coated with DB-5. The GC operating parameters were identical with those of GC analysis described above.

The identification of components present in the various active fractions of the plants' extracts was based on direct comparison of the retention times and mass spectral data with those for standard compounds, and by computer matching with the Wiley 229 and Nist 21 Library, as well as by

comparison of the fragmentation patterns of the mass spectra with those reported in the literatures (Adams, 2001; Setzer *et al.,* 2007).

2.8. Statistical Analysis and Data Evaluation

Data obtained from this work were analyzed statistically using Students' *t*-test and ANOVA (One-way) followed by a post test (Tukey-Kramer multiple comparison test). Differences between means will be considered significant at 1% and 5% level of significance i.e. $p \leq 0.01$ and 0.05.

3. Results

3.1. Cellular Antioxidant Activity

Ethanolic seed extract of *Telfairia occidentalis* was observed to exhibit pro-oxidant effect at lower doses and weak antioxidant effect at higher dose especially in the whole blood, while different degrees of inhibitory effect on the oxidative burst activities in neutrophils and macrophages was also recorded and were in dose-dependent manner. The extract produced -30.4 – 0.2 % inhibition in whole blood, 0.00 – 89.0 % in neutrophils when activated with zymosan-A, 33.40 – 85.90 % in neutrophils when activated with PMA and 9.90 – 93 % in macrophages (Table 1).

Table 1. Immunomodulatory activity of ethanolic seed extract of *Telfairia occidentalis*

Cell Type	Dose (µg/mL)	% Inhibition (RLU)
Whole blood	1	-30.4±3.81
	10	-1.90±1.84
	100	0.2±6.70
Neutrophils (intracellular)	0.5	0.00±0.00
	5	6.80±5.08
	50	89.90±1.44
Neutrophils (extracellular)	0.5	33.40±7.80
	5	40.30±2.25
	50	85.90±2.54
Macrophages	0.5	9.90±1.56
	5	11.70±0.20
	50	93.0±2.08

Note: Data are represented as mean ± SEM of three independent experiments.

3.2. Anticancer Activity against HeLa Cells

The results of anticancer activity of crude seed extract and fractions of *Telfairia occidentalis* shows significant activity with the hexane fraction exerting the highest activity than other fractions and crude extract (Table 2). The potency order was hexane > dichloromethane > ethyl acetate > butanol > acqueous > crude extract.

Table 2. Anticancer activity of crude extract and fractions of seed of *Telfairia occidentalis* against HeLa cells

Extract/Fraction	GI_{50} (µg/mL)	LC_{50} (µg/mL)	TGI (µg/mL)
Crude extract	171.3±1.24	244.0±1.29	216.4±3.23
Hexane	14.6±1.22	72.6±1.16	21.6±1.05
Dichloromethane	25.0±0.57	81.3±1.52	46.3±1.04
Ethyl acetate	43.3±0.73	88.6±1.38	-
Butanol	55.3±0.57	78.3±1.15	-
Aqueous	51.3±0.83	92.0±1.18	-
Doxorubucin (µM)	0.61±0.03 µM	7.80±0.80 µM	3.60±0.30 µM

Note: Data are represented as mean ± SEM of three independent experiments; Values in the table are concentrations of extract/fraction expressed as µg/mL; GI_{50} = Concentration of the drug causing 50% growth inhibition of the cells; TGI = Concentration of the drug causing total growth inhibition of the cells; LC_{50} = Lethal concentration of the drug that killed 50% of the cells.

3.3. DNA Interaction Study

Gel electrophoresis results shows that treatment of *E. coli* DNA with various concentrations of the hexane fraction of *Telfairia occidentalis* seed did not produce any effect on the DNA. Similar effect was also observed with the standard drug used, paclitaxel (Fig. 1).

3.4. Egg Albumin-induced Oedema

Administration of seed extract of *Telfairia occidentalis* on egg albumin-induced oedema in mice caused a significant ($p < 0.05 – 0.001$) dose-dependent anti-inflammatory effect against oedema caused by egg albumin. The effect was comparable to that of standard drug, ASA (100 mg/kg) (Table 3).

3.5. Xylene-induced Ear Oedema

Anti-inflammatory effect of seed extract of *Telfairia occidentalis* against xylene-induced ear oedema in mice is shown in Table 4. The extract exerted a dose-dependent anti-inflammatory effect

which was significant ($p < 0.0\ 01$) when compared to control. The effect was incomparable to that of the standard drug, dexamethasone (4.0 mg/kg).

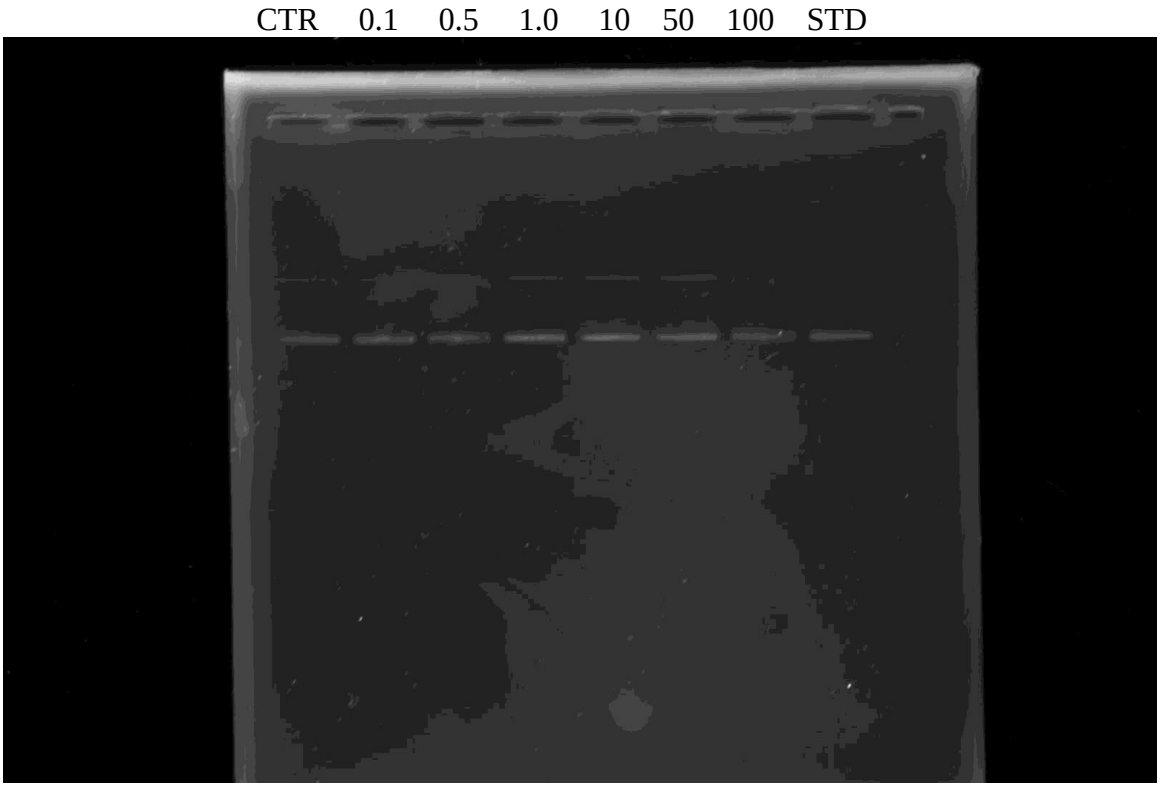

Figure 1: The effect of various concentrations of *n*-hexane fraction of *Telfairia occidentalis on* DNA interaction using gel electrophoresis.

Table 3. Effect of *Telfairia occidentalis* seed extract on egg-albumin induced oedema in mice

Treatment/	**Time Intervals (hr)**						
Dose (mg/kg)	**0**	**0.5**	**1**	**2**	**3**	**4**	**5**
Control	0.25±0.01	0.33±0.01	0.35±0.01	0.34±0.01	0.33±0.01	0.32±0.01	0.31±0.01
Extract 450	0.26±0.01	0.33±0.01	0.33±0.01	0.32±0.01	0.31±0.01 [a]	0.29±0.01 [a]	0.28±0.01 [a]
Extract 900	0.26±0.01	0.34±0.01	0.32±0.01	0.31±0.01 [a]	0.28±0.01 [a]	0.28±0.01 [b]	0.27±0.01 [b]
Extract 1350	0.24±0.01	0.30±0.01	0.31±0.01	0.29±0.01 [a]	0.27±0.01 [b]	0.26±0.01 [b]	0.25±0.01 [b]
ASA 100	0.25±0.01	0.30±0.01[a]	0.28±0.01 [a]	0.27±0.01[b]	0.26±0.01[b]	0.26±0.01[b]	0.25±0.01[b]

Note: Data are expressed as mean ± SEM; Significant at [a] $p < 0.01$, [b] $p < 0.001$ when compared to control; n = 6.

3.6. GC-MS Analysis

The GCMS analysis of the hexane and dichloromethane fractions of *Telfairia occidentalis* seed revealed the presence of 11 bioactive compounds each as represented in Tables 5 and 6.

Table 4. Effect of *Telfairia occidentalis* seed extract on xylene-induced ear oedema in mice

Treatment/Dose (mg/kg)	Weight of Right Ear (g)	Weight of Left Ear (g)	Increase in Ear Weight (g)	% Inhibition
Control (normal saline) 0.2 mL	0.09±0.00	0.043±0.00	0.05±0.00	
Extract 450	0.082±0.01	0.036±0.01	0.043±0.01	14
Extract 900	0.07±0.01	0.036±0.01	0.036±0.01[a]	28
Extract 1350	0.076±0.01	0.043±0.01	0.033±0.00[a]	34
Dexamethasone 4.0	0.026±0.01	0.049±0.01	0.023±0.00[a]	54

Note: *significant at [a] $p < 0.001$ when compared with control; n = 6.

Table 5. GC-MS analysis of dichloromethane fraction of *Telfairia occidentalis* seed

S/No.	Name of Compound	Mol. Wt.	Chemical Formula	RI
1	Pentadecanoic acid, 14-methyl-,methyl ester	270	$C_{17}H_{34}O_2$	619
2	Hexadecanoic acid	256	$C_{16}H_{32}O_2$	648
3	8,11-Octadecadienoic acid, methyl ester	294	$C_{19}H_{34}O_2$	703
4	16-Octadecenoic acid, methyl ester	296	$C_{19}H_{36}O_2$	706
5	Heptadecanoic acid,6-methyl, methyl ester	298	$C_{19}H_{38}O_2$	719
6	9, 12-Octadecadienoyl chloride (Z,Z)-	298	$C_{18}H_{31}ClO$	735
7	9-Octadecadienoic acid (Z)-,2,3-dihydroxypropyl ester	356	$C_{21}H_{40}O_4$	741
8	Octadecanoic acid	284	$C_{18}H_{36}O_2$	749
9	Hexadecanoic acid, 2,3-bis[(trimethylsilyl)oxy] propyl ester	474	$C_{25}H_{54}O_4Si_2$	876
10	Cyclohexanespiro-5'-(4'-methyl-2'-phenyl-2'-oxazoline	229	$C_{15}H_{19}NO$	947
11	9-Octadecenoic acid (Z)-,2-hydroxy-1-(hydroxymethyl) ethyl ester	356	$C_{21}H_{40}O_4$	966

4. Discussion

The seed of fluted pumkin, *T. occidentalis* is used in Nigeria basically for nutritional purposes, especially in the Southern part where it is consumed raw, cooked or used in making soup. Very little is known about its medicinal values. In this study, the seed extract was found to exhibit antioxidative burst, anticancer, and anti-inflammatory activities. The anticancer mechanism of action was found to be unrelated to DNA interaction and is likely to involve interference with cell division processes. However, the GC-MS analysis revealed the presence of some pharmacologically active compounds such as hexadecanoic acid methyl ester, 9,12,15-octadecatrienoic acid ethyl ester (Z,Z,Z)-,8,11,14-eicosatrienoic acid (Z,Z,Z) and monoterpenes such as terpinen-4-ol, trans-β-ocimene, phellandrene, and borneol (found in the hexane fraction) which have been implicated in the anticancer activity of

plants ((Sarumathy *et al*., 2011; Tan *et al.*, 2001; Wu *et al*., 2012). Kumar *et al.*, (2010) also reported the activities of some phyto-components with compound nature of flavonoids; palmitic acid (hexadecanoic acid ester and n-hexadecanoic acid), unsaturated fatty acid and linolenic (docosatetraenoic acid and octadecatrienoic acid) as antimicrobial, anti-inflammatory, antioxidant, hypocholesterolemic, cancer preventive, hepatoprotective, antiarthritic, antihistimic, antieczemic and anticoronary. These compounds could have contributed to the observed anticancer and antioxidant activity of the seed extract. Besides, borneol and terpen-4-ol present in the extract have also been reported to have antioxidant activity (Chen *et al*., 2011; Wu *et al*., 2012). Similarly, phytosterols have been reported to have preventive effects on the development of diseases due to reactive oxygen species (Vivacons and Moreno, 2005). Moreover, Yoshida and Niki (2003) showed the antioxidant effects of the phytosterols against lipid peroxidation. Stigmastan-3-ol, 5-chloro-, acetate, (3a', 5a')-, a phytosterol, have been found to be present in the hexane fraction of the seed extract. This compound could have also contributed to the observed anticancer activity. Compounds with antioxidant activity have been known to prevent cancer diseases. This radical scavenging activity of the phytochemical components of this extract could have accounted for the anticancer activity observed in this study and may be the mechanism of anticancer activity of the seed extract.

Table 6. GC-MS analysis of *n*-hexane fraction of *Telfairia occidentalis* seed

S/No.	Name of Compound	Mol. Wt.	Chemical Formula	RI
1	2,4-Heptadien-6-ynal,(E,E)-	106	C_7H_6O	190
2	Benzoic acid	122	$C_7H_{14}O$	195
3	Dodecanoic acid	200	$C_{12}H_{24}O_2$	201
4	Linoleic acid ethyl ester	308	$C_{20}H_{36}O_2$	729
5	Hexadecanoic acid, methyl ester	284	$C_{18}H_{36}O_2$	756
6	α-phellandrene	136	$C_{10}H_{16}$	1005
7	α-campholene aldehyde	152	$C_{19}H_{16}O$	1123
8	Terpinen-4-ol	154	$C_{10}H_{18}O$	1137
9	Trans-β-ocimene	136	$C_{10}H_{16}$	1150
10	Borneol	154	$C_{10}H_{18}O$	1164
11	Stigmastan-3-ol, 5-chloro-,acetate,(3a',5a')-	492	$C_{13}H_{53}ClO_2$	1175

The seed extract was also observed to exert anti-inflammatory activity against egg-albumin induced paw oedema and xylene-induced ear oedema. Egg albumin-induced oedema is suggested to be similar to carrageenan-induced oedema and associated with pro-inflammatory agents such as histamine, 5-HT and prostaglandin (Akah and Nwanbie, 1994; Nwafor *et al*., 2007). The prostaglandins, especially prostaglandin E_2 (PGE_2) amplify the pain mechanism and enhance vascular

permeability whereas the leukotrienes contract the smooth muscles of blood vessels, enhance vascular permeability and mediate pro-inflammatory and allergic responses (Gonzalez *et al*., 2000).

Monoterpenes are reported to inhibit lipoxygenase (LOX) (Wei and Shibamoto, 2010). Terpinen-4-ol which has been found in the seed extract has been reported to suppress production of prostaglandin and *in vitro* of TNF-α, IL-1β, as well as IL-8, IL-10 and PGE_2 by LPS-activated human blood monocytes (Hart *et al.,* 2000; Miguel, 2010). This compound may in part be responsible for the observed activity. Similarly, the seed extract exerted considerable inhibition of ear oedema caused by xylene in a dose-dependent manner. This suggests the inhibition of phospholipase A_2 which is involve in the pathophysiology of inflammation due to xylene (Lin *et al.,* 1992). Antioxidants are reported to prevent inflammation by scavenging radical oxygen species (Miguel, 2010). The seed extract has been reported above to contain some anti-oxidant compounds. These compounds may have been responsible for the observed activity. However, the anti-inflammatory activity of plants' extracts may be attributed not only to their antioxidant activities but also to their interactions with signalling cascades involving cytokines and regulatory transcription factors, and on the expression of pro-inflammatory genes. Moreover, the GC-MS of the hexane fraction has revealed the present of a-phellandrene, an acyclic monoterpene which has been reported for significant anti-inflammatory activity (Lima *et al.,* 2012). Its activity may in part have contributed to this anti-inflammatory activity. Triterpene-fatty acid esters and free fatty acids including long chain C16-C20 unsaturated have been suggested to be responsible for the anti-inflammatory activity in the extract from *Tinospora smilacina* (Li *et al*., 2004). The GC-MS of dichloromethane fraction showed the presence of long chain fatty acids. These fatty acids may have contributed to the anti-inflammatory activity of this extract.

5. Conclusions

The results of this study shows that the seed extract of *Telfairia occidentalis* possesses immunomodulatory, anticancer, and anti-inflammatory properties which are due to its phytochemical components

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