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Antioxidant activity and identification of bioactive compounds in *Telfairia occidentalis* leaves using GC-MS analysis

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Abstract

Telfairia occidentalis belongs to the family cucurbitaceae. It is a plant with dark green leaves that also bears a pod containing edible seeds. It is widely cultivated in Western Africa, having high commercial importance in the eastern part of Nigeria. It is primarily used in the preparation of soup in the form of vegetable and in herbal medicines for production of blood tonic for weak and ill person, and also for the treatment of other ailments. *Telfairia occidentalis* leaves extract and fractions were evaluated for its phytochemical constituents, antioxidant activities and GC-MS analysis on the active extract and fractions to identify the bioactive compounds conferring these activities using standard procedures. The phytochemical screening of the ethanol extract and different fractions revealed the presence of flavonoids, alkaloids, tannins, saponin, cardiac glycoside and anthraquinones. The antioxidant activity was carried out using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) model; among the fractions ethyl acetate fraction (84.91%;100 µg/mL) demonstrated the best antioxidant activities in DPPH assay followed by the extract (80%;100 µg/mL), dichloromethane(79.51%;100 µg/mL) and n-hexane fractions (46.63%;100 µg/mL) with IC50 of 22.56 µg/mL, 37.43 µg/mL, 37.59 µg/mL, and 91.11 µg/mL. Ferric reducing antioxidant power (FRAP) recorded highest activity at 100 µg/mL with ethyl acetate having the highest antioxidant effect (1.574 nm) compared to ascorbic acid (1.750 nm; 100 µg/mL) followed by DCM fraction (1.040 nm), crude extract (0.966 nm), and n-hexane fraction (0.724 nm). The GC-MS analysis recorded a total of 39 compounds from extract; 9 major constituents; oleic acid, (10.27%), acetic acid (9.66%), hexanoic acid (7.66%), phytol acetate (7.22%), 18,19-seccoyohimban-19-oic acid (6.69%), iso-propyl 9,12,15-octadecatrienoate(6.20%), n-hexadecanoic acid, methyl ester (5.83%), trans-geranyl geraniol (4.21%) and 9,12- octadecadienoic acid (4.11%),39 compounds from DCM with 8 major constituents; (1,1-bicyclopropyl)-2-octanoic acid (25.87%), quinic acid (9%), acetic acid (6.66 %), 9-octadecadienoic acid (z,z) (5.63 %), benzofuran (5.34%), oxirane (4.11%), phytol, acetate (2.52 %) and vitamin E (2.37%), and 37 compounds from ethyl acetate fraction with 7 major constituents; stigmasterol (17.09%), vitamin E (14.73%), stigmasterol (11.25%), 4,22-stigmastadiene-3-one (9.40%), 7,22-ergostadienol (5.84%), dotricontane (3.88%) and eicosane (3.07%). From these results T. occidentalis extract and fractions shows high antioxidant activity. These compounds present in T. occidentalis extract and fractions could provide a rationale for the ethnomedicinal use of the plant in the management of several pathological conditions associated with oxidative stress including type 2 diabetes and inflammatory conditions.

Keywords: *Telfairia occidentalis*; Phytochemicals, Antioxidant activity; Bioactive compounds; GC-MS

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1. Introduction

Biological system undergoes metabolic processes, a redox reaction that generates reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2), nitric oxide (NO), hydroxyl radical (·OH), peroxyl radical (ROO·), and superoxide radical (O_2^-). In a balanced biological system, ROS generated during metabolic process are detoxified by the antioxidant enzymes in the system; thereby creating an equilibrium between the ROS generation and its detoxification by antioxidants within the system [1].Reactive oxygen species (ROS) are responsible for oxidative stress and so many other degenerative diseases such as diabetes, inflammatory and cardiovascular disorders. Antioxidants are substances that can prevent or slow down damage to cells caused by free radicals [2]. Antioxidants are believed to play a very important role in the body defense system against ROS [3]. A variety of free radical scavenging antioxidants is found in dietary sources like fruits, vegetables and tea etc. Secondary metabolites such as flavonoids, coumarins, phenolic acids among others are responsible for these properties possessed by medicinal plants [4].

Telfairia occidentalis is a perennial plant that grows through climbing by means of bifid and tendrils which are usually coiled. The stem has ridges often covered with multi-cellular hairs. The leaves of the plant are compound dark green with 3-5 foliate. The seed which are embedded within a bright yellow fibrous endocarp are large, non-endospermic and usually dark red [5]. *T. occidentalis* are commonly called fluted pumpkin, oyster nut, oil nut, fluted gourd (English), Ugwu (Igbo-Nigeria), Aworoko/Eweroko (Yoruba-Nigeria) and Ikong ubong (Efik/Ibibio-Nigeria) (6).

According to Okonwu and Ariaga, [7], nutritional compositions of *T. occidentalis* are vitamins (including vitamin C and E), minerals such as Zinc, Magnesium and Copper; proximate analysis of *T. occidentalis*.

Studies that have been carried out on the plant include anti-convulsant activity (8); antidiabetic activity [9;10]; renal protection activity [11] and anti-cancer activity [12; 13; 14]. There is no systematic report on the antioxidant activity of *T. occidentalis* in literature and also there is paucity of information on the identification of bioactive component of *T. occidentalis*. The aim of the present study was to find out antioxidant activity of the leave extract and fractions and also the bioactive compounds conferring this activity.

2. Material and methods

2.1. Plant material and extraction

The leaves of T. *occidentalis* were collected from a farm in Uyo Local Government Area, Akwa Ibom State, Nigeria in April 2021. The plant was identified by a Taxonomist, Faculty of Pharmacy, University of Uyo, the Herbarium of which a voucher specimen (voucher no. UUPH28(d)) was prepared and deposited. The leaves were air dried and pulverized. The pulverized sample (2.5 kg) was macerated in ethanol (70%,) for 72 h and re-macerated for another 72 h. The filtrate was concentrated using rotary evaporator at 30 ^{0}C to obtain the crude extract. Sixty grams (60 g) of the extract were dissolved in distilled water and fractionated successively and exhaustively with n-hexane, dichloromethane (DCM), and ethyl acetate, to obtain their respective fractions. The fractions were concentrated in vacuo at 30 ^{0}C.

2.2. Phytochemical screening

Telfairia occidentalis was subjected to qualitative phytochemical investigation for alkaloids, flavonoids, tannins, glycosides, saponnin, and steroids according to the methods described by [15 ;16].

2.3. Antioxidant assays

2.3.1. Diphenyl-1-picrylhydrazyl (DPPH) Radical Scavenging Activity

The free radical scavenging activity of the extract was determined using the modified method of Blois [17]. The different concentration (0.2-1 mg/mL) of the extract (5 mL) in different test tubes, and standard drug (ascorbic acid) in a test tube was added to 1 mL of 0.3% DPPH in methanol. The mixture was vortexed and then incubated in a dark chamber for 30 minutes after which the absorbance was measured (in triplicates) at 517 nm against a DPPH control containing only 5 mL of methanol in place of extract. Percentage scavenging activity was calculated using the expression below:

$$\% \text{ Scavenging activity} = \frac{\text{absorbance of control} - \text{absorbance of sample} \times 100\%}{\text{absorbance of control}}$$

2.3.2. Determination of ferric reducing antioxidant power (FRAP) assay

The reducing power was determined according to the method of Yen and Chen (18). Various concentrations of *T. occidentalis* extract, fractions, and ascorbic acid (2 mL each) were mixed with sodium phosphate buffer (pH 6.6, 200 mM, 2 mL) and 30 mM potassium ferricyanide (2 mL). The mixture was incubated at 50 ◦ C for 20 min, followed by the addition of trichloroacetic acid (10% w/w, 2 mL); the mixture was centrifuged at 3000 rpm for 10 min. The upper layer of the resultant solution (5 mL) was mixed with deionized water (5 mL) and ferric chloride (0.1% w/v, 1 mL). The absorbance was measured at 700 nm. The assays were carried out in triplicate and the results were expressed as mean ± SEM.

2.4. Identification of bioactive compounds using GC-MS

The ethanolic extract, DCM and ethyl acetate fractions of the leaves were analyzed using Gas Chromatography Mass Spectroscopy (GC-MS) for the identification of the bioactive component present in the extract and fractions. This analysis was carried out on a GC system comprising a Gas Chromatograph interfaced to a Mass Spectrometer (GC-MS) instrument; Schimadzu GCMS-QP2010SE, employing the following conditions: helium (99.999%) as carrier gas at a constant flow of 1 mL/ minute and a sample injection volume of 1 µL was employed (split ratio of 1:1); injector temperature 250 °C; ion-source temperature 230 °C. The oven temperature was programmed from 60°C (isothermal for 1 minutes), with an increase of 10°C/minute to 240 ^{0}C, then 5°C/minute to 300°C, ending with a 9 minutes isothermal at 300°C.Mass spectra were taken at 70 eV; a scan interval of 0.5 S, total run time was 29.90 min. The compounds were then identified from the GC-MS peaks, using library data of the corresponding compounds. GC-MS was analyzed using electron impact ionization at 70 eV and data was evaluated using total ion count (TIC) for compound identification and quantification. The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library using NISP Search. The relative % amount of each component was calculated by comparing its average peak area to the total areas. Measurement of peak areas and data processing were carried out by Turbo-Mass-OCPTVS-Demo SPL software.

3. Results

Preliminary qualitative phytochemical analysis of ethanol extract and fractions (n-hexane, DCM and ethyl acetate) revealed the presence of secondary metabolites such as alkaloids, saponins, flavonoids, anthraquinones, tannins, Cardiac glycosides, as presented in Table 1.

3.1. Antioxidant activity

Different concentrations of leaf extract and fractions of *T. occidentalis* revealed maximum DPPH activity of; extract (80%), hexane (46.63%), DCM (79.51%) and ethyl acetate (84.91%) while vitamin C (91.41%) (Table 2). The ethyl acetate fraction of the leaf extract exhibited the highest DPPH radical scavenging activity with an IC_{50} of 22.56 µg/mL, followed by DCM 37.43 µg/mL, ethanol extract 37.59 µg/mL, and n-hexane with the least activity with IC_{50} of 91.11 µg/mL, whereas the vitamin C (standard) exhibited IC_{50} of 10.26 µg/mL. Among the extracts and fractions, ethyl acetate leaf extract revealed a maximum Ferric reducing antioxidant power assay (FRAP) activity with an absorbance of 1.574 nm followed by DCM (1.040 nm), extract (0.966 nm) and n-hexane (0.724 nm) with vitamin C (1.750 nm) used as standard (Table 3).

3.2. Bioactive component present in extract and fractions of *T. occidentalis*

The GC-MS analysis was carried out on the ethanol extract, DCM and ethyl acetate fractions. The ethanol extract recorded39 components as shown in Figure 1 and presented in Table 4; with 9 major compounds (Table 5) with high standard index and peak area percent of these compounds which include, oleic acid (10.27 %), acetic acid (9.66 %), hexanoic acid (7.66 %), phytol acetate (7.22 %), 18,19-seccoyohimban-19-oic acid (6.69 %), isopropyl 9,12,15-octadecatrienoate(6.20 %), n-hexadecanoic acid, methyl ester (5.83 %), trans-geranyl geraniol (4.21 %) and 9,12-octadecadienoic acid (4.11 %).

Table 1 Phytochemical analysis of the leaves extract and fractions of *T. occidentalis*

Phytochemicals	**Test**	**Ethanol**	**Fractions**		
			n-hexane	**DCM**	**EtOAc**
Alkaloids	Dragendoff'sreagent	++	-	+	+
	Meyer's reagent	++	-	-	+
Tannins	Ferric chloride	+++	+	+	++
Saponins	Frothing test	+++	+	+	-
Flavonoids:	Magnesium metaltest	+++	-	+	++
	Sodium hydroxide test	++	-	+	++
Anthraquinones	Combine anthraquinone	++	-	+	+
	Free anthraquinone	++	-	+	+
Cardiac glycosides	Salkwoski's test	++	-	+	+
	Keller Killiani test	++	+	+	++
	Lieberman's test	++	-	+	++

Keys:++ = moderately present;+++ = heavily present;- = absent

Table 2 DPPH radical scavenging activity of *T. occidentalis* extract, and fractions Blank = 0.815 ± 0.01

S/N	**Conc.µg/mL**	**Absorbance and % inhibition**				
		EtOH	**n-hexane**	**DCM**	**EtOAc**	**Vitamin C**
		abs **% inhi.**	**abs** **% inhi.**	**abs** **% inhi.**	**abs** **% inhi.**	**abs** **% inhi.**
1	20	0.323±0.00 (60.37%)	0.566±0.01 (30.55%)	0.365±0.00 (55.21%)	0.213±0.01 (73.87%)	0.075±0.01 (90.79%)
2	40	0.318±0.00 (60.98%)	0.512±0.01 (37.18%)	0.255±0.01 (68.71%)	0.149±0.00 (81.72%)	0.073±0.01 (91.04%)
3	60	0.233±0.01 (71.41%)	0.478±0.01 (41.35%)	0.241±0.00 (70.43%)	0.144±0.00 (82.33%)	0.073±0.01 (91.04%)
4	80	0.188±0.00 (76.93%)	0.447±0.00 (45.15%)	0.199±0.00 (75.58%)	0.127±0.01 (84.42%)	0.070±0.00 (91.41%)
5	100	0.163±0.00 (80.00%)	0.435±0.01 (46.63%)	0.167±0.00 (79.51%)	0.123±0.01 (84.91%)	0.070±0.01 (91.41%)

Values in parenthesis represents percentage inhibition

Total of 39 constituents were identified from the DCM fraction shown in Figure 2 and Table 6 with good standard index and peak area percent, with 8 major compounds presented in Table 7; (1,1-bicyclopropyl)-2-octanoic acid (25.87 %), quinic acid (9%), acetic acid (6.66 %), 9-octadecadienoic acid (z,z) (5.63 %), benzofuran (5.34 %), oxirane (4.11 %), phytol acetate (2.52 %) and vitamin E (2.37 %). Total of 37 components were obtained from the ethyl acetate fraction

presented in Table 8 and Figure 3, with 7 major compounds shown in Table 9 obtained with high standard index and peak area percent which include; stigmasterol (17.09 %), vitamin E (14.73 %), stigmasterol (11.25 %), 4,22-stigmastadiene-3-one (9.40 %), 7,22-ergostadienol (5.84 %), dotricontane (3.88 %) and eicosane (3.07 %).

Table 3 Absorbance for FRAP assay of *T. occidentalis* extract, and fractions (Blank = 0.577 ± 0.001**)**

Conc.µg/mL	**Absorbance (nm) in different solvent**				
	extract	**n-hexane**	**DCM**	**EtOAc**	**Vitamin C**
20	0.788±0.01	0.646±0.01	0.874±0.01	0.981±0.02	0.929±0.01
40	0.790±0.01	0.655±0.02	0.899±0.02	0.989±0.02	1.260±0.01
60	0.865±0.01	0.689±0.02	0.934±0.01	1.330±0.01	1.613±0.02
80	0.894±0.02	0.689±0.01	0.979±0.01	1.561±0.02	1.649±0.01
100	0.966±0.01	0.724±0.01	1.040±0.01	1.574±0.01	1.750±0.01

Table 4 GC-MS analysis of the ethanol extract of *T. occidentalis*

Peak	**Retention Time**	**Area%**	**Molecular Formula**	**Molecular Weight**	**Standard Index**	**Name of compounds**
1	7.683	2.52	$C_6H_8O_4$	144	85	4H-pyran-4-one,2,3-dihydroxy-6-methyl
2	11.626	0.42	$C_{14}H_{22}O$	206	86	Phenol, 2,6-bis(1,1-dimethylethyl)-
3	12.894	2.94	$C_{18}H_{36}O_2$	284	68	Octadecanoic acid
4	14.086	0.21	$C_{14}H_{28}O_2$	228	70	Tetradecanoic acid
5	14.839	2.01	$C_{20}H_{40}O$	296	88	3,7,11,15-tetramethyl-2-hexadecen-1-ol
6	15.039	0.44	$C_{20}H_{40}O$	296	79	3,7,11,15-tetramethyl-2-hexadecen-1-ol
7	15.199	0.82	$C_{13}H_{22}O$	296	86	3,7,11,15-tetramethyl-2-hexadecen-1-ol
8	15.276	0.40	$C_{17}H_{24}O_3$	276	58	7,9-di-tent-butyl-1-oxaspiro(4,5)deca-6,9-diene
9	15.456	1.21	$C_{17}H_{34}O_2$	270	90	Hexadecanoic acid, methyl ester
10	15.601	0.23	$C_{22}H_{33}NO_6$	407	55	Glutaric acid, 3-nitrophenethyl nonyl ester
11	15.781	5.83	$C_{16}H_{32}O_2$	256	92	n-Hexadecanoic acid
12	16.592	0.41	$C_{13}H_{24}O_2$	212	57	2-Tridecanoic acid
13	16.835	1.63	$C_{19}H_{34}O_2$	294	91	9,12-octadecadienoic acid(Z,Z), methyl ester
14	16.909	1.92	$C_{19}H_{36}O_2$	296	91	9-octadecenoic acid, methyl ester, Elaidic acid ester
15	16.958	0.19	$C_{13}H_{22}N_2O$	222	48	Pyridine,2,3,4-Tetrahydro-1-(1-oxopropyl)-5-(2-piperidinyl)

16	17.159	0.45	$C_{19}H_{38}O_2$	298	81	Methylstearate, methylester, octadecenoic acid
17	17.214	4.11	$C_{18}H_{32}O_2$	280	93	9,12-octadecadienoic acid, cis-9,cis-12-octadecadienoic acid
18	18.286	10.27	$C_{18}H_{34}O_2$	282	93	Oleic acid,
19	17.515	2.49	$C_{18}H_{36}O_2$	284	93	octadecanoic acid
20	18.195	0.62	$C_{16}H_{30}O_2$	254	71	13-tetradecen-1-ol acetate
21	19.515	0.43	$C_{22}H_{44}O_2$	340	60	Docosanoic acid
22	19.750	0.20	$C_{10}H_{15}BrO_4S$	310	45	(+)-3-bromocamphor-8-sulfonic acid
23	20.691	2.04	$C_{26}H_{52}O_2$	396	69	2-ethylbutyric acid,eicosyl ester
24	21.00	0.38	$C_{23}H_{46}O_2$	354	60	octadecanoic acid, 5,9,13,17-tetramethyl
25	21.733	1.58	$C_{29}H_{48}O$	412	61	Stigmasterol
26	22.075	1.86	$C_{24}H_{39}NO_2Si$	401	31	Androsta-1,4-dien-3-one, 17-methyl-17-[(trimethylsilyl
27	22.225	6.69	$C_{12}H_{24}N_2O_3$	352	56	18,19-Secoyohimban-19-oic acid, 16,17,20,21
28	22.329	7.66	$C_{24}H_{48}O_2$	368	72	Hexanoic acid, octadecyl ester
29	22.510	6.20	$C_{21}H_{36}O_2$	320	78	i-Propyl 9,12,15-octadecatrienoate
30	22.942	9.66	$C_{17}H_{28}O_2$	264	79	Acetic acid, [(Z,Z)-3,7,11-trimethyl-2,6,10-dod
31	23.017	3.66	$C_{20}H_{42}O_2S$	346	43	Di-n-decylsulfone
32	23.050	2.35	$C_{19}H_{16}O_4S$	220	42	5-(2-Oxo-[1,2]oxathiolan-3-yl)pentanoic acid.
33	23.106	7.22	$C_{22}H_{42}O_2$	338	85	Phytol acetate
34	23.300	0.74	$C_{10}H_3F_{17}O_2$	478	29	Heptadecafluorononanoic acid, methyl ester
35	23.811	4.21	$C_{20}H_{34}O$	290	77	trans-Geranylgeraniol
36	24.103	1.78	$C_{12}H_{16}NO_3$	236	58	Cyclobarbital
37	24.285	2.01	$C_{30}H_{52}O$	428	80	2,2,4-Trimethyl-3-(3,8,12,16-tetramethyl-heptane
38	24.600	0.72	$C_{14}H_{24}O$	208	43	2,5,5,6,8a-Pentamethyl-trans-4a,5,6,7,8,8a-hexa
39	24.877	1.51	$C_{24}H_{32}O_6$	416	35	9,12,15,18,21,24-Hexaoxa(2,16)[30]paracycloph

Table 5 Major compounds contained in the GC-MS analysis of extract

S/N	Standard index(SI)	Peak area percent (%)	Name of compounds
1	93	10.27	oleic acid, stearic acid
2	79	9.66	acetic acid
3	72	7.66	hexanoic acid
4	85	7.22	Phytol acetate
5	56	6.69	18,19-seccoyohimban-19-oic acid
6	78	6.20	i-propyl 9,12,15-octadecatrienoate
7	92	5.83	n-hexadecanoic acid, methyl ester
8	77	4.21	trans-geranyl geraniol
9	93	4.11	9,12- octadecadienoic

Table 6 GC-MS analysis of the DCM fraction of *T. occidentalis*

Peak	Retention Time	Peak Area %	Molecular Formula	Molecular Weight	Standard Index	Name of compounds
1	6.324	1.17	$C_{22}H_{24}N_2O_8S_2$	508	85	N,N'-Dicarbobenzyloxy-L-cystine
2	7.204	1.26	$C_8H_{10}O$	122	91	Phenylethyl alcohol
3	7.570	1.38	$C_6H_8O_4$	144	90	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methy
4	8.134	1.58	$C_8H_6O_3$	150	91	Methanol, oxo-, benzoate
5	8.748	5.34	C_8H_8O	120	89	Benzofuran, 2,3-dihydro-
6	9.493	0.62	$C_9H_{10}O_2$	150	83	2-Methoxy-4-vinylphenol
7	11.622	3.32	$C_{14}H_{22}O$	206	72	Phenol, 2,4-bis(1,1-dimethylethyl)-
8	13.042	9.00	$C_7H_{12}O_6$	192	76	Quinic acid
9	14.839	25.87	$C_{21}H_{38}O_2$	322	79	[1,1'-Bicyclopropyl]-2-octanoic acid, 2'-hexyl-, meth
10	15.042	0.57	$C_{19}H_{36}$	264	82	7-Octadecyne, 2-methyl-
11	15.200	0.58	$C_{20}H_{40}O$	296	88	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
12	15.454	0.34	$C_{17}H_{34}O2$	270	89	Hexadecanoic acid, methyl ester
13	15.782	2.55	$C_{16}H_{32}O_2$	256	91	n-Hexadecanoic acid
14	16.834	1.14	$C_{19}H_{34}O_2$	294	92	9,12-Octadecadienoic acid (Z,Z)-, methyl ester
15	16.909	0.91	$C_{19}H_{36}O_2$	296	92	6-Octadecenoic acid, methyl ester, (Z)-

16	17.086	0.68	$C_{24}H_{47}NO_4$	413	50	Glycine, N-propoxycarbonyl-, octadecyl ester
17	17.157	0.70	$C_{19}H_{38}O_2$	298	72	Methyl stearate
18	17.217	2.31	$C_{18}H_{32}O_2$	280	94	9,12-Octadecadienoic acid (Z,Z)-
19	17.297	5.63	$C_{18}H_{34}O_2$	282	94	9-Octadecenoic acid, (E)-
20	17.518	1.45	$C_{18}H_{36}O_2$	284	92	Octadecanoic acid
21	17.708	1.18	$C_{29}H_{50}O_2$	430	44	Vitamin E
22	17.825	2.37	$C_{29}H_{50}O_2$	430	88	Vitamin E
23	19.067	3.35	$C_{30}H_{50}O$	426	83	Friedelan-3-one
24	19.174	0.59	$C_9H_{16}O_3$	172	47	Methyl 8-oxooctanoate
25	19.267	0.53	$C_6H_{10}N_2O_2$	142	49	Acetic acid, 2-(2-pyrrolidinylideneamino)-
26	19.508	0.69	$C_{20}H_{40}O_2$	312	62	Eicosanoic acid
27	20.691	0.76	$C_{18}H_{36}O_2$	284	69	Hexanoic acid, dodecyl ester
28	21.755	1.03	$C_{29}H_{48}O$	412	78	Stigmasterol
29	21.942	0.84	$C_{20}H_{40}O$	296	76	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
30	22.406	4.11	$C_{18}H_{36}O$	268	77	Oxirane, hexadecyl-
31	22.442	2.52	$C_{18}H_{36}O$	268	78	Phytol acetate
32	22.716	1.32	$C_{29}H_{50}O$	414	77	gamma-Sitosterol
33	22.862	0.98	$C_{25}H_{36}O_2$	368	44	Phenol, 2,2'-methylenebis[6-(1,1-dimethylethyl)-4-et
34	23.009	3.06	$C_{23}H_{36}O_2$	344	59	1,4-Epoxynaphthalene-1(2H)-methanol, 4,5,7-tris (1,
35	23.295	1.64	$C_{32}H_{52}O_2$	468	56	9,19-Cycloergost-24(28)-en-3-ol, 4,14-dimethyl-, ace
36	23.733	0.55	$C_{30}H_{50}O$	426	56	Lanost-7-en-3-one, (9.beta,13.alpha.,14.beta.,17.alp
37	24.192	0.89	$C_{20}H_{22}N_2O_3$	338	46	2H-1,5-Benzodiazepin-2-one, 5-benzoyl-1,3,4,5-tetra
38	24.364	6.66	$C_{17}H_{28}O_2$	264	86	Acetic acid, [(Z,Z)-3,7,11-trimethyl-2,6,10-dodecatri
39	24.735	0.53	$C_{12}H_{22}Cl_2O_2$	268	71	Chloromethyl 5-chloroundecanoate

Table 7 Major compounds contained in the GC-MS analysis of DCM fraction

S/N	Standard index (SI)	Peak area percent (%)	Name of compounds
1	79	25.87	(1,1-bicyclopropyl)-2-octanoic acid
2	76	9.0	quinic acid
3	86	6.66	acetic acid
4	94	5.63	9-octadecadienoic acid (z,z)
5	89	5.34	benzofuran
6	77	4.11	oxirane
7	78	2.52	phytol, acetate
8	88	2.37	vitamin E

Table 8 GC-MS analysis of the ethyl acetate fraction of *T. occidentalis*

Peak	Retention Time	Peak Area %	Molecular Formula	Molecular Weight	Standard Index	Name of compounds
1	14.842	2.03	$C_{20}H_{38}$	278	88	3-Eicosyne
2	15.040	0.35	$C_{20}H_{40}O$	296	90	3,7,11,15-Tetramethyl-2-hexadecen-1-ol
3	15.201	0.61	$C_{19}H_{36}$	264	88	7-Octadecyne, 2-methyl-
4	15.466	0.17	$C_{17}H_{34}O_2$	270	84	Hexadecanoic acid, methyl ester
5	15.784	0.68	$C_{16}H_{32}O_2$	256	89	n-Hexadecanoic acid
6	17.090	0.24	$C_{20}H_{40}O$	296	90	Phytol
7	17.218	0.65	$C_{18}H_{32}O_2$	280	81	9,12-Octadecadienoic acid (Z,Z)-
8	17.278	1.25	$C_{18}H_{34}O_2$	282	79	9-Octadecenoic acid, (E)-
9	17.442	3.07	$C_{20}H_{42}$	282	90	Eicosane
10	17.981	14.73	$C_{29}H_{50}O2$	430	93	Vitamin E
11	18.783	0.43	$C_{21}H_{36}O_4$	352	68	9,12,15-Octadecatrienoic acid, 2,3-dihydroxypropyl est
12	19.691	1.00	$C_{22}H_{42}O_2$	338	85	Phytol acetate
13	20.485	1.98	$C_{24}H_{48}O_2$	368	64	Tetracosanoic acid
14	21.165	1.41	$C_{28}H_{48}O$	400	57	5-Cholestene-3-ol, 24-methyl-
15	21.333	0.50	$C_{21}H_{38}O_5$	370	64	Fumaric acid, 2-methoxyethyl tetradecyl ester
16	21.525	0.65	$C_{19}H_{38}O_3$	314	81	Methoxyacetic acid, 4-hexadecyl ester
17	21.825	11.25	$C_{29}H_{48}O$	412	87	Stigmasterol
18	21.916	17.09	$C_{29}H_{48}O$	412	88	Stigmasterol

19	22.344	0.51	$C_{30}H_{50}O$	426	67	Obtusifoliol
20	22.473	1.17	$C_{21}H_{44}O_3S$	376	74	Sulfurous acid, octadecyl 2-propyl ester
21	22.655	5.84	$C_{28}H_{46}O$	398	79	7,22-Ergostadienol
22	22.761	2.28	$C_{29}H_{50}O$	414	80	gamma-Sitosterol
23	22.883	1.81	$C_{15}H_{24}$	204	74	1,1,4a-Trimethyl-5,6-dimethylenedecahydronaphthalen
24	22.950	1.71	$C_{32}H_{66}$	450	65	Dotriacontane
25	23.044	3.88	$C_{32}H_{66}$	450	61	Dotriacontane
26	23.165	1.71	$C_{30}H_{48}O$	424	69	4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8
27	23.292	1.19	$C_{24}H_{37}NO_2$	371	48	Octadeca-9,12-dien-1-ol, nicotinate
28	23.372	0.98	$C_{15}H_{22}O$	218	70	Solavetivone
29	23.510	3.29	$C_{29}H_{50}O$	414	80	beta-Sitosterol
30	23.775	9.40	$C_{29}H_{46}O$	410	80	4,22-Stigmastadiene-3-one
31	23.967	2.09	$C_{29}H_{48}O$	412	58	(E)-14.alpha.-Methyl-5.alpha.-ergosta-8,23-dien-3.beta.
32	24.075	0.90	$C_{29}H_{46}O$	410	67	Stigmasta-3,5-dien-7-one
33	24.134	0.84	$C_{24}H_{50}S_2$	402	54	Disulfide, didodecyl
34	24.183	0.66	$C_{20}H_{20}O_5$	340	36	2,4-dimethyl-3,5-bis(3,4-methylenedioxyphenyl)tetrahy
35	24.360	0.69	$C_{31}H_{52}O_3$	472	67	alpha.-Tocopheryl acetate
36	24.481	1.34	$C_{17}H_{26}O_2$	262	70	Bicyclo[4.4.0]dec-6-en-9.beta.-ol, 1,7-dimethyl-4.alpha
37	24.748	1.65	$C_{31}H_{52}O$	440	83	9,19-Cyclolanostan-3-ol, 24-methylene-, (3.beta.)-

Table 9 Major compounds contained in the GC-MS analysis of ethyl acetate fraction

S/N	Standard index (SI)	Peak area percent (%)	Name of compounds
1	88	17.09	stigmasterol
2	93	14.73	vitamin E
3	87	11.25	stigmasterol
4	80	9.40	4,22-stigmastadiene-3-one
5	79	5.84	7,22-ergostadienol
6	61	3.88	dotricontane
7	90	3.07	eicosane

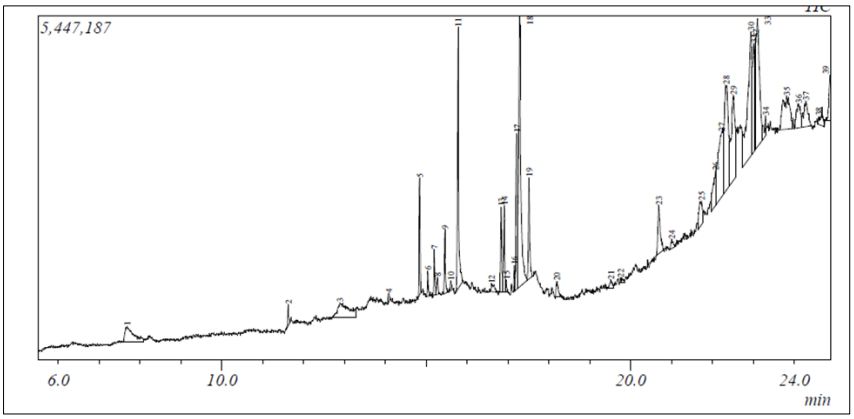

Figure 1 GC-MS chromatogram of, ethanol extract of *T. occidentalis* leaves

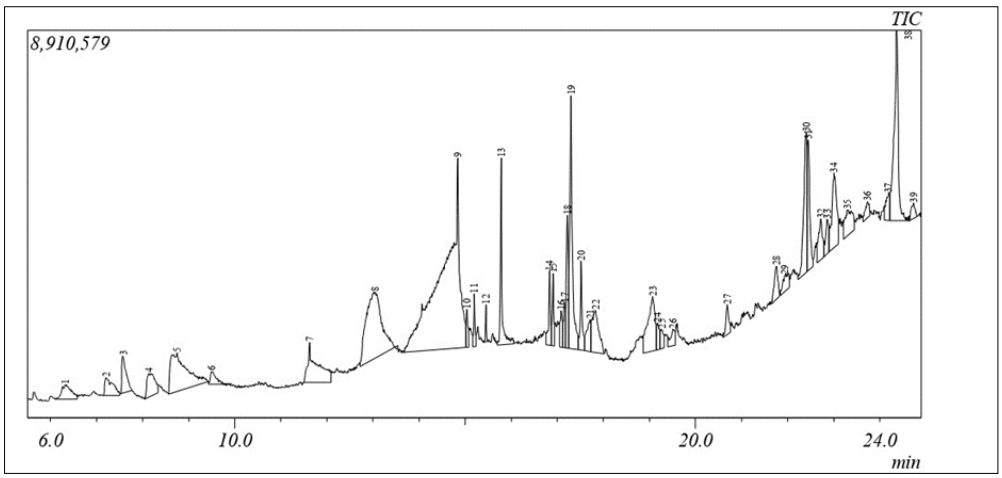

Figure 2 GC-MS chromatogram of, DCM fraction of *T. occidentalis* leaves

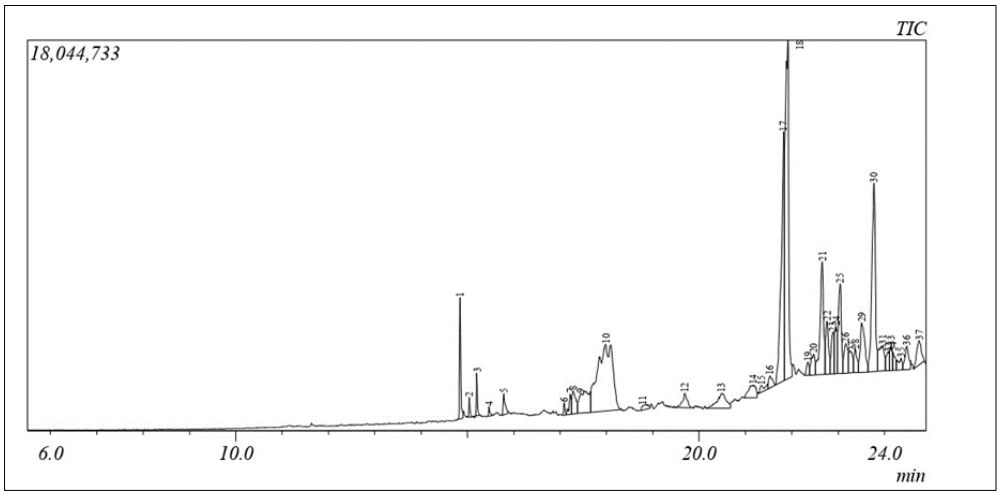

Figure 3 GC-MS chromatogram of, ethyl acetate fraction of *T. occidentalis* leaves

4. Discussion

The phytochemical analysis of *T. occidentalis* leaf extract and fractions revealed the presence of flavonoids, alkaloids, tannins, saponin, cardiac glycoside and anthraquinones with exception of n-hexane fraction that shows only the presence of tannins, saponins and cardiac glycosides in a very low proportion.

Phytochemicals are nonessential nutrients and mainly produced by plants to provide them protection; these non-nutritive plant chemicals have either defensive or disease protective properties. Dietary intake of phytochemicals may promote health benefits, protecting against chronic degenerative disorders such as cancer, cardiovascular diseases and neurodegenerative diseases etc [19].

Free radicals and oxidative stress are associated with several disease conditions such as cancer, cardiovascular diseases in biological system [20]. Antioxidants agents prevents/reduces the damaging effect of free radicals, the mechanism through which they elicit their actions are by preventing the formation of new free radicals, capturing of free radicals and repairs of bimolecular damaged by free radicals. The ethanol extract and fractions of *T. occidentalis* leaves have shown high antioxidant activities in their capacity to scavenge DPPH free radicals and also their ability to reduce Fe^{3+} to Fe^{2+} in the ferric reducing antioxidant power (FRAP) method presented in Table 2. The different concentrations of leaf extract and fractions of *T. occidentalis* were subjected to 2,2-diphenyl-1-picryl- hydrazyl (DPPH) free radical scavenging model and ferric reducing antioxidant power (FRAP) method. At 100 µg/mL the ethanol extract revealed maximum DPPH scavenging activity of 80 % while n-hexane, DCM, ethyl acetate fractions and vitamin C (standard) recorded 46.63 %, 79.51 %, 84.91 % and 91.41 % respectively. Among the fractions ethyl acetate recorded the maximum scavenging activity of 84.91 %, this might be due to fat that it is moderately polar and therefore can extract moderately and partly polar compounds which were indicated by the high percentage presence of stigmasterol and vitamin E which have been implicated for their antioxidant activities in literature [21; 22]. Analytical methods for determining bioavailability and bio-accessibility of bioactive compounds from fruits and vegetables: A review. (Comprehensive Reviews in Food Science and Food Safety, 13, 155–171). The IC_{50} of 37.43 µg/mL, 37.59 µg/mL, 22.56 µg/mL and 10.26 µg/mL were obtained for DCM, ethanol extract, ethyl acetate and vitamin C respectively. IC_{50} is the half maximum inhibitory concentration of the extract/fractions, the lower the value the better the antioxidant activity [23].The ferric reducing antioxidant power (FRAP) activity of leaf extract and fractions recorded the highest value at 100 µg/mL with ethyl acetate (1.574) having the highest value followed by DCM (1.040), ethanol extract (0.966) compared to vitamin C (1.750) used as standard, with n-hexane (0.724) with least activity.

GC-MS analysis was carried out on the most active fractions (DCM and ethyl acetate) and extract to further identify the bioactive compounds conferring the antioxidant activities. A total of 39 components were identified from the ethanol extract of *T. occidentalis* with 9 major compounds; oleic acid (10.27 %), acetic acid (9.66 %), hexanoic acid (7.66 %), phytol acetate (7.22 %), 18,19-seccoyohimban-19-oic acid (6.69 %), isopropyl 9,12,15-octadecatrienoate(6.20 %), n-hexadecanoic acid, methyl ester (5.83 %), trans-geranyl geraniol (4.21 %) and 9,12- octadecadienoic acid (4.11 %). A total of 39 compounds were identified from DCM fraction of *T. occidentalis* with 8 major constituents; (1,1-bicyclopropyl)-2-octanoic acid (25.87 %), quinic acid (9%), acetic acid (6.66 %), 9-octadecadienoic acid (z,z) (5.63 %), benzofuran (5.34 %), oxirane (4.11 %), phytol acetate (2.52 %) and vitamin E (2.37 %). Finally a total of 37 constituents were identified from the ethyl acetate fraction of *T. occidentalis* with 7 major compounds with high standard index; stigmasterol (17.09 %), vitamin E (14.73 %), stigmasterol (11.25 %), 4,22-stigmastadiene-3-one (9.40 %), 7,22-ergostadienol (5.84 %), dotricontane (3.88 %) and eicosane (3.07 %). The identified phytochemicals including; n-hexadecanoic acid, methyl ester (palmitic acid), hexanoic acid, vitamin E, oleic acid, phytol acetate, acetic acid and so on have been implicated for their antioxidant and other pharmacological activities [24;25].These findings have several differences and similarities in the nature and number of phytocompounds identified through GC-MS analysis of the leaf extract and fractions of *T. occidentalis*. Some of the major compounds are in tandem with the work of Ezekwe [26] which reported the presence of hexanoic acid (methyl ester) etc via GC-MS analysis of methanol extract of *T. occidentalis*; similarly, Igwe *et al*.,[27] reported the presence of phytocompounds such as n-hexadecanoic acid (palmitic acid), octadecanoic acid (stearic acid), linoleic acid and so on in the GC-MS analysis of methanol extract of *T. occidentalis*. Most of the major compounds are uniquely different from the previous GC-MS analysis performed on *T. occidentalis* extract in literature, this may be due to the difference in age of the plant, concentration of ethanol (solvent) and extraction time

[28]. Also, this is the first attempt to identify bioactive components in the active (antioxidant) fractions obtain from the leaf extract of *T. occidentalis* which contains unique compounds with high standard index (SI) and peak area percentage. These compounds include trans-geranyl geraniol, i-propyl 9,12,15-octadecatrienoate, 18,19-secoyohimban-19-oic acid, quinic acid, (1,1-bicyclopropyl)-2-octanoic acid, vitamin E, oxirane, stigmasterol, 4,22-stigmastadiene-3-one, 7,22-ergostadienol, dotricontane and so on. These compounds with high standard index and peak area percent maybe responsible for the observed antioxidant activities of the extract and fractions.

5. Conclusion

The presence of phytochemicals (tannins, saponins, alkaloids, flavonoids etc.) and bioactive compounds (hexanoic acid, n-hexadecanoic acid, vitamin E etc.) in the *T. occidentalis* leaf extract is an indication that this vegetable is rich in phytochemicals with characteristic therapeutic potentials such as antioxidant activities etc. Therefore T. *occidentalis* could be beneficial for the control of oxidative stress related diseases including cancer, cardiovascular diseases etc. Structures of the major compounds identified by GC-MS analysis could be docked for molecular modeling and charting a new avenue for drug discovery.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare no conflict of interest.

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