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Original Research Article

Antioxidant Activity of Column Fractions and Caryatin Isolated from the Ethyl acetate Extract of *Dioscorea hirtiflora* tuber

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ABSTRACT

The tubers of *Dioscorea hirtiflora*Benth. are widely used as food during famine and as medicine in South-western Nigeria. The aim of this study was to identify bioactive compound(s) from the tuber of *Dioscorea hirtiflora*. Bioactive constituents from the ethyl acetate extract and fractions of the tubers were identified using gas chromatography-mass spectrometry (GC-MS), and by reverse phase-high performance liquid chromatography (RP-HPLC). The extract was subjected to column chromatography (CC) and preparative thin layer chromatography (PTLC) for the isolation of constituent(s). Fractions from the column chromatography of the extract as well as an isolated compound from the sub-fraction of the extract were evaluated for their antioxidant activity using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. The GC-MS of the derivatized ethyl acetate extract showed the presence of phenolics (benzoic acid, 4-hydroxybenzoic acid and 3,4-dihydroxybenzoic acid) while the RP-HPLC confirmed the presence of gallic acid, protocatechuic acid, catechin, isovanillic acid and quercetin in the fractions. CC and PTLC of the extract led to the isolation of compound I-24characterised as 3,5-dimethoxy-7, 3′, 4′-trihydroxy flavone (caryatin) by a combination of nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS), and by comparison of spectra data with literature. Fraction I and sub-fraction I-2 had the highest antioxidant activity with IC_{50} values of 2.11 ± 0.09 and 4.67 ± 0.06 µg/mL, respectively,while the isolated compound had IC_{50} value of 49.4 ± 2.18 µg/mL.The effect of these compounds as antioxidant could give credence to their traditional use as food and medicine.

Keywords: Antioxidant activity,Caryatin, *Dioscorea hirtiflora*, Phenolics, Nigeria.

Introduction

Dioscorea hirtiflora Benth.belongs to the family Dioscoreaceae. They are widely distributed across Namibia, Zambia, Malawi, Mozambique, Southern Congo (Kinshasa), Western Tanzania and Uganda.[1]Morphologically, the leaves are usually opposite, while the tuber is elongated and cylindrical. In South-western Nigeria, *Dioscorea hirtiflora* (Dh) is usually found in open woodland, riverine vegetation and wild grassland. The tubers are usually found in the wild by farmers during land clearing and tilling in preparatory for the planting season between February and April.

Alkaloids, diterpenoids, steroids, glycosides, flavonoids, phenolics, amongst others are examples of classes of compounds which have been reported in the family Dioscoreacea.[2-6] Some of the reported chemical constituents from the family Dioscoreacea include; *β*-sitosterol, palmitic acid, succinic acid, kaemferol, protocatechuic acid, catechin and quercetin.[5,7] The ethyl acetate extract of the tuber has been reported to have high antioxidant activity and brine shrimp lethality.[8] The tubers have also been reported to be used traditionally in the treatment of constipation, boil, haemorrhoids, measles, small pox, snake bite and for wound dressing.[9]

Due to the extensive application of the tubers of Dh as food and medicine in South-western Nigeria, without previous report of bioactive compounds from it, the present investigation focuses on: (i) identifying bioactive constituents from the derivatised ethyl acetate extracts of Dh using gas chromatography-mass spectrophotometry (GC-MS), (ii) monitoring of fractions and sub-fractions obtained from the column chromatography of the ethyl acetate extract using antioxidant activity and reverse phase-high performance liquid chromatography with a view to identifying any bioactive compound(s).

Materials and Methods

General experimental procedure

Nuclear Magnetic Resonance (NMR)spectra was recorded on a Bruker AV500 spectrometer (Bruker Instruments Incorporation, Billerica, MA USA), ^{1}H- and ^{13}C-NMR chemical shift (δ) was expressed in parts per million (ppm) with deuterated dimethyl sulphoxide (DMSO-*d*6) as the solvent and tetramethylsilane (TMS) as the internal standard. The FTIR spectra of compound I-24 was measured on PerklinElmer Fourier Transform Infrared spectrometer (PerklinElmer, U.S.A) within an absorption spectral range of 400-4000 cm^{-1}. Mass spectra data was obtained using Time of flight electrospray ionization mass spectrometer (TOFESI-MS) (Bergmann Messgeraete Entwicklung, Germany) in Positive ionisation mode (Es^{+}) with a mass range 100-1000 amu and voltage of 70 eV.

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Chemicals and reagents
All solvents and reagents used for this study were products of Sigma-Aldrich, (Germany). These include; analytical/HPLC grade Methanol, HPLC grade Water, HPLC grade Acetonitrile, analytical n-Hexane, Dichloromethane, Chloroform, Ethyl acetate, N-Methyl-N-tertButyldimethylsilyltrifluoroacetamide(MTBSTFA), Dimethylformamide, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Isovanillic acid, Quercetin, Protocatechuic acid, Gallic acid, Ascorbic acid, Catechin, Deuterated dimethyl sulphoxide (DMSO-*d*6).

Plant collection
The fresh tubers and leaves of *Dioscorea hirtiflora* (Dh) were collected from Ekiti State (7.67° N, 5.31° E) South-western, Nigeria between October 2015 and May 2016. It was identified and authenticated by Mr. A. Adeyemo at the Forest Herbarium Ibadan (FHI) of the Forestry Research Institute of Nigeria, Ibadan, Nigeria. The voucher specimen was prepared and deposited at FHI as *Dioscorea hirtiflora*Benth. -FHI 108911.

Extraction
The method of Adeniran and Sonibare[2] was used for the extraction of the dried powder tubers. Briefly, 1 kg was successively extracted using *n*-hexane, dichloromethane, ethyl acetate and methanol. Distilled solvent (10-15 L) was used for maceration for a period of 72 h with intermittent stirring. Extraction was repeated 3-5 times for each solvent. The extracts were filtered under pressure using a Buchner funnel connected to a vacuum pump. The filtrates were concentrated using Rotary evaporator at 45°C. The dried extract of ethyl acetate was used for the gas chromatography-mass spectrometry and column chromatography in the present study.

Sample preparation and silylation by derivatisation
The method of Fiehn*et al*.[4] was used for the sample preparation. Briefly, the ethyl acetate extract was dissolved in HPLC grade methanol to a concentration of 1 mg/mL. From the prepared concentration, 1 mL of ethyl acetate extract was incubated at 60ºC until completely dried. Derivatization by silylation was achieved by the addition of 50 µL of N-Methyl-N-tert-Butyldimethylsilyltrifluoroacetamide (MTBSTFA) and 50 µL of Dimethylformamide incubated for 1 h at 60ºC with steady shaking up to 4 times. It was then centrifuged at 4ºC for 1 minute and the supernatant was transferred into a clean GC-MS tube for analysis.[10]

Identification of constituents by GC-MS analysis using MTBSTFA as derivatizing reagent
The GC-MS (Agilent Technologies 7890 Inc., USA) was used for identification of compounds from the ethyl acetate extract of Dh tubers. The column size film thickness used was 30 m x 250 µm x 0.25 µm. The initial temperature was programmed at 60ºC for 1 min followed by 20ºC/min to 220ºC and then 2ºC/min to 310ºC for a total run of 57 min. Samples (1 µL), were injected using Agilent automatic liquid sampler. The injection was performed at a spiltless (Split ratio 100:1) mode temperature of 250ºC. The carrier gas was helium, with a static flow of 2 mL/min at a pressure of 11.446 psi. The mass scan range was 20-600 atomic mass units (amu).[11, 12]The MS was operated at ionization voltage of 70 eV. The metabolites in the active fractions were recognised by comparison with known spectra of compounds stored in the data base of National Institute of Standards and Technology (NIST) with MS library version 2011.

Fractionation and isolation
Column was packed using silica gel (70-230 mesh; 30 g) in n-hexane. About 7.0 g of the dried ethyl acetate extract of *D. hirtiflora* was chromatographed on the packed column (2.5 x 80 cm) and eluted with n-hexane containing increasing amount of ethyl acetate (up to 100%) and then with ethyl acetate/methanol in increasing amounts of methanol (up to 60%). Fractions (25-30 mL) were monitored and pooled using TLC on pre-coated silica gel 60G F_{254} plates (Merck) and visualized under UV lamp and spraying with 50% H_2SO_4 and heated at 110ºC. The 243 fractions were pooled into 12 fractions (A-L). Fraction I (1.50 g) was re-chromatographed on silica gel column and eluted with chloroform/methanol in increasing polarity (up to 60% methanol) to afford four sub-fractions (I-1, I-2, I-3 and I-4). Sub-fraction I-2 was subjected to preparative thin layer chromatography [chloroform (9): Methanol (1)] which led to the isolation of compound I-24.

Reverse phase-high performance liquid chromatographic Analysis of fractions and isolated compound
The twelve fractions (A-L) and isolated compound (I-24) were further monitored by RP-HPLC for chromatogram profiling of compound(s) present in comparison with the retention time of mixture of standard compounds at 25 µg/mL (quercetin, isovanillic acid, catechin, protocatechuic acid, gallic acid and ascorbic acid) as reported by Adeniran*et al*.[9] The standard and sample solutions were filtered prior to injection into HPLC for analysis. The methods reported by Seal[13] and Ahmadi-Sakha*et al.*[14] were used for the HPLC analyses of mixture of standards, fractions and the isolated compound.

DPPH radical scavenging activity assay
The free radical scavenging activity of the fractions and isolated compound were evaluated according to the method of Bursal and Gulcin,[15] with slight modifications. In brief, 50 µL methanol solution of test sample (standard-catechin, fractions A-L and isolated compound I-24) at concentrations ranging from 1.56 - 50 µg/mL were mixed separately with 200 µL (0.004%) of freshly prepared 1,1-diphenyl-2-picrylhydrazyl (DPPH) in a 96 well plate. In the control, 50 µL methanol replaced the test sample. The reaction mixtures were incubated at 27°C and allowed to react for 30 min in the dark. After 30 min, the absorbance was measured at 517 nm using a spectrophotometer (SpectraMax M3). The results were recorded in triplicates. The concentration of sample required to scavenge 50% of the DPPH free radical (IC_{50}) was calculated from a calibration curve by linear regression. The percentage of inhibition of DPPH (%) was calculated as follows:

$$\text{Percentage Inhibition} = \frac{Ab_c - Ab_{se}}{Ab_c} \times 100$$

Ab_c = Absorbance of DPPH without sample extract
Ab_{se} = Absorbance of sample extract/standard

Statistical analysis
Statistical analysis was performed using GraphPad prism (version 7.0). Statistical significance level was by one-way analysis of variance (ANOVA), followed by Tukey's Multiple Comparison test. Values of $p < 0.05$ were considered statistically significant. Results are expressed as mean of three replicates

Results and Discussion

The compounds identified by GC-MS from the ethyl acetate extracts of *Dioscoreahirtiflora* (Dh) using MTBSTFA as derivatizing reagent are presented in Table 1. Specifically, the presence of Propane-1,2,3-tris[(tert-butyldimethylsilyl)oxy]-Butanedioic acid, 2-[(tert-butyldimethylsilyl)oxy]- bis(tert-butyldimethylsilyl) ester (Succinic acid), Hexadecanoic acid, tert-butyldimethylsilyl ester (palmitic acid) and Octadecanoic acid, tert-butyldimethylsilyl ester (stearic acid) were observed in the EtOAc extract of Dh (Table 1). Phenolics present in the EtOAc extract of Dh include 3, 4-dihydroxybenzoic acid bis(tert-butyldimethylsilyl)ether tert-butyldimethylsilyl ester (protocatechuic acid), 4-Hydroxybenzoic acid tert-butyldimethylsilyl ether tert-butyldimethylsilyl ester (p-salicylic acid) and benzoic acid 2-(tert.-butyldimethylsilyloxy)-methyl ester (Table 1). The GC-MS of the derivatized ethyl acetate extract of Dh showed the presence of phenolics (benzoic acid, 4-hydroxybenzoic acid and 3,4-dihydroxybenzoic acid- protocatechuic acid), poly unsaturated and saturated fatty acids (hexadecanoic acid, octadecanoic acid, trans-9-octadecenoic acid, 9,12-octadecadienoic acid). The detected compounds from the ethyl acetate extract of Dh tuber have also been

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reported in the leaf and stem of *Dioscoreaalata* and *Dioscorearotundata* using GC-MS analysis.[16,17]

A total of 243 fractions (25 – 30 mL each) were collected from the column chromatography (CC) of the ethyl acetate extract of *D. hirtiflora*. After TLC analysis, twelve (12) fractions (A - L) were obtained. The antioxidant activities of fractions A – L are presented in Table 2. Fraction I with the highest antioxidant activity (IC_{50} = 2.11 µg/mL) was re-chromatographed over silica gel column eluted with chloroform:methanol in increasing polarity to afford four sub-fractions (I-1 to I-4) with sub-fraction I-2 having the highest antioxidant activity (IC_{50}= 4.67 ± 0.06 µg/mL), while the isolated compound I-24 had $IC_{50}$of 49.4 ± 2.18 µg/mL (Table 3). The HPLC chromatograms of the selected standards and fractions E, F, G, H, I and isolated compound (I-24) are presented in Figure 1.

The characteristic colour (pasty-sticky yellowish) of fractions A–D obtained from the column chromatography of ethyl acetate extract suggest the presence of fatty acids which were detected from the derivatized ethyl acetate extract of *Dioscoreahirtiflora* using GC-MS. Fractions A-D did not show antioxidant activity when reacted with DPPH at the tested concentrations. However, fractions E-L indicated varying antioxidant properties which could be partly attributed to the presence of ascorbic acid, gallic acid, protocatechuic acid, catechin, and quercetin as detected by the HPLC analysis of these fractions in comparison with the retention time from the standard mixtures. It is also worthy of note that these standard compounds were not detected in fractions A-D. The presence of protocatechuic acid as indicated in the HPLC chromatogram of fractions E-I supports its presence in the GC-MS derivatised ethyl acetate extract of *Dioscorea hirtiflora* where it was identified as 3, 4-dihydroxybenzoic acid. Specifically, fraction I with the highest antioxidant activity showed a prominent unidentified peak at 22.756 min in addition to the identified gallic acid, catechin and isovanillic acid from HPLC chromatogram. Hexadecanoic acid (palmitic acid), succinic acid, 3,4-dihydroxybenzoic acid (protocatechuic acid), vanillic acid and isovanillic acid identified via GC-MS and RP-HPLC from the ethyl acetate extract of Dh in the present investigation are in agreement with compounds previously identified from the family Dioscoreaceae.[7] Specifically, compounds reported in the present study have also been isolated from the ethyl acetate and n-butanol fractions of rhizhomes of *Dioscorea bulbifera*.[7] This justifies their chemotaxonomy of being in the same family. In our previous study, ethyl acetate extract had earlier showed antioxidant activity to DPPH with $IC_{50}$of 11.9 µg/mL.[8] The present varying antioxidant activities of fractions E-L obtained from the column chromatography could be attributed to the synergistic effect of the identified phenolic compounds and may have contributed to the antioxidant properties in the present study.

The isolated compound (I-24) was obtained as a white amorphous powder (11 mg) and identified as 3,5-dimethoxy-7,3′,4′-trihydroxy flavone (Figure 2). The TOF EIS-MS of compound I-24 displayed base peak $[M + Na]^+$ corresponding to molecular weight 353.0621. Its positive mode showed a molecular ion peak at m/z 331.0776 $[M + H]^+$(Calcd. 331.0818 for $C_{17}H_{14}O_7$). FTIR (υ_{max}) cm^{-1}: showed bands indicating hydroxyl (O-H), carbonyl (C=O) and aromatic (C=C) at 3405.1 cm^{-1}, 1728.8 cm^{-1} and 1572.6 cm^{-1}, respectively. These functional groups are known to stretch within a range, especially hydroxyl group which stretches between 3400-3000 cm^{-1}.

The ^{1}H-NMR spectrum (supplementary material, Table 4) indicated the presence of a trisubstituted B-ring by an ABX coupling system at δ_H 7.21 (d, *J* = 1.6 Hz), 7.31 (dd, *J* = 8.4, 1.6 Hz) and 6.68 (d, *J* = 8.4 Hz) for H-2′, H-6′and H-5′, respectively. In furtherance, spin coupling system of two meta-coupled proton signals at (δ_H 6.13 and 6.08, each δ, *J* = 2.5 Hz) were typified for H-8 and H-6, respectively in a tetrasubstituted A ring. Also, the presence of two methyl ether groups was indicated by two singlet signals at δ_H 3.60 and 3.77 ppm, each integrated for three protons (supplementary material). The confirmation of methyl ether at position C-5 of ring A was evidenced by the absence of a H-bonded hydroxyl signal at around 12.00 ppm or higher.

The ^{13}C-NMR spectrum (supplementary material, Table 4) indicated the presence of two methoxy groups (-OCH_3) at positions C-5 and C-3 was evidenced by signals at 55.6 and 59.1 ppm. Etherification at C-3 position was confirmed by the downfield signal of C-3 (~ + 3 ppm) relative to that of a C-3 substituted -OH and the presence of a downfield methyl ether at δ_C 59.5 ppm as compared to δ_C 59.7 ppm reported by Agrawal.[18] The ^{13}C-NMR spectrum also showed a carbonyl signal at δ_C (172.0 ppm). The ^{1}H- and ^{13}C-NMR data were consistent with a 3, 5-dimethoxy-3′, 4′-dihydroxyflavone.[19] It also agrees with isolated compound caryatin-3′-sulphate reported by Abdallah*et al.*[20] The signals obtained from ^{1}H- and ^{13}C-NMR, functional chemical properties from FTIR, molecular ion peak from TOFEIS-MS together with literature data, led to the characterization of compound I-24 as 3,5-dimethoxy-7,3′,4′-trihydroxyflavone (caryatin). This is an initial report of the isolation of 3, 5-dimethoxy-7, 3′, 4′-trihydroxyflavone from the tubers of *Dioscoreahirtiflora*.

Table 1: Compounds identified from the GC-MS analysis of ethyl acetate extract of *Dioscoreahirtiflora*tubers

S/N	Compounds	R_t	% Area
1	6-Azauracil, bis(tert-butyldimethylsilyl)	19.125	2.503547
2	Lactic acid	23.899	10.3359
3	Benzoic acid, 2-(tert.-butyldimethylsilyloxy)-, methyl ester	27.685	2.339567
4	Succinic acid	30.158	5.14309
5	Propane, 1,2,3-tris[(tert-butyldimethylsilyl)oxy]-	32.567	18.54321
6	Phosphoric acid, tris(tert-butyldimethylsilyl) ester	33.321	0.637211
7	4-Hydroxybenzoic acid, tert-butyldimethylsilyl ether, tert-butyldimethylsilyl ester	36.414	1.43127
8	Hexadecanoic acid, tert-butyldimethylsilyl ester	38.349	22.10316
9	9,12-Octadecadienoic acid (Z,Z)-, 2,3-bis[(trimethylsilyl)oxy]propyl ester	38.433	0.873051
10	9,12-Octadecadienoic acid, tert-butyldimethylsilyl ester	39.914	6.089217
11	trans-9-Octadecenoic acid, tert-butyldimethylsilyl ester	39.956	7.758961
12	Octadecanoic acid, tert-butyldimethylsilyl ester	40.181	10.87967
13	3,4-Dihydroxybenzoic acid, bis(tert-butyldimethylsilyl) ether, tert-butyldimethylsilyl ester	40.35	3.138075
14	3,11,20,21-Tetrakis[(trimethylsilyl)oxy]pregnane	41.362	3.648654
15	Zearalenone, bis(tert-butyldimethylsilyl) ether	49.178	4.575414

Rt – Retention time

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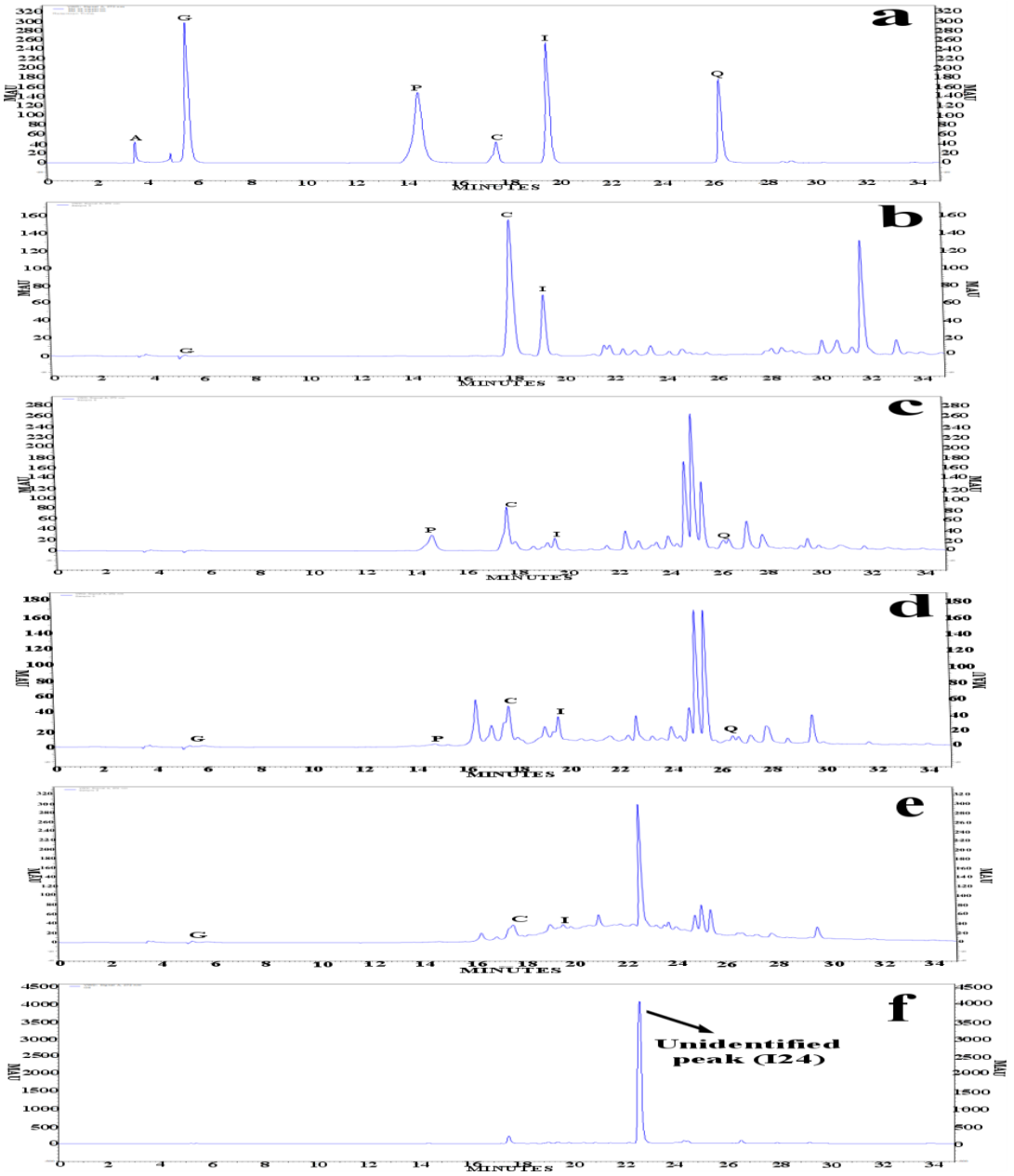

Figure 1: HPLC chromatogram profiling showing: (a) Mixture of six standards at 25 µg/mL (A-Ascorbic acid, G-Gallic acid, P-Protocatechuic acid, C-Catechin, I-Isovanillic acid, Q-Quercetin); (b)Fraction E; (c) Fraction F; (d) Fraction H; (e) Fraction I; (f) Isolated compound from fraction I.

The low antioxidant activity of compound I-24 (IC_{50} = 49.4 ± 2.18 µg/mL) isolated from fraction I could be attributed to the molecular structure in which positions 3 and 5 contain methoxyl group. The hydroxyl group at positions 3´, 4´ and 7 could have been responsible for the free radical scavenging activity observed in the present study by donating protons to the DPPH. Specifically, phenolic hydroxyl groups of flavonoids, especially 3-OH, 5-OH, and 3´-OH have been reported to play pivotal role in DPPH scavenging activity.[21] The structure of I-24- 3,5-dimethoxy-7, 3´, 4´-trihydroxy flavone (caryatin) clearly lacks hydroxyl group at positions 3 and 5, hence the low antioxidant activity observed could be attributed to the molecular structure.

However, in a study on the antitumour enhancing compounds from the rhizomes of *Dioscorea bulbifera*L., the 75% ethyl acetate soluble fraction of ethanol extract showed prevention against the tumour of JB6 mouse epidermal cells.[22]Studies on the constituents of the ethyl acetate fraction from the rhizomatous tubers indicated the presence of the following compound; kaempferol-3, 5-dimethyl ether, caryatin, catechin amongst others.[22] These compounds characterised as flavonoids with the two hydroxyl groups at C-7 and C-4´, showed remarkable anti-tumour promoting activity.[22] In the present investigation, catechin as well as caryatin isolated from *Dioscorea hirtiflora* tubers may also have antitumour properties. Generally, catechin has been reported as a flavonol antioxidant which possesses pharmacological properties such as antiviral, antidepressant and antitumour activities.[23,24] Similarly, the presence of catechin, caryatin, gallic acid and protocatechuic acid detected via RP-HPLC could also have played synergistic role in the antioxidant activities of the extract and fractions of *Dioscoreahirtiflora* tubers.

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Table 2: Antioxidant activity of fractions A – L of the ethyl acetate extract of *Dioscoreahirtiflora* tubers

Name of fraction/ Standard	Yield (mg)	Solvent system	Characteristic colour of fraction	DPPH scavenging activity (IC_{50} µg/mL)
A	151	hex (100%)	Pasty light yellow	ND
B	24	hex (100%)	Sticky light yellow	ND
C	9	hex(9): EtOAc(1)	Crystalline light yellow	ND
D	170	hex(9): EtOAc(1)	Sticky whitish yellow	ND
E	124	hex(7.5): EtOAc(2.5)	Yellow-whitish powder	48.30 ± 4.92^{d}
F	384	hex(1): EtOAc(1)	Sticky brownish	25.13 ± 0.63^{b}
G	141	hex(1): EtOAc(4)	Sticky brownish	21.97 ± 0.43^{b}
H	365	EtOAc (100%)	Sticky brownish	4.11 ± 0.20^{a}
I	3411	EtOAc(9): MeOH(1)	Crispy light brown	2.11 ± 0.09^{a}
J	102	EtOAc(7): MeOH(3)	Crispy brownish	5.83 ± 0.00^{a}
K	201	EtOAc(7): MeOH(3)	Crispy brownish	4.67 ± 0.44^{a}
L	17	EtOAc(7): MeOH(3)	Brownish	35.83 ± 1.77^{c}
Catechin	-	-	-	4.08 ± 0.16^{a}

ND- Not detected. Different alphabet represents significant difference at $P < 0.05$. Each value for the antioxidant activity represents Mean±S.E.M for three replicates.

Table 3: Antioxidant activity of sub-fractions I-1 to I-4

Sub-fractions from I/Compound/ Standard	Solvent system	Characteristic colour	Yield (mg)	DPPH scavenging activity (IC_{50} µg/mL)
I-1	CH_3Cl (9): MeOH (1)	Sticky whitish brown	151	ND
I-2	CH_3Cl (7): MeOH (3)	Crispy light brown	432	4.67 ± 0.06^{a}
I-3	CH_3Cl (5): MeOH (5)	Crispy dark brown	881	12.73 ± 0.06^{b}
I-4	CH_3Cl (4): MeOH (6)	Light brown	9	15.33 ± 0.35^{b}
I-24	-	White amorphous powder	11	49.4 ± 2.18^{c}
Catechin	-	-	-	4.08 ± 0.16^{a}

ND- Not detected. Different alphabet represents significant difference at $P < 0.05$. Each value for the antioxidant activity represents Mean±S.E.M for three replicates.

Table 4: NMR spectra data of compound I-24 (500 MHz, DMSO- *d*6)

Position	^{1}H (ppm) J (Hz)	^{13}C (ppm)
1	-	-
2	-	151.5
3	-	139.3
4	-	172.0
5	-	160.7
6	6.08, d (2.5)	95.4
7	-	160.5
8	6.13, d (2.5)	98.1
9	-	158.6
10	-	107.3
1´	-	121.3
2´	7.21, d (1.6)	114.8
3´	-	140.9
4´	-	145.9
5´	6.68, d (8.4)	115.9
6´	7.31, dd (8.4, 1.6)	119.9
5-OMe	3.77, S	55.6
3-OMe	3.60, S	59.1

d - doublet, dd- double of doublet, s - singlet

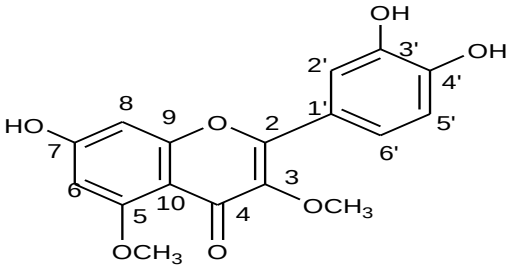

Figure 2: Chemical structure of 3,5-dimethoxy-7,3´,4´-trihydroxyflavone (caryatin)

Conclusion

The present study revealed the presence of phenolics, polyunsaturated and saturated fatty acids in the derivatized ethyl acetate extract of *Dioscorea hirtiflora*. Of the twelve fractions (A –L), fraction I had the highest antioxidant activity (IC_{50}= 2.11 µg/mL) while caryatin (3,5-dimethoxy-7, 3´, 4´-trihydroxy flavone) had the least antioxidant activity (IC_{50} = 49.4 µg/mL). The effect of these compounds as antioxidant could give credence to the traditional use *Dioscorea hirtiflora*tubers as food and medicine in South-western Nigeria.

Conflict of interest

The authors declare no conflicting interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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