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Original Research Article

Isolation and characterization of antioxidant constituents of the fruit of *Telfairia occidentalis* Hook F (Cucurbitaceae)

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Abstract

Purpose: *To evaluate the antioxidant property of the fruit of Telfairia occidentalis and isolate the components responsible for the antioxidant activity.*

Methods: *The fruit pericarp was macerated with methanol and the extract obtained successively partitioned with n-hexane, dichloromethane and ethyl acetate. The in vitro antioxidant activity of the extract and fractions was evaluated using 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging, reducing power, nitric oxide scavenging, total antioxidant and hydrogen peroxide scavenging assays. The n-hexane fraction, which had the highest DPPH scavenging and total antioxidant activities, was subjected to column and thin layer chromatography to isolate the components. The isolated compounds were identified by ultraviolet-visible (UV), nuclear magnetic resonance (NMR), Fourier transform infra-red (FTIR) spectroscopy and mass spectrometry.*

Results: *Among the fractions tested, n-hexane had the best total antioxidant activity of 99.44 % at 20 mg/ml ($p < 0.05$) compared to ascorbic acid at 99.71 % of 20 mg/ml. This fraction also had the highest DPPH radical scavenging activity of all the fractions ($p < 0.05$) at all test concentrations. For nitric oxide scavenging activity, the whole extract, and the chloroform and aqueous fractions exhibited activity ranging from 92.29 to 97.66 % compared to 98.93 % for ascorbic acid. The hydrogen peroxide scavenging activity of the extract and fractions ranged from 92.60 to 96.23 % compared with of the standard, ascorbic acid (101.68 %). The major components of the n-hexane fractions were α- amyrin and β-amyrin.*

Conclusion: *The fruit pericarp of Telfairia occidentalis possesses good DPPH radical scavenging activity. This is the first time the antioxidant activity of the fruit (pericarp) and the presence of α- and β-amyrins in Telfairia occidentalis have been reported.*

Keywords: *Telfairia occidentalis, Fluted pumpkin, Amyrin, Antioxidants*

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INTRODUCTION

Telfairia occidentalis is popularly known mainly because of its nutritionally valuable leaves [1]. The leaves have high nutritive value compared with other vegitables grown in the tropics. The seed is also edible. *Telfairia occidentalis* is popular among herbal practitioners. A review of the medicinal properties of the plant was written by Eseyin *et al* [2]. *Telfairia occidentalis* possesses antioxidant property [3].

Not much work has been done on the fruit pericarp of the plant which is usually discarded as it is often considered not useful. There is therefore no available report on the antioxidant activity or isolated component of the fruit pericarp of *Telfairia occidentalis*. Since the leaf and seed of *Telfairia occidentalis* have been found to possess significant antioxidant properties, the authors decided to screen the fruit pericarp for antioxidant property and isolate the antioxidant components of the pericarp.

EXPERIMENTAL

Plant collection and identification

The fruit of *Telfairia occidentalis* (Fluted pumpkin) was obtained from the medicinal plant farm of the Faculty of Pharmacy, University of Uyo, Akwa Ibom State, Nigeria in June 2015. The fruits were identified by Professor Margaret Bassey in the Department of Botany and Ecological Studies University of Uyo, Akwa Ibom State, Nigeria and it was assigned voucher number UUPH28 (d). A voucher specimen was kept in the faculty of Pharmacy, University of Uyo, herbarium.

Preparation and extraction of plant material

The freshly collected and identified fruits of *Telfairia occidentalis* were sliced opened and the pulp and the seed removed. The pericarp was chopped into small bits and air dried. Methanol (95 %, 5 litres) was poured into a container containing 500 g of the dried fruit material and macerated for 72 h at ambient temperature with intermittent shaking. The liquid extract was filtered and concentrated *in vacuo*. The extract was further dried in a desiccator containing self-indicating silica gel orange (Sigma-Aldrich, Germany).

Partitioning of the extract

The dried methanol extract (85 g) was dissolved in 500 mL of distilled water and partitioned successively with n-hexane, dichloromethane and ethyl acetate to obtain their respective fractions. Each of the fractions was concentrated in a rotary evaporator to dryness.

Evaluation of antioxidant properties

The DPPH antioxidant capacity of the extracts, vitamin C and E were evaluated by the method of Enujiugha [4]. A dose of 0.2 mL of the extracts was added to 3.8 mL ethanol solution of DPPH radical until a final concentration of 0.1 mM was obtained. The mixture was agitated vigorously for 1 min and left to stand at room temperature for 30 min. The absorbance of each sample (As) was measured on a UV spectrophotometer at 517 nm against ethanol blank. Negative control (A) was taken after adding DPPH solution to 0.2 mL of the extracts. DPPH scavenging activity (D) of the sample was calculated as in Eq 1.

$$D\ (\%) = \{(Ac - As)/Ac\} \quad \ldots\ldots\ldots\ldots\ (1)$$

where Ac and As are the absorbance of control and test compound, respectively.

The reducing power assay was estimated by the method of Athukorala *et al* [5]. 1.0 mL extract was mixed with 2.5 mL of phosphate buffer (20 mM, pH 6.6) and 2.5 mL of potassium ferricyanide (30 mM) and incubated at 50 ^{o}C for 20 minutes. Thereafter, 2.5 mL of trichloroacetic acid (600 mM) was added to the reaction mixture, centrifuged for 10 minutes at 3000 rpm. The upper layer of the solution (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of $FeCl_3$ (6 mM) and absorbance was measured at 700 nm. Nitric oxide (NO) scavenging activity was estimated by the method of Green *et al* [6]. Sodium nitroprusside (10 mM, 3 mL) in phosphate buffer was added to 2.0 mL of extract and reference compound in different concentrations (20 - 100 g/ml).

The resulting solutions were then incubated at 25 ^{o}C for 60 minutes. A similar procedure was repeated with methanol as blank, which served as control. To 2.0 mL of the incubated sample, 5.0 mL of Griess reagent (1 % sulphanilamide, 0.1 % naphthyethylene diamine dihydrochloride in 2 % H_3PO_3) was added and absorbance of the chromophore formed was measured at 540 nm. The total antioxidant activity was evaluated using the method of Lingnert [7]. Each extract (0.1-20 mg/ml) in water or ethanol (100 µl) was mixed with 2.0 mL of 10 mM linoleic acid emulsion in 0.2 M sodium phosphate buffer (pH 6.6) in a test tube and kept in the dark at 37 ^{o}C to accelerate oxidation. After incubation for 15 h, 0.1 mL from each tube was mixed with 7.0 mL of 80 % methanol in deionized water and the absorbance

of the mixture was measured at 234 nm against a blank in a spectrophotometer. Antioxidant activity (D) was calculated as as in Eq 2.

D (%) = {(Ac – As)/Ac} ……. (1)

where Ac and As are the absorbance of control and test compound, respectively.

Hydrogen peroxide scavenging activity was evaluated according to the method of Ruch *et al* [8]. A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (50 mM, pH 7.4). The concentration of hydrogen peroxide was determined by absorption at 230 nm using a spectrophotometer. Extract (20 - 60 g/mL) dissolved in distilled water was added to hydrogen peroxide and absorbance at 230 nm was determined after 10 min against a blank without hydrogen peroxide. Hydrogen peroxide scavenging H (%) was calculated as in Eq 3.

H (%) = {(Ac – As)/Ac} ……. (1)

where Ac and As are the absorbance of control and test compound, respectively.

Isolation and purification of compounds in n-hexane fraction

The dried n-hexane fraction (9.13 g) which had the highest DPPH and total antioxidant activities was subjected to open column chromatography (Rotaflo glass column (Quick-fit, England) measuring 3 x 80 cm; silica gel-G mesh 60 - 120 (Burgoyne, India). The flow rate of the eluent was kept constant at about 1 mL/min and the fractions were collected in 20 mL test tube. The sample was eluted using solvent systems that consisted of varying percent of two of the following solvents at a time in the specified order Pentane, n - hexane, $CHCl_3$, EtOAC and, MeOH to obtain 291 eluates. The eluates were monitored by TLC (silica gel; solvent system: pentane 10: n - hexane 9: ethyl acetate 1) and visualized using Ultraviolet lamp (366 nm) (Allen, London) and appropriate detecting reagents such as 1 % anisaldehyde in 10 % H_2SO_4, 5 % $FeCl_3$ and conc. H_2SO_4.

Eluates with same R_f values were pooled together to obtain fractions A, B, C, D, E, F and G. Fraction D (3500 mg) was further purified using open column chromatography (silica gel-G; 60 - 120 mesh). The fraction was separated using n-hexane and $CHCl_3$ (starting with 100 % n-hexane, varying ratio of the two solvents and 100 % $CHCl_3$) to obtain 40 eluates which were pooled together based on similarity in Rf values to obtain different fractions. Fraction D2 (eluate 12 – 36; 3300 mg) gave a spot tailing on TLC (silica gel; solvent system: pentane 10: n - hexane 5: ethyl acetate 1) under UV: 366 nm. Fraction D2 was purified further using Sephadex LH-20 (Pharmacia Fine Chemicals, Sweden) as the stationary phase while ethanol (100 %) was used to elute the fraction to obtain BEN- 4 (pure white compound, 1951 mg) which gave a single spot on TLC.

Fraction E (2570 mg) was also subjected to further purification using column chromatography with silica gel-G (60 – 120 mesh) using n - hexane (100%), mixtures of n-hexane and $CHCl_3$ (varying ratios), $CHCl_3$ (100%), different ratios of $CHCl_3$ and EtOAC, and EtOAC (100%). The fraction was separated using appropriate solvent systems to obtain four fractions. Eluate 1-48 gave Fraction E1 (one spot tailing, white amorphous, 1730 mg) which was subjected to further purification with column chromatography (Sephadex LH-20, solvent system: starting with 100% n - hexane, varying ratio of the two solvents and 100% $CHCl_3$) to obtain BEN-7 (1343 mg) which was a pure compound with R_f values of 0.38 (silica gel; solvent system: n-hexane 10: ethyl acetate 2), 0.74 (silica gel; solvent system: n-hexane 10: ethyl acetate 4), 0.40 (silica gel; solvent system: n-hexane 4: chloroform 8) and 0.54 (silica gel; solvent system: n-hexane 4: chloroform 10).

Spectroscopic analyses

Ultraviolet spectroscopy

The UV spectra of isolated compounds were obtained using a UV spectrophotometer (Unico UV-2100 Spectrophotometer, Shanghai Instruments Co, Ltd., China).

Nuclear magnetic resonance

The isolates where weighed (10 mg) and dissolved in deuterioted chloroform ($CDCl_3$). The solution was introduced into sample tubes, and was inserted into the NMR spectrometer (Bruker Avance AV 400, 400.033 MHz) equipped with a 5mm DUL 13C-1 z-gradient probe head. The machine was operated by means of a computer to obtain the different NMR spectra. The proton and Carbon NMR (including 2-dimensional Carbon NMR: COSY and NOESY) spectra were acquired.

Fourier transform infra-red spectroscopy (FTIR)

The isolates where weighed (10 mg) and dissolved in dichloromethane. The solution was

introduced into sample tubes, and was inserted into the infra-red spectrometer (JASCO 302-A) to obtain the IR spectra.

Mass spectrometry (MS)

The isolates were weighed (10 mg) and dissolved in dichloromethane. The solution was introduced into sample tubes, and was inserted into the mass spectrometer (ionization voltage 70 eV; JEOL JMS-600H). The machine was operated by means of a computer to obtain the MS spectra.

Statistical analysis

The data obtained are expressed as mean ± SEM and were subjected to one-way analysis of variance (ANOVA) using GraphPad Prism 5.01 (USA). Values of $p < 0.05$ were considered significant.

RESULTS

The yield of methanol extract, n - hexane, chloroform, ethyl acetate and aqueous fractions are 15.7, 11.87, 5.49, 26.60 and 51.63 %, respectively. There were significant variations in the antioxidant activity of the different fractions based on the method used. Some fractions showed very high antioxidant activity in one or more methods but less in other models. Table 1 shows the results for DPPH scavenging property of extract and fractions of the pericarp of *Telfairia occidentalis.* Table 2 and Table 3 show the results for reducing power and nitric oxide scavenging activities for extract and fractions, respectively, while those for hydrogen peroxide scavenging and total antioxidant activities of extract and fractions are depicted in Table 4 and Table 5, respectively. Table 6 displays the results for antioxidant activity of the isolated compounds based on DPPH-radical scavenging.

The n - hexane fraction which had the highest total antioxidant and DPPH-radical scavenging activities was purified further on column chromatography to obtain two compounds coded BEN-4 and BEN-7. From the proton NMR spectra, COSY, NOESY, MS and IR spectra, BEN-4 was identified as alpha-amyrin (1) and BEN-7 as beta-amyrin (2).

Table 1: DPPH-radical scavenging activity of extract and fractions of the fruit of *T. occidentalis*

Antioxidant	Antioxidant concentration (g/mL)				
	20	***40***	***60***	***80***	***100***
	Absorbance				
Vitamin C	1.194 ±0.689 (74.65%)	1.222 ± 0.706 (78.26%)	1.232 ± 0.711 (79.52%)	1.239 ± 0.715 (80.41%)	1.244 ± 0.718 (81.01%)
Extract	0.640 ± 0.370* (68.34%)	0.681 ± 0.393* (70.75%)	0.683 ± 0.394* (70.84%)	0.758 ± 0.438* (75.67%)	0.794 ± 0.458* (78.18%)
N-hexane	0.782 ± 0.451 (77.37%)	0.822 ± 0.475 (80.23%)	0.898 ± 0.518* (86.05%)	0.902 ± 0.521* (86.40%)	0.913 ± 0.527 (82.30%)
Ethyl acetate	0.374 ± 0.216* (52.26%)	0.390 ± 0.225* (56.80%)	0.514 ± 0.297* (61.72%)	0.628 ± 0.363* (67.62%)	0.645 ± 0.373* (68.60%)
Chloroform	0.535 ± 0.309* (53.58%)	0.510 ± 0.294* (45.62%)	0.326 ± 0.188* (45.26%)	0.307 ± 0.177 (38.37%)	0.283 ± 0.163* (37.21%)
Aqueous	0.634 ± 0.366* (67.98%)	0.553 ± 0.319* (63.69%)	0.457 ± 0.264* (55.01%)	0.089 ± 0.051 (49.64%)	0.647 ± 0.374* (31.31%)

Absorbance values are expressed as mean ± standard deviation; % scavenging activity is expressed in bracket. *Significant difference compared to ascorbic acid ($p < 0.05$)

Table 2: Reducing power activity of extract and fractions of the fruit of *T. occidentalis*

	Antioxidant concentration (g/mL)		
	10	***20***	***30***
Antioxidant	***Absorbance***		
Vitamin C	0.553 ± 0.319	1.866 ± 1.077	1.867 ± 1.078
Extract	0.500 ± 0.287*	0.583 ± 0.337*	0.587 ± 0.339*
N-hexane	0.540 ± 0.312	0.598 ± 0.345*	0.603 ± 0.348*
Ethyl acetate	0.520 ± 0.300	0.588 ± 0.339*	0.640 ± 0.370*
Chloroform	0.613 ± 0.354	0.607 ± 0.350*	0.567 ± 0.327*
Aqueous	0.580 ± 0.335	0.531 ± 0.307*	0.520 ± 0.300*

Absorbance values are expressed as mean ± standard deviation. *Significant difference compared to ascorbic acid ($p < 0.05$)

Table 3: Nitric oxide scavenging assay of extract and fractions of the fruit of *T. occidentalis*

	Antioxidant concentration (g/mL)		
	20	***40***	***60***
Antioxidant		***Absorbance***	
Vitamin C	1.671 ± 1.247 (98.93 %)	1.694 ± 0.978 (95.49 %)	1.694 ± 0.978 (95.19 %)
Extract	1.643 ± 0.949 (95.15 %)	1.642 ± 0.948 (95.08 %)	1.642± 0.948 (95.08 %)
N-hexane	1.666 ± 0.962 (96.39 %)	1.664 ± 0.961 (96.29 %)	1.644 ± 0.949 (95.22 %)
Ethyl acetate	1.675 ± 0.967 (96.89 %)	1.670 ± 0.930 (96.62 %)	1.667 ± 0.962 (96.49 %)
Chloroform	1.682 ± 0.971 (97.29 %)	1.680 ± 0.970 (97.19 %)	1.675 ± 0.967 (96.92 %)
Aqueous	1.688± 0.975 (97.66 %)	1.674 ± 0.966 (96.86 %)	1.670 ± 0.964 (96.62 %)

Absorbance values are expressed as mean ± standard deviation; % scavenging activity is expressed in bracket.
*Significant difference compared to ascorbic acid ($p < 0.05$)

Table 4: Hydrogen peroxide scavenging activity of the extract and fractions of the fruit of *T. occidentalis*

	Antioxidant concentration (g/mL)		
	20	***40***	***20***
Antioxidant		***Absorbance***	
Vitamin C	1.922 ± 1.110 (101.68 %)	1.897 ± 1.095 (99.09 %)	1.890 ± 1.091 (98.38 %)
Extract	1.817 ± 1.049* (95.46 %)	1.832 ± 1.058* (96.17 %)	1.840± 1.062 (96.59 %)
N-hexane	1.674 ± 0.966* (88.58 %)	1.718 ± 0.992* (90.61 %)	1.763 ± 1.018* (92.76 %)
Ethyl acetate	1. 759 ± 1.016* (92.60 %)	1.797 ± 1.037* (94.44 %)	1.860 ± 1.074 (97.61 %)
Chloroform	1.833 ± 1.058* (96.23 %)	1.843 ± 1.064* (96.72 %)	1.859 ± 1.073 (97.55 %)
Aqueous	1.804 ± 1.042* (94.77 %)	1.826 ± 1.054* (95.90 %)	1.828 ± 1.055 (96.01 %)

Absorbance values are expressed as mean ± standard deviation; % scavenging activity is expressed in bracket.
*Significant difference compared to ascorbic acid ($p < 0.05$)

Table 5: Total antioxidant assay of the extract and fractions of the fruit of *T. occidentalis*

	Antioxidant concentration (g/mL)				
	20	***40***	***60***	***80***	***100***
Antioxidant			***Absorbance***		
Vitamin C	1.861 ± 1.074 (99.71 %)	1.857 ± 1.072 (99.31 %)	1.847 ± 1.066 (98.27 %)	1.837 ± 1.061 (97.15 %)	1.825 ± 1.054 (95.88 %)
Extract	1.734 ± 1.001* (93.29 %)	1.727 ± 0.997* (92.92 %)	1.714± 0.990* (92.28 %)	1.699 ± 0.981* (91.59 %)	1.632 ± 0.969* (90.56 %)
N-hexane	1.757 ± 1.014* (94.44 %)	1.739 ± 1.004* (93.52 %)	1.731 ± 0.999* (93.15 %)	1.708 ± 0.986* (92.00 %)	1.704 ± 0.984* (91.79 %)
Ethyl acetate	1.692 ± 0.977* (91.22 %)	1.681 ± 0.971* (90.70 %)	1.663 ± 0.962* (89.81 %)	1.667 ± 0.962* (89.35 %)	1.633 ± 0.943* (88.45 %)
Chloroform	1.683 ± 0.972* (90.79 %)	1.671 ± 0.965* (90.21 %)	1.671 ± 0.965* (89.43 %)	1.642 ± 0.948* (88.80 %)	1.637 ± 0.945* (88.60 %)
Aqueous	1.700 ± 0.981* (91.59 %)	1.682 ± 0.971* ((90.73 %)	1.672 ± 0.965* (90.24 %)	1.651 ± 0.953* (89.26 %)	1.632 ± 0.942* (88.37 %)

Absorbance values are expressed as mean ± standard deviation. % scavenging activity is expressed in bracket.
* Significant difference compared to ascorbic acid ($p < 0.05$)

Table 6: DPPH-radical scavenging assay of the isolated compounds

	Antioxidant concentration (g/mL)				
	20	***40***	***60***	***80***	***100***
			Absorbance		
Antioxidant					
Vitamin C	0.060 ± 3.600 (94.77 %)	0.065 ± 3.594 (94.33 %)	0.070 ± 3.588 (93.90 %)	0.076 ± 3.581 (93.37 %)	0.080 ± 3.576 (93.03 %)
α-amyrin (BEN-4)	0.635 ± 2.896* (44.64 %)	0.656 ± 2.870* (42.81 %)	0.660 ± 2.866* (42.46 %)	0.663 ± 2.862* (42.20 %)	0.672 ± 2.851* (41.41 %)
β- amyrin (BEN-7)	0.613 ± 2.923* (46.56 %)	0.629 ± 2.904* (45.16 %)	0.632 ± 2.900* (44.90 %)	0.637 ± 2.885* (44.46 %)	0.641 ± 2.889* (44.12 %)

Absorbance values are expressed as mean ± standard deviation. % scavenging activity is expressed in bracket. * Significant difference compared to Ascorbic acid ($p < 0.05$)

Spectral data

BEN – 4 was obtained as a white solid amorphous compound, melting point 180 –186 ˚C; IR (Vmax, cm-1) 3342 (OH stretch), 2947 (CH_3, CH_2; asymmetric stretch) 1639 (C=C stretch) 1379 (CH_3, CH_2; symmetric bending), 1460 (CH_3, CH_2; asymmetric bending) 1035 (CO stretch). UV: λmax 500 nm (C = 0.263); EI-MS m/z, 426 (M+), 411 (M-CH_3), 218 ($C_{15}H_{32}$; base peak), 203 ($C_{16}H_{34}$), 189 ($C_{13}H_{28}$). H NMR diagnostic peaks occurred at 5.15 (IH, J = 3.6 Hz, H-12), 3.16 (1H, m, H-3). The H NMR analysis also showed the presence of 8 methyl groups corresponding to H-23 - H-30 assigned as follows 1.51 (H, d, J = 3.2; H-30), 1.12 (3H, s, H-26), 0.98 (3H, s, H-23), 0.95 (3H, s, H-25), 0.85 (3H, d, J = 3.0 Hz, H-29), 0.92 (3H, s, H-27), 0.81 (3H, s, H-28), 0.77 (3H, s, H-24).

In HMBC spectrum, the olefinic proton at δH 5.15 (H-12) was coupled to δC 145.0 (C-13) and δC 23.5 (C-11), this was supported by COSY coupling between δH 5.15 (H-12) and protons at δH 1.96 (H-11) to establish the presence of double bond at H-12. The peak at δH 3.16 assigned to H-3, also correlated with δC 79.2 (C-3) HSQC spectrum showing that the proton is attached to C-3. In HSQC spectrum the following correlations were also observed: δH 5.15 (H-12) to 122.0 (C-12), 0.73 (H-5) to 55.2 (C-5) 3.16 (H-3) to 79.2 (C-3). In the HMBC analysis the correlation between signal at δH 3.16, (H-3) with δC28.0 (C-2), δH 0.98(H-23) and 0.77 (H-24) with 55.4 (C-5) and 79.2 (C-3) confirmed the presence of a gem-dimethyl arrangement at C-4. In COSY analysis δH 3.16 (H-3) correlates with δH 1.66 (H-2), 5.15 (H-12) with 1.96 (H-11).

These NMR features are consistent with the established features of triterpenoid amyrins. The occurrence of a doublet at 2.0 (J = 4.4 Hz, H-18) indicated the presence of only one proton attached to H-19 which then coupled with the proton at H-18 to give the doublet at H-18. This implies that the other position on C-19 is occupied by CH_3¬presenting a vicinal dimethyl arrangement at H-19 and H-20. This suggests an α - amyrin structure for the isolated compound. A doublet occurs at H-18 in triterpenoids if structure is α - amyrin. The structure of α - amyrin is also confirmed by the presence of doublet at H-29 (J = 6.2 Hz) and H-30 (J = 7.0 Hz) with the molecular formula $C_{30}H_{50}O$ which is consistent with molecular ion peak at m/z 426.2 (M+) in EI-MS spectrum [10]. The chemical structure of α - amyrin (3β - hydroxy - urs – 12 – en – 3 - ol) is shown in Figure 1.

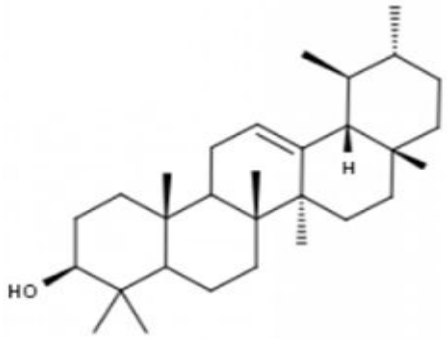

Figure 1: Structure of α-amyrin (3β – hydroxyl – urs – 12 – en – 3 - ol)

BEN –7 was obtained as a white amorphous compound, melting point 190-192 ˚C. The spectral data of BEN 7 resemble very closely that of BEN 04. IR (Vmax, cm-1) 3303 (OH str.) 2941 (CH_3, CH_2 str.) 2860 (CH_3, CH_2 asym str.) 1593 (C=C, str.), 1460 (CH_3, CH_2 sym. bend) 1035 C-O str. The IR analysis indicated the presence of one OH group and one olefinic bond in the molecule. UV: λmax 500 nm (c = 0.363); EI-MS m/z 426.3 (M+); 411 (M-CH_3), 218.1 ($C_{15}H_{32}$; base peak) 203.1 ($C_{16}H_{34}$), 189.1 ($C_{13}H_{28}$) consistent with fragmentation patterns of amyrin triterpenoid.

H-NMR spectrum is similar to that of Ben - 4 showing the presence of 8 methyl groups, a hydroxyl group and an olefinic bond. The signals were assigned as follows δH 5.15 (1H, t, J = 3.6 Hz, H-12), 3.19 (1H, t, J = 5.2 Hz, H-3), 1.53 (3H, s, H-30), 1.12 (3H, s, H-26), 0.98 (3H, s, H-23),

0.95 (3H, s, H-25), 0.92 (3H, s, H-27), 0.85 (3H, s, H-29), 0.81 (3H, s, H-28) and 0.77 (3H, s, H-24). In the HMBC analysis the olefinic proton at 5.15 (H-12) was linked in J13 coupling to 48.0 (C-9) and 41.5 (C-14) and J12 coupling to 24.9 (C-11), 1.12 (H-26) correlated with 41.5 (C-14) and 145.0 (C-13); 0.98 (H-23) with 18.6 (C-6), 39.9 (C-4), 55.2 (C-5), 79.0 (C-3) and 0.85 (H-29) with 23.9 (C-30), 47.8 (C-18); 0.81 (H-28) with 32.0 (C-17), 47.8 (C-18), 0.77 (H-24) with 55.2 (C-5) and 79.0 (C-3).

The correlation between H-11 and H-12 was supported by the COSY experiment which confirmed the position of the olefinic bond at position C-12 (δC 121.8). The COSY correlation between) 0.98 (H-23) and 0.77 (H-24) indicated that they are nearby groups. The HSQC correlations helped to assign the protons to carbon as follows: 0.98 (H-23) to 28.2 (C-23), 0.77 (H-24) to 15.5 (C-24), 1.12 (H-26) to 16.9 (C-26), 1.56 (H-9) to 47.7 (C-9), 3.19 (H-3) to 79.0 (C-3), 1.68 (H-1) to 38.7 (C-1), 0.73 (H-5) to 55.2 (C-5), 5.15 (H-12) to 121.8 (C-12). The occurrence of singlets at H-29 (0.85) and H-30 (1.53) and comparison with the published values of the spectroscopic data established the structure of Ben - 7 to be the β - amyrin with the formula $C_{30}H_{50}O$, molecular mass 426 (cacld calculated), confirmed by EI-MS molecular ion peak (M+) at m/z 426.3. The chemical structure of β - amyrin (3β – hydroxyl – olean – 12 – en – 3 - ol) is also depicted in Figure 2 and its melting point is 190-192 ^{o}C [11].

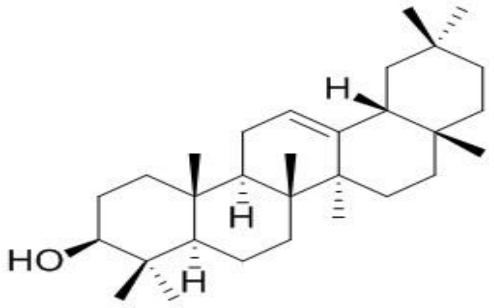

Figure 2: Structure of β-amyrin (3β – hydroxyl – olean – 12 – en – 3 - ol)

DISCUSSION

N - hexane had the highest DPPH inhibitory activity (Figure 1). The activity was comparable ($p < 0.05$) to that of the reference compound (Vitamin C). This correlates with the report of Nkereuwem *et al* [9] on the DPPH inhibiting activity of fractions of the leaf extract of *Telfairia occidentalis*.

None of the fractions had significant reducing power activity (Table 2). For Nitric Oxide (NO) radical scavenging model, the whole extract had the least activity of 95.15 % at 20 mg/mL while the chloroform and aqueous fraction had similar activity of 92.29 and 97.66 % at 20 mg/mL compared to ascorbic acid of 98.93 % at 20 mg/mL (Figure 3). Hydrogen peroxide (H_2O_2), although not very reactive, but it may be toxic to cell due to increase in hydroxyl radical concentration in the cells. Thus, removal of H_2O_2 as well as superoxide anion leads to survival of the cell life and its components. The scavenging ability of the extract (95.46 %), chloroform (96.23%), ethyl acetate (92.60 %) and aqueous (94.77 %) on hydrogen peroxide is comparable with that of the standard ascorbic acid (101.68 %) at 20 mg/mL (Figure 4). n - hexane had the best total antioxidant activity of 99.44 % at 20 mg/mL compared to the ascorbic acid at 99.71 % of 20 mg/mL.

Amyrins are abundant naturally occurring two isomeric pentacyclic triterpenoids which are isolated from the leaves and barks of plants. Plants from which the amyrins have been reported since 2008 to possess α-amyrin, β-amyrin and α, β-amyrin mixture in minor amounts include *Boswellia carterii*, *Populus euramericana*. The amyrins have been isolated from the n-hexane extract of *Melastoma malabathricum* L, *Pocirus trifoliate*, *Antiaris africana*, *Amelanchier alnifolia*, *Nelumbo nucifera Ficus cordata*, *Ficus cordata* and *Byrsonima crassa*.

Alpha- and β-amyrins showed *in vitro* and *in vivo* antihyperglycemic, hypolipidemic [12] and antioxidant [13] activities. Biological activities of the amyrins and the pharmacological potential of their modified products has been reviewed [14].

Supplementary data: Details on the antioxidant assays and results, FTIR, NMR and MS data are available from the corresponding author on request.

CONCLUSION

The findings of the present study indicate that the pericarp of *Telfairia occidentalis* is a potential source of natural antioxidants and a rich source of alpha- and beta- amyrins.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

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