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Phytochemical Analysis and Antioxidant Evaluation of Crude Extracts from the Roots, Stem and Leaves of *Dictyandraarborescens* (Welw.)

Uchechi E. Enenebeaku[1]*, Chidi E. Duru[2], Evangeline N. Okotcha[3], Odangowei I. Ogidi[4], Ifeyinwa C. Mgbemena[1], Harriet C. Nwigwe[1], Conrad K. Enenebeaku[5]

[1] *Department of Biotechnology, Federal University of Technology, Owerri, Nigeria*
[2]*Department of Chemistry, Imo State University, Owerri, Nigeria*
[3]*Department of Integrated Science, Federal College of Education (Technical) Asaba, Nigeria*
[4]*Department of Biochemistry, Federal Polytechnic, Ekowe, Bayelsa State, Nigeria*
[5]*Department of Chemistry, Federal University of Technology Owerri, Nigeria*

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ABSTRACT

Antioxidants are compounds which play important roles in the prevention of many diseases by removing free radical intermediates and inhibit other oxidation reactions by being oxidized themselves. *Dictyandra arborescens*, a tropical plant, is locally used by natives of Mbaise, Imo State, Nigeria for the treatment of various ailments. Phytochemical analysis and *in vitro* antioxidant activities of parts of the plant were evaluated. Gas chromatography flame-ionization detector (GC-FID) was used to determine the phytochemicals present in the leaves and roots while *in vitro* antioxidant potentials of extracts of the leaves, stem and roots were determined by their abilities to scavenge 2, 2-diphenyl-1- picrylhydrazyl (DPPH), nitric oxide (NO), and hydrogen peroxide radicals (H_2O_2). Ferric reducing antioxidant power (FRAP) and total antioxidant capacity were also determined using standard methods. GC-FID analysis of the leaves and roots identified phytochemicals such as; rutin, ribalinidine, naringin, flavan-3-ol, epihedrine, sapogenin, spartein, flavonones, epicatechin, phytate, oxalate, naringenin, resveratol, quinine, catechin, lunamarin, kaempferol etc. Scavenging of the extracts were concentration-dependent. Activities of the extracts for DPPH, nitric oxide and H_2O_2 differed significantly ($p<0.05$) from the standard antioxidant. Methanolic extract of *D. arborescens* roots showed the best reducing ability while methanolic extract of *D. arborescens*root also showed the highest antioxidant activity (2352.94 ±164.87 mg AA/g extract). Findings showed that extracts of the leaves, and roots of *D. arborescens* were rich in phytoconstituents which could serve as potential sources of natural antioxidants. Antioxidant and therapeutic activities of this plant could therefore be linked to the synergy between the various phytochemicals.

Keywords: *Dictyandra arborescens*, Gas-chromatography, Phytochemical, Antioxidants.

Introduction

Plants are sources of medicinal compounds, and many modern clinical drugs are from plant origin. The secondary metabolites in plants include alkaloids, saponins, phenols, tannins, terpenoids, flavonoids, etc[1]. These phytochemicals play essential roles in drug discovery, particularly in the pharmaceutical industry.[2] Due to the health benefits of these bioactive compounds, there has been increased interest and curiosity in health improvement and general well-being via the use of these natural products.[3] The renewed interest in plant-derived drugs is anchored on the belief that green medicine is safer than synthetic ones.[4] These natural products have been used for decades, either as herbs or spices, to improve aroma, taste, colour, flavour, and food nutrition. They are also patronized continuously for their medicinal values and preservative potentials[3]. In many developing countries, particularly Africa, people rely on these medicinal plants to treat different ailments.[5,6]

*Corresponding author. E mail: ucheodionye@yahoo.com
Tel+2348036515665

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Antioxidants are substances that prevent cells from being damaged by the effects of reactive oxygen species (ROS) known as free radicals. They have sufficient electrons, which they easily donate to electron-deficient compounds or molecules. These electron-deficient compounds have unpaired valence electrons.[7] In the bid to get more electrons, they may attack cells or biomolecules in the human body, resulting in many diseases.[7] The reactive oxygen species include peroxyl radicals, hydroxyl radicals, superoxide, and peroxynitrite, whose presence leads to oxidative stress and cellular damage.[8] Natural antioxidants serve to maintain good health and well-being and prevent certain chronic and degenerative diseases. Plant resources are major sources of natural antioxidants.

Dictyandra arborescens (Welw.), a wild plant belonging to the Rubiaceae family, is found in west tropical Africa. It is a shrub of about 8 m high with dry deciduous forest and a hard stem. It is locally called '*edo*' in Igbo language. As a result of their wide distribution, plants in this family have been used worldwide as ornaments and food.[9]Two popular members of this family are *Coffea camphora* (also called *Coffea robusta*) and *Coffea arabica* used in the production of coffee. The medicinal uses of *D. arborescens* are scarce and nearly unavailable in literature. However, in some local communities, such as Amuzi in Imo State Nigeria, traditional healers have affirmed that this plant's root and leaf extracts are used to treat malaria. In this study, the phytochemical composition of the crude extracts of the leaves and roots of *D. arborescens* and the *in vitro* antioxidant activities of the aqueous and methanolic extracts of its leaves stem, and roots were evaluated.

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Materials and Methods

Collection of plant materials

D. arborescens leaves, stem, and roots were collected in October 2019 from a fallow farmland in Amuzi community in Imo State, Nigeria. They were identified by Dr. C.M. Duru of the Department of Biological Sciences, Federal University of Technology, Owerri, Nigeria, with voucher number - FUTOH-2021/005. The plant parts were transported to the laboratory, washed, and air-dried at room temperature (30±0.5 ^{o}C) for two weeks to a constant weight. The plant materials were pulverized to a coarse powder using an electric blender (Kenwood-BL440A-UK), and a 2 mm sieve was thereafter used to obtain samples of uniform particle size.

Preparation of ethanol extract of D. arborescens

Ethanol extracts of the leaves and roots of *D. arborescens* were obtained using a soxhlet extraction method.[10] Exactly 200 g of the pulverized leaves and roots were carefully wrapped in a thimble, loaded in the soxhlet extractor, and extracted completely using redistilled ethanol. This extraction method was chosen because it allows for an exhaustive extraction and ensures that all the analytes are removed from the plant matrix. After the extraction, the solvent was separated from the extract using a rotary evaporator (RE-52A, Searchtech Instruments).

GC-FID analysis

GC-FID analysis and quantification of phytochemical components were carried out as described by Duru& Duru.[11]The phytochemicals in the crude extract were identified using a BUCK M910 Gas Chromatograph fitted with a flame ionization detector (GC-FID). All processed data were automatically compiled and reported through a data report function incorporated in the data station of the instrument. The reported data includes the name of the compound, retention time, and concentration.

Radical scavenging activity of extracts against 2,2-diphenyl-1-picryihydrazyl radical (DPPH)

The method of Lin & Quah[12] was used to determine the free radical scavenging activity of the plant extracts against 2,2-diphenyl-1-picryl-hydrazyl hydrate using various concentrations (0.5, 1.0, 1.5, 2.0, and 2.5 mg/mL) of the extracts. The concentrations were mixed vigorously in 5 ml of DPPH solution (0.1 mM in methanol) in test tubes. These were allowed to stand for 30 minutes in a dark chamber at room temperature. A standard solution of antioxidant (ascorbic acid) was treated in the same manner and incubated together with a reagent blank. At the end of the incubation process, the absorbance of the standard and extract was read with a spectrophotometer. Ability to decolourize DPPH was measured against blank at a wavelength of 517 nm. Percentage inhibition was then calculated thus:

$$\%\ \text{inhibition} = \frac{(Abs\ blank\ -\ Abs\ sample)\ \times 100}{Abs\ blank}$$

Where Abs blank is the absorbance of blank solution, and Abs sample is the absorbance of the sample.

Determination of nitric oxide scavenging activity

The methods of Panda *et al.* [13] and Alisi*et al.*[14] were used to measure the nitric oxide scavenging activities of 0.5, 1.0, 1.5, 2.0, and 2.5 mg/mL concentrations of the plant extracts. 4 mL of each concentration of the extract was added to 1 mL of sodium nitroprusside (SNP) solution (5 mM) in test tubes. The mixtures were incubated at 27 ^{o}C for 2 h. An aliquot (2 mL) of the incubated solution was taken and diluted with 1.2 mL of Griess reagent (1 % sulphanilamide in 5 % H_3PO_4 and 0.1 % naphthylethylenediamine-dihydrochloride). Absorbance of the chromophore, which was formed during diazotization of the nitrite with sulfanilamide and subsequent coupling with naphthylethylenediamine-dihydrochloride, was immediately read at 550 nm and compared with the absorbance of standard ascorbic acid.

$$\text{Nitric oxide scavenging activity}\ (\%) = \frac{Abs(control)\ -\ Abs(sample)}{Abs\ (control)} \times 100$$

Where Abs (control) is the absorbance of the control and Abs (sample) is the absorbance of the extract/standard.

Determination of hydrogen peroxide scavenging activity

This was determined according to Ngoda[15]. A solution of hydrogen peroxide (2 mmol/L) was prepared in phosphate buffer (pH 7.4). Different concentrations of the extracts (0.5, 1.0, 1.5, 2.0 and 2.5 mg/mL) were each added to hydrogen peroxide solution (0.6 mL) in test tubes. The absorbance of hydrogen peroxide at 230 nm was read after 10 min against a blank solution (only phosphate buffer) and compared with the standard (ascorbic acid). The hydrogen peroxide activity of the extracts was then calculated using the equation:

$$H_2O_2\ \text{activity}\ (\%) = \frac{Abs(control) - Abs(sample)}{Abs\ (control)} \times 100$$

Where, Abs (control) is the absorbance of the control and Abs (sample) is the absorbance of the extracts/standard.

Determination of reducing power

The reducing power of the plant extracts was determined using the ferric reducing antioxidant power (FRAP) assay as outlined by [16]. Various concentrations (0.5, 1.0, 1.5, 2.0, and 2.5 mg/mL) of the extracts were each added to 1 mL of deionized water, and were mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferrocyanide. The mixture was left to incubate at 50 ^{o}C for 20 min, and then 2.5 mL of 10 % trichloroacetic acid was added, and it was centrifuged at 3000 rpm for 10 min. Thereafter, 2.5 mL of the decant was mixed with 0.5 mL of 0.1 % $FeCl_3$. Absorbance was measured at 700 nm. The reducing power of the extracts was determined from the graph of optical density against the concentration of the extracts. Reducing power (RP 0.5_{AU}) was determined as the concentration that gave 0.5 absorbance reading.

Total antioxidant activity

The total antioxidant capacities of the extracts were evaluated according to the phosphomolybdenum method described by [17]. Aliquots of 0.3 mL of sample solutions (100 mg/mL in ethanol) were each combined in 4 mL vials with 3 mL of reagent solutions (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The vials were capped and incubated at 95 ^{o}C for 90 minutes in a water bath. Samples were cooled down at room temperature, and their absorbance was measured at 695 nm against the standard.

Statistical analysis

Data obtained for antioxidant activities were fitted into kinetic equations (2-5) which include Sigmoid a,b,c, logistic dose response a,b,c and a,b,c,d and Weibull cumulative a,b,c,d respectively using table curve 2D systat version 5.01, USA and Sigma plot 10.0 Systat USA. Significance level was set at $p > 0.05$.

Results and Discussion

The therapeutic effects of plants are due to a complex mixture of phytochemicals that possess varying biological activities.[18] The only known traditional medicine-based application of *D. arborescens* is the use of its leaf and root extracts in the treatment of malaria. The gas chromatograms of the phytochemicals extracts in the leaf and root of *D. arborescens*are shown in Figures 1 and 2. The identified phytochemicals from these plant parts are summarized in Tables 1 and 2. Most of the phytoconstituents present in the leaves of the plant were absent in the roots.Proanthocyanin, rutin, ribalinidine, naringin, quinine, flavan-3-ol, cathecin, anthocyanin, lunamarin, epihedrine, sapogenin, spartein, phenols, flavonones, steroids, epicatechin, kaempferol, phytate, flavones, oxalate, narigenin, resveratol, and tannins were identified in the leaves. In the roots, ribalinidine,

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naringin, quinine, cathechin, lunamarin, sapogenin, phenol, steroids, kaepmpferol, phytate, flavones, naringenin, and tannin were the prominent phytochemicals. Flavan-3-ol was the most abundant (24.502 %) phytochemical in the leaves, while cathechin was the most abundant (30.361 %) in the roots of this plant.

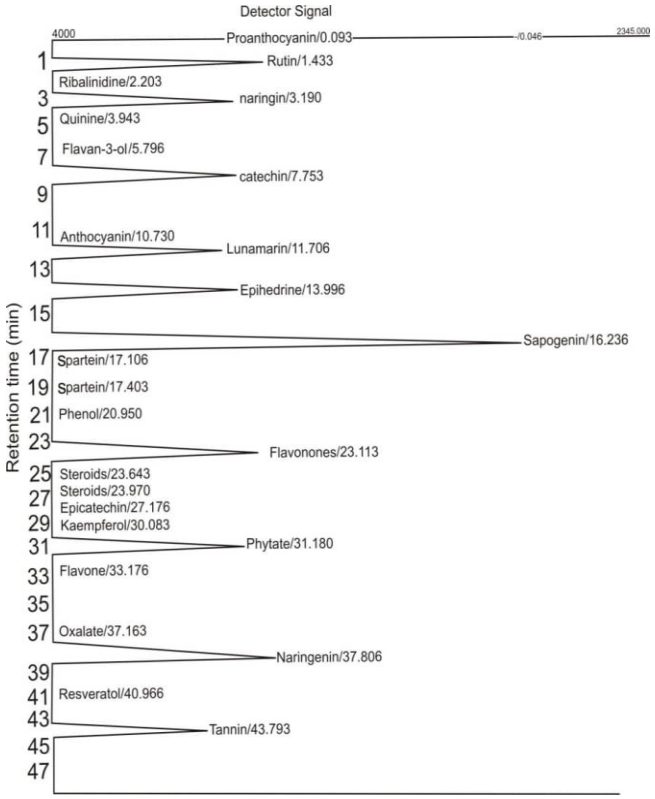

Figure 1: Chromatogram showing phytochemicals in the leaf extract of *D. arborescens*

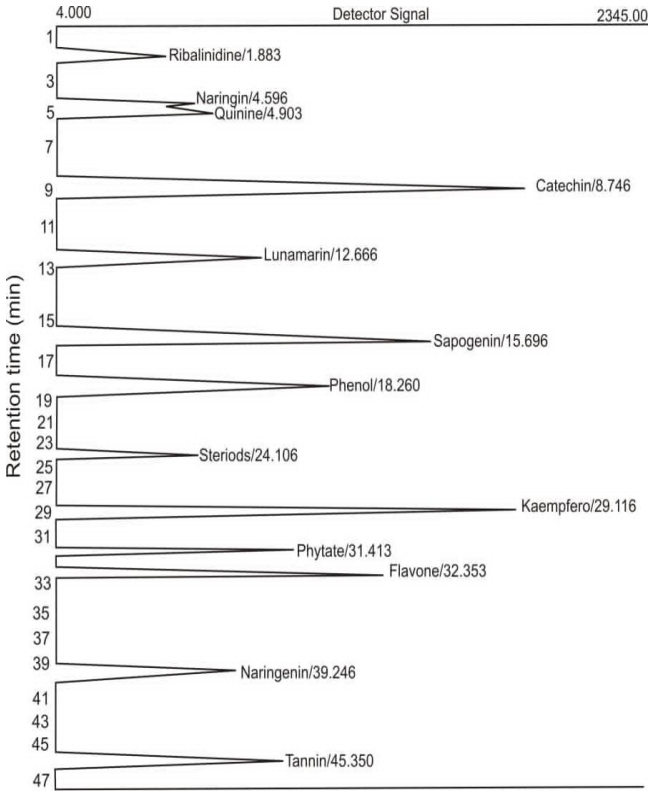

Figure 2: Chromatogram showing phytochemicals in the root extract of *D. arborescens*

Anthocyanins have been reported to play significant roles in managing heart-related diseases and cancer.[19]Ribalinidine, spartein, and lunamarine are quinoline alkaloids reported to have antimicrobial, anti-inflammatory, and antimalarial activities.[20,21] A report has shown that ribalinidine and lunamarine also possess antioxidant properties.[22]Catechin (the major phytoconstituent identified in the root of *D. arborescens*) is hemostatic [23]while epicatechin, has strong radical scavenging activity.[24] In the body, rutin is readily converted to an antioxidant (quercetin), with antimicrobial activity.[25] Studies have shown that kaempferol has antimicrobial activity [26-27]. Phytate or phytic acid is found in plant seeds, and they serve as the primary storage form of phosphorus in all grains, vegetables, and certain fruits.[28] Their antioxidant activities have also been reported.[29] Tannins are polyphenols with several therapeutic activities, including antioxidant, antibacterial, and antimalarial properties.[30]Naringenin is a flavourless, colourless flavone reported to have anti-cancer and cholesterol-lowering effects.[31,32] Quinine is an important antimalarial compound used in treating severe and uncomplicated malaria.[33] It was initially identified in the stem bark of the *cinchona* tree and used to treat malaria and diarrhea.[34,35] Quinine and other cinchona alkaloids such as quinidine, cinchonine, and cinchonidine are effective against malaria.[36] Quinine has fast schizonticidal activity against intra-erythrocytic malaria parasites.[37]

Table 1: Phytochemical components in the leaf extract of *D. arborescens*

Phytochemicals	Concentration (µg/mL)	% Composition
Proanthocyanin	7.38	1.87
Rutin	54.09	13.73
Ribalinidine	0.40	0.10
Naringin	32.23	8.18
Quinine	0.18	0.05
Flavan-3-ol	96.54	24.50
Catechin	31.71	8.05
Anthocyanin	4.65	1.18
Lunamarin	4.37	1.11
Epihedrine	12.98	3.29
Sapogenin	33.80	8.58
Spartein	16.89	4.29
Phenol	35.43	8.99
Flavonones	42.53	10.80
Steroids	0.30	0.08
Epicatechin	0.10	0.02
Kaempferol	0.11	0.03
Phytate	3.88	0.99
Flavones	5.16	1.31
Oxalate	0.19	0.05
Naringenin	2.28	0.58
Resveratol	3.78	0.96
Tannin	5.03	1.28

The therapeutic properties of plants are credited to the antioxidant compounds and phytochemicals present in medicinal plants.[38] Antioxidant compounds can chelate and bind free radicals into removable forms such that they can easily be excreted from the body. This property of antioxidant compounds is attributed to their redox properties, giving them the ability to act as reducing agents, electron donors, or metal chelators.[39] Extracts of the leaves, stem, and roots of

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D. arborescens showed appreciable antioxidant activities. The more the decolourization of DPPH, the more the reducing ability of the extracts.[40] Figure 3 shows the DPPH radical scavenging properties of crude aqueous and methanolic extracts of leaves, stem and roots of *D. arborescens* and ascorbic acid (AA) standard within the tested concentrations 0.5 mg/mL–2.5 mg/mL. Activities were dose-dependent as an increase in the concentration of extracts increased DPPH scavenging activities. Aqueous extract of *D. arborescens* root (DARA) exhibited the highest DPPH scavenging activity, with an inhibitory concentration (IC_{50}) value of 1.19 ± 0.08 mg/mL. Activities of the extracts were significantly ($p < 0.05$) lower than the standard antioxidant. Results obtained showed that DPPH radical scavenging activities of the aqueous and methanol extracts mainly were sigmoidal. Mathematical modeling of results is presented in Table 3.

Table 2: Phytochemical components in the root extract of *D. arborescens*

Phytochemicals	Concentration (µg/mL)	% Composition
Ribalinidine	1.24	0.99
Naringin	0.89	0.71
Quinine	1.73	1.38
Catechin	38.10	30.36
Lunamarin	17.36	13.83
Sapogenin	6.87	5.48
Phenol	26.47	21.10
Steroids	5.98	4.77
Kaempferol	6.34	5.05
Phytate	1.13	0.90
Flavones	6.22	4.96
Naringenin	0.73	0.58
Tannin	12.41	9.89

Nitric oxide (NO) is reported to be involved in many biological functions, such as neurotransmission, antimicrobial and antitumor activities, and vascular homeostasis. [41] Results obtained from this study show that crude aqueous and methanolic extracts of *D. arborescens* were able to inhibit nitrite formation by competing with oxygen to react with nitric oxide, which led to the reduction of nitrite concentration. Percentage inhibition of nitric oxide by the extracts was comparable to that recorded for ascorbic acid (standard). However, the activity of ascorbic acid was higher than those of the extracts against nitric oxide radical, with IC_{50} value of 0.65 ± 0.07 mg/mL. Nitric oxide radical scavenging activities were concentration-dependent (Figure 4). The highest activity, with inhibitory concentration of 0.72 ± 0.11 mg/mL was recorded for the aqueous extract of *D. arborescens* root. Figure 4 shows the nitric oxide radical scavenging activities of aqueous and methanolic extracts of the leaves, stem, and roots of *D. arborescens* and ascorbic acid (AA). Mathematical modeling of results is presented in Table 4. The results showed that nitric oxide radical scavenging activities of the extracts were sigmoidal, indicating that the crude aqueous and methanolic extract of the plant parts could inhibit nitrite formation by competing with oxygen to react with NO, which led to the reduction of nitrite concentration. Nitric oxide scavenging activities recorded in this study could be attributed to secondary metabolites present in the plant.

Hydrogen peroxide easily decomposes into oxygen and water, producing hydroxyl radicals that can initiate lipid peroxidation causing damage to DNA. Figure 5 represents hydrogen peroxide scavenging activities of the aqueous and methanol extracts of leaves, stem, and roots of *D. arborescens* with the standard antioxidant (ascorbic acid). Mathematical modeling of the results is presented in Table 5. The results obtained showed that the extracts inhibited H_2O_2 compared with ascorbic acid. Methanol extract of *D. arborescens* root recorded the highest activity (IC_{50} = 0.42 ± 0.00 mg/mL) at the highest concentration of 2.5 mg/mL. This differed significantly ($p < 0.05$) with the standard antioxidant (ascorbic acid) whose IC_{50} was 0.38 ± 0.01 mg/mL at the same concentration. IC_{50} values showed that hydrogen peroxide radical scavenging activity was in the order AA > DARM >DALM > DARA > DASM> DALA > DASA. From the results obtained, hydrogen peroxide radical scavenging activities of the extracts were largely sigmoidal while the standard antioxidant followed a logistic dose-dependent pattern. Both the aqueous and methanolic extracts from *D. arborescens* parts scavenged this compound.

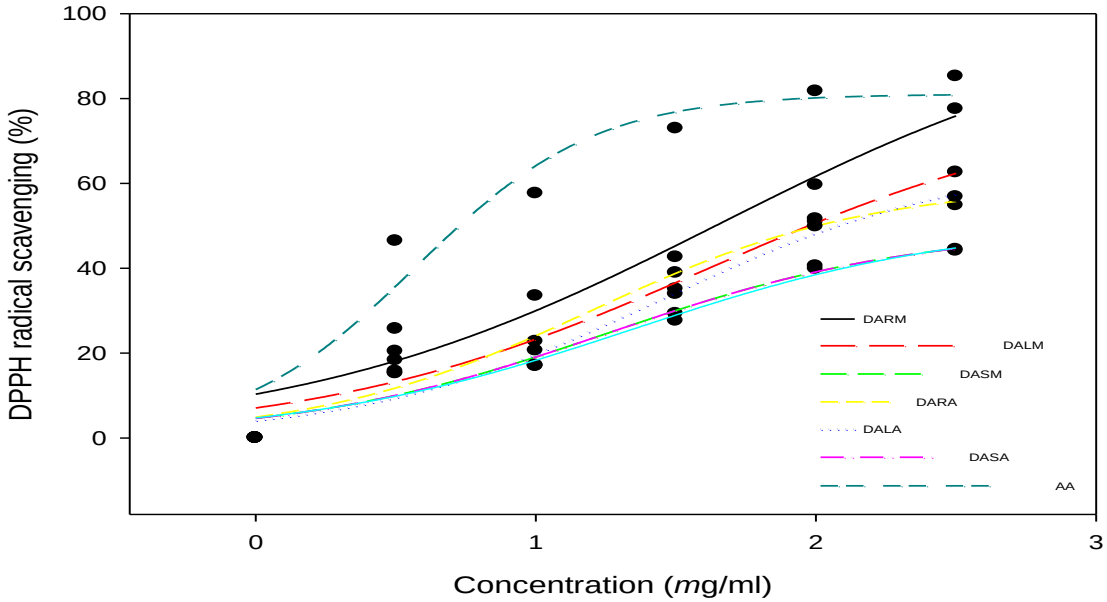

Figure 3: Effects of aqueous and methanol extracts of leaves, stem and roots of *D. arborescens* on scavenging of DPPH

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Table 3: Threshold Inhibitory concentration (IC_{50}) of aqueous and methanolic extracts of leaves, stem and roots of *D. arborescens* on DPPH radicals

Extracts	Threshold inhibitory concentration (*IC_{50})	Mathematical Model	Equation	R^2
DARM	1.66 ± 0.27	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.948
DALM	1.61 ± 0.19	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.959
DASM	1.26 ± 0.10	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.956
DARA	1.19 ± 0.08	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.964
DALA	1.45 ± 0.09	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.975
DASA	1.34 ± 0.12	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.959
AA	0.58 ± 0.05	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.934

* IC_{50} is the concentration of the extract or standard that scavenged 50% of the generated free radicals.
DARM= *D. arborescens* root methanol, **DALM** = *D.arborescens*leaves methanol, **DASM** = *D.arborescens* stem methanol, **DARA** =*D.arborescens* root aqueous, **DALA** = *D.arborescens* leaves aqueous, **DASA** = *D.arborescens* stem aqueous, AA =Ascorbic acid (standard)

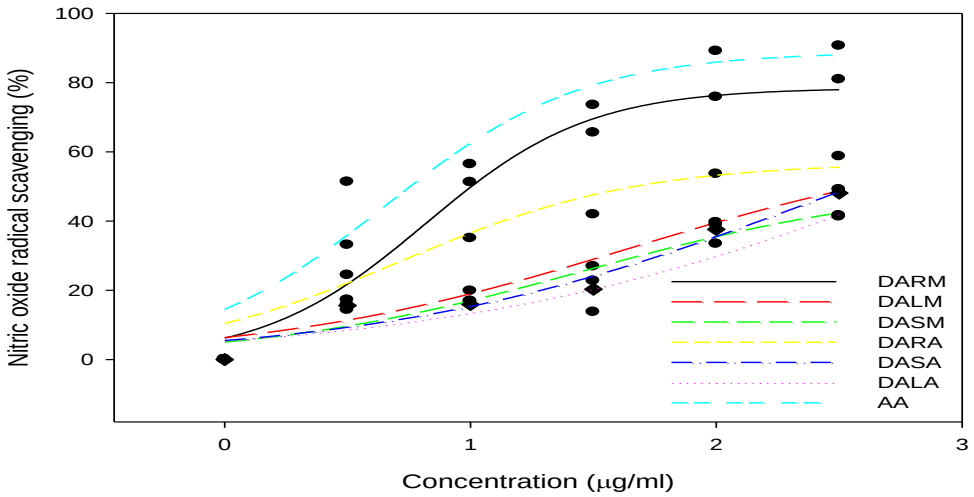

Figure 4: Nitric oxide radical scavenging activities of aqueous and methanol extracts of leaves, stem and roots of *D. arborescens*

The ferric reducing antioxidant power of crude aqueous and methanolic extracts of leaves, stem, and roots of *D. arborescens* and ascorbic acid is shown in Figure 6. Ascorbic acid was a more potent reducing agent, with $RP_{0.5}$ of 0.18±0.00 mg/mL. Results showed that root extracts of *D. arborescens* in aqueous and methanol solvents possessed reducing power activity comparable to the standard as they did not differ significantly ($p < 0.05$) from ascorbic acid. The data obtained fitted into logistic dose-response (a,b,c,d) and Logistic dose-response (a,b,c) models (Table 6). The reducing power of extracts indicates the presence of reducing agents whose atoms can donate electrons or react with free radicals and convert them into stable metabolites, terminating radical chain reactions. [42] The reductants present in the extracts promote the reduction of ferric ion (Fe^{3+}) to ferrous ion (Fe^{2+}).[43] As the concentration of Fe^{2+} increases, absorbance

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value increases, indicating the extract's electron-donating ability as recorded in this study. The plant extracts' possession of ferric reducing power indicates the ability of the bioactive components contained in the extracts to donate electrons. Many antioxidants and pharmacological agents used in treating diseases associated with oxidative stress have been reported to have high reducing power.[44]
Table 7 shows results of total antioxidant capacity (TAC) of crude aqueous and methanolic extracts of leaves, stem, and roots of *D. arborescens* expressed as mg AA/g extract. The highest value (2352.94 ± 164.87 mg AA/g extract) was recorded for methanolic extract of *D. arborescens* root. The activities of these extracts suggest that they have antioxidant properties, which could be compared to those of ascorbic acid used as a standard in this study. Concentration-dependent activity recorded in this study agrees with the report by [45] in evaluating the antioxidant activity of the secondary metabolites from *Piliostigma reticulatum*. It also corroborates the findings of [14] who reported free radical scavenging and *in vitro* antioxidant effects of ethanol extract of *Chromolaena odorata* Linn.

Table 4: Threshold Inhibitory concentration (IC_{50}) of aqueous and methanolic extracts of leaves, stem and roots of *D. arborescens* on nitric oxide radicals

Extracts	Threshold inhibitory concentration (*IC_{50})	Mathematical Model	Equation	R^2
DARM	0.82 ± 0.03	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.99
DALM	1.64 ± 0.26	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.95
DASM	1.46 ± 0.21	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.93
DARA	0.72 ± 0.11	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.87
DALA	3.1 ± 0.21	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.88
DASA	2.40 ± 0.32	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.94
AA	0.65 ± 0.07	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.91

* IC_{50} is the concentration of extract or standard that scavenged 50% of the generated free radicals.
DARM= D. arborescens root methanol, **DALM** = D.arborescens leaves methanol, **DASM** = D.arborescens stem methanol, **DARA** =D.arborescens root aqueous, **DALA** = D.arborescens leaves aqueous, **DASA** = D.arborescens stem aqueous, AA = Ascorbic acid (standard)

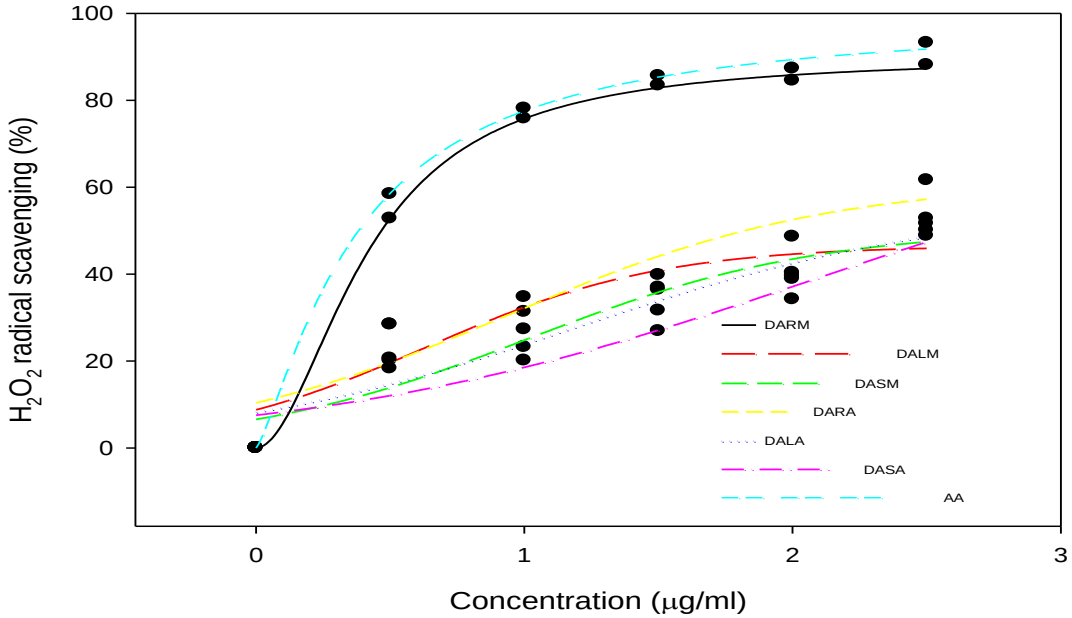

Figure 5: Hydrogen peroxide radical scavenging activities of aqueous and methanol extracts of leaves, stem and roots of *D. arborescens*

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Table 5: Threshold inhibitory concentration (IC_{50}) of aqueous and methanol extracts of *D. arborescens* on hydrogen peroxide radicals

Extracts	Threshold inhibitory concentration (IC_{50})	Mathematical Model	Equation	R^2
DARM	0.42 ± 0.00	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.999
DALM	0.64 ± 0.10	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.845
DASM	1.02 ± 0.11	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.931
DARA	0.94 ± 0.15	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.888
DALA	1.19 ± 0.21	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.910
DASA	2.08 ± 0.35	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.920
AA	0.38 ± 0.01	Logistic dose response (a,b,c)	$y = \frac{a}{1 + \left(\frac{x}{b}\right)^c}$	0.999

* IC_{50} is the concentration of extract or standard that scavenged 50% of the generated free radicals.
DARM= D. arborescens root methanol, **DALM** = D.arborescens leaves methanol, **DASM** = D. arborescens stem methanol, **DARA** =D. arborescens root aqueous, **DALA** = D. arborescens leaves aqueous, **DASA** = D. arborescens stem aqueous, AA = Ascorbic acid (standard)

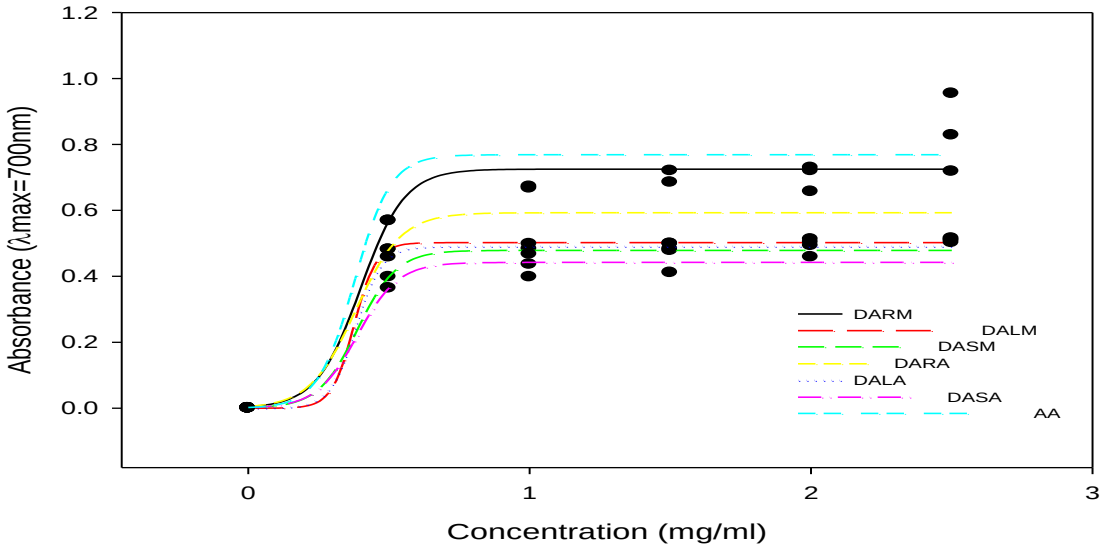

Figure 6: Ferric reducing antioxidant power (FRAP) of aqueous and methanolic extracts of leaves, stem and roots of *D. arborescens*

Table 6: Ferric reducing antioxidant power (FRAP) of aqueous and methanol extracts of leaves, stem and roots of *Dictyandra arborescens*

Extracts	Reducing power ($RP_{0.5}$)	Mathematical Model	Equation	R^2
DARM	0.30 ± 0.02	Logistic dose response (a,b,c,d)	$y = a + \frac{b}{1 + \left(\frac{x}{c}\right)^d}$	0.992
DALM	1.38 ± 0.16	Logistic dose response (a,b,c,d)	$y = a + \frac{b}{1 + \left(\frac{x}{c}\right)^d}$	0.999

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DASM	2.16 ± 0.05	Logistic dose response (a,b,c)	$y = \frac{a}{1 + \left(\frac{x}{b}\right)^c}$	0.999
DARA	0.84 ± 0.02	Logistic dose response (a,b,c)	$y = \frac{a}{1 + \left(\frac{x}{b}\right)^c}$	0.958
DALA	1.99 ± 0.09	Sigmoid (a,b,c)	$y = \frac{a}{1 + exp\left(-\left(\frac{x-x_0}{b}\right)\right)}$	0.994
DASA	2.82 ± 0.48	Logistic dose response (a,b,c)	$y = \frac{a}{1 + \left(\frac{x}{b}\right)^c}$	0.994
AA	0.18 ± 0.00	Logistic dose response (a,b,c)	$y = \frac{a}{1 + \left(\frac{x}{b}\right)^c}$	0.953

Table 7: Total antioxidant content (TAC) of aqueous and methanolic extracts of *D. arborescens*

Extract	TAC (mg AA /g extract)
DARM	2352.94 ± 164.87
DALM	1050.42 ± 20.61
DASM	1046.22 ± 37.78
DARA	1050.42 ± 13.74
DALA	920.17 ± 17.17
DASA	201.68 ± 13.74

DARM= D. arborescens root methanol, **DALM** = D.arborescens leaves methanol, **DASM** = D.arborescens stem methanol, **DARA** =D.arborescens root aqueous, **DALA** = D.arborescens leaves aqueous, **DASA** = D.arborescens stem aqueous

Conclusion

Phytochemical and antioxidant evaluation of crude extracts from the roots, stem, and leaves of *D. arborescens* (Welw.) was carried out. The findings showed that extracts of the leaves and roots of *D. arborescens* were rich in various phytochemicals, which could serve as potential sources of natural antioxidants that can prevent or slow down the progress of degenerative diseases. Furthermore being rich sources of secondary metabolites with antioxidant potentials, a synergy between these phytochemicals could be responsible for the therapeutic properties of this plant, justifying its use in ethnomedicine for the management of different ailments. This preclinical information on the leaves, stem, and roots of *D. arborescens* is of great importance given the plant's availability, scanty information in literature, and its use in folk medicine.

Conflict of Interest

The authors declare no conflict of 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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