ANTIRADICAL ACTIVITY OF 1,4-AND 1,2-BENZENE DICARBOXYL ESTERS FROM DEINBOLLIA PINNATA LEAVES

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Abstract
The family Sapindaceae are tropical and sub-tropical continental plants. In this study, 1,4-Benzene dicarboxyl ester (1) and 1,2-Benzene dicarboxyl ester (2) from methanolic extracts of Deinbollia pinnata leaves. The successful separation of the isomeric mixture is by freezeing the sub-fraction with methanol for seventy-two hours; followed by filtration, isolation and purification. The compounds were characterized spectroscopically (1H & 13C NMR, FTIR and GC-MS). The methanolic extracts showed good DPPH scavenging activity with percentage inhibition greater than 70% at 125 ppm, an excellent antioxidant activity towards ABTS assay with the IC50 value of 13.68 µg/mL and also exhibits most potent ferric ion reducer at 3.45 ± 1.30 mM FRAP equivalent compared to other extracts. The 1,4-Benzene dicarboxyl ester (1) displayed high antioxidant radical scavenging activity towards DPPH assay with IC50 value of (IC50 32.99; 73.491%) and 1,2-Benzene dicarboxyl ester (2) at (IC50 33.99; 71.11%);ABTS activity with SC50 value at 48.81µg/mL; 87.98% (1) and 49.55µg/mL; 83.85% (2) and a good potential as ferric ion reducer ranging from 0.05±0.00 to 1.37±0.02 (1) and 0.16±0.04 to 1.29±0.03 (2) mM FRAP equivalent respectively.

Keywords: Deinbollia pinnata, 1,4-and 1,2-benzene dicarboxyl esters, Phenolics, Antiradical activity

Introduction
Plants were known to have been created before mankind as their reliance; source of food, shelter, human health/ wellbeing and income inspiration. Their extracts serve as alternative health care dependent for more than 80% world population[1]. Herbal medicine is often used side by side with modern medicine, while herbal medicine has taking upper hand when the cost of modern medicine is beyond reach [2]. Great attention has been on the family Sapindaceae especially D. pinnata plant because of their often use as medication by most traditionalist for various diseases in Africa. It’s common name (water willow), in Yoruba (Ogiri-egba) and Egbo (Ekusi-oloko). The Roots and leaves of D. pinnata are used in folkloric medicine as remedy for febrifuge, analgesic, bronchiassintercostals, intestinal pains, jaundice, cough, asthma, and infections [3]. Leaf extracts is used in foetus positioning during child birth [4]. Identification of percentages of the volatile constituents [5] and the leaf essential oils for insecticidal activity were reported [6]. The root acts as antibacterial [3]. Isolated compounds include quercetin and stigmasterol [3], 4,8,12,16-tetramethylheptadecan-4-olide, squalene, Phytol palmitate, lupeol, taraxasterol, myristic acid, palmitic acid, campesterol, λ-sitosterol, stigmastan-3, 5-diene, stignasta-5,22-diene-3-ol acetate [7], characterization with antioxidation of pyrogallol methyl gallate, ethyl gallate, 3,4-dihydroxyl benzoic acid, catechol and scopoletin [8]. However, D. pinnata species phytochemicals have been exhorted despite the on-going research of the plant parts especially leaves and root. The elucidation of isomeric esters coincided with present search for virgin plant phytochemicals identification, effective microbial degradation and bioremediation [10], [11]. Plants have developed mechanisms of defence (antioxidant system) to neutralize oxidative stress, even in relatively small concentrations for body physiological actions. Antioxidant inhibits lipids oxidation, lowers autoxidation and assist in pero-oxidants removal. It furthers protect reactive photolysis from damaging cells membrane, disintegration and DNA mutation [12], [13]. Thus, phytochemicals which are naturally synthesized by plant were recognized as basis for traditional medicine [14]. More so,
due to massive consumption of *D. pinnata* extracts for the management of diseases, the isolation of more active compounds and their antioxidant evaluation with different methods (DPPH, FRAP and ABTS) become vital as investigated in this present study.

### Materials and Methods

The *D. pinnata* (Poir.) Schumach. & Thonn leaves were collected during flowering period, at dry season, early January for one week from Okehi Local Government Area of Kogi State, Nigeria. The plant part was identified and confirmed at the Biological Department, Federal College of Education Okene Kogi State by Mrs. Aniama S.O.A. a botanist. The plant material was authenticated at Forestry Research Institute of Nigeria Ibadan through comparison with the voucher specimen under the accession number FHI 3251 by Mr. Michael. The leaves were collected, washed and air dried at room temperature for one month.

#### Chemicals and Reagents

Solvents used were of general-purpose grade and the reagents used were of analytical grade. The solvents used were *n*-hexane (HEX), diethyl ether (DEE), dichloromethane (DCM), chloroform (CHCl₃), ethyl acetate (EtOAc), acetone (ACE), methanol (MeOH), Deuterated acetone (CD₃₂CO) and Deuterated chloroform (CDCl₃). TLC plates were sprayed with vanillin sulphuric acid reagent. Fehling’s A solution; Feh-ling’s B solution, boiling water; Benedict’s reagent; Molisch’s reagent; Biurrett reagent; dil./conc. H₂SO₄; benzene; dilute ammonia solution; FeCl₃ solution; 10% NaOH; distilled water; chloroform; dil./conc. HCl and Hager’s reagent; Mayer’s reagent; Dragendorff’s reagent; Wagner’s reagent; and lead acetate. 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2’-azino-bis-3-ethylbenzthiazolin-6-sulphonic acid (ABTS), (+) 6-hydroxy-2,5,7,8-tetramethoxychroman-2-carboxylic acid (trolox) and butylatedhydroxyanisole (BHA) pyrogallol, quercetin, butylated hydroxyl (BHT), toluene gallic acid, and ascorbic acid were obtained from Sigma-Aldrich. tripyridyl-s-triazine (TPTZ) and potassium persulfate were purchased from Fluka. Gravity column chromatography (CC) was carried out on Merck silica 60 (230-400 mesh size), VLC on Silica gel Merck silica 60 (70-230 mesh size) and TLC on 0.20 mm precoated gel aluminium plate (DC Kieselgel 60 F254). The ¹H and ¹³C of nuclear magnetic resonance spectra data were recorded on Bruker Avance AMX (400 MHz). UV spots were detected on UVI泰CE Cambridge CB4 IQB (254 nm and 365 nm). FTIR (Neat) analysis were conducted on Perkin-Elmer series 1600 FT-IR spectrophotometer. GC-MS analysis of isolates was performed on Agilent 7820A (G4350) instrument coupled with S9877E. The HP- 5MS column with dimension of 30 m × 0.25 μm × 0.25 μm was used. Initial temperature was 100°C, maintained for 10 min, while final temperature 300°C kept for 10 mins, pressure at 10.686 psi, septum purge flow at 3.5 mL/min, split ratio (26.8: 0.1) and split flow of 24.228 mL/min. Helium gas was used as a carrier gas with ionization energy of 70eV was maintained for MS detection. Mass spectral data were obtained from Mass Spectrometry Laboratory, National Institute of Standards and Technology. Melting points was determined using capillary tube on electrothermal 9100 apparatus.

### Extraction and Isolation

Powdered plant material of *D. pinnata* (Poir.) Schumach. & Thonn was extracted using Rufa-zah method [7]. Briefly, leaves sample was taken into several conical flasks (30 g each) and extracted three times with organic solvents (150 mL) such as *n*-hexane, dichloromethane, ethyl acetate, methanol in a sonicator using ultrasonic assisted extraction method at interval of ten minutes’ agitation, which was filtered into bottles and allowed for 24 hr; then filtered using Whatman No. 1. The filtrates were concentrated in vacuo at 40°C to obtained *n*-hexane (48.25 g, 3.22%), dichloromethane (10.01 g, 0.80%), ethyl acetate (20.91 g, 1.39%) and methanol (73.92 g, 4.93%). The methanolic extracts was further fractionated using VLC and fraction M001.
was subjected to CC to obtain pure compounds.

**Determination of Antioxidant Activity**

The ability of *D. pinnata* leaves extracts for inhibiting oxidants were evaluated. The antioxidant activity was determined using three assays, DPPH free radical scavenging, ferric reducing antioxidant potential (FRAP) and 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid (ABTS).

**2,2'-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) Free Radical Scavenging Assay**

The free radical scavenging assay was conducted based on method described in [15] with little changes. Briefly, 100 µM DPPH (1 mL) dissolved in MeOH was added to the MeOH solution (3 mL) of the tested samples. An equal volume of MeOH was added into the control test. The mixture was shaken vigorously and allowed to stand at room temperature for 30 min. Then the absorbance at 517 nm was measured with Epoch microplate reader. The percentage of scavenging of DPPH was calculated using the following equation: S% = (A<sub>blank</sub> - A<sub>sample</sub> / A<sub>blank</sub>) × 100; where A<sub>blank</sub> is the absorbance value of the control reaction (containing all reagents except the test compound) and A<sub>sample</sub> is the absorbance value of the test compound. The sample concentration providing 50% scavenging/inhibition (SC<sub>50</sub>/IC<sub>50</sub>) was calculated by plotting scavenging percentages against concentrations of the sample. All tests were carried out in triplicate and scavenging values were reported as means (scavenging percentage).

**2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulphonic acid (ABTS) Assay**

The ABTS assay was determined as described [16] with some modification. ABTS and potassium persulphate were dissolved with distilled water to obtain concentration for 7 mM and 4.9 mM, respectively. The two solutions were mixed and allow to stand in the dark for 12-16 hours at room temperature. After incubation time, the ABTS radical was added with distilled water until the absorbance is 0.7 at 734 nm. 10 µL of sample and 190 µL of ABTS solutions were added to the 96-well plates. The absorbance was recorded after 30 mins incubation in the dark at room temperature. The percentage of antioxidant activity was calculated using the following formula: Scavenging Percentage = Abs (ABTS)=Abs (ABTS+Sample)/ Abs (ABTS) × 100.

**Ferric Reducing Antioxidant Potential (FRAP) Assay**

The ferric reducing antioxidant potential (FRAP) assay was carried out according to [17] with little changes. FRAP reagent was freshly prepared, consist of stock solution with ratio 10:1:1 of 300 mM acetate buffer, 10 mM TPTZ in 40 mM HCl and 20 mM FeCl<sub>3</sub>,6H<sub>2</sub>O solutions. 5 µL of sample, 15 µL of MeOH and 150 µL of FRAP reagent were added to the 96well plates. The absorbance at 573 nm was measured after 10 min of incubation at 37°C. FeSO<sub>4</sub>.7H<sub>2</sub>O solution (0.06 mM - 1.0 mM) was used to build up calibration curves of standard antioxidant as displayed in Figure 7.

**Statistical Analysis**

The data obtained from biological activities were performed in triplicates and expressed as mean and mean ± standard deviation. The statistical analyses were carried out with one-way ANOVA (p < 0.05). A statistical package (Origin Lab version 2019b) was used for the data analysis.

**Results and Discussions**

The agita-sonication extraction yielded *n*-hexane (48.25 g, 3.22%), dichloromethane (10.01 g, 0.80%), ethyl acetate (20.91 g, 1.39%) and methanol (73.92 g, 4.93%) compared to residual yield from Soxhlet method; *n*-hexane (1.3 g, 0.09%), dichloromethane (0.3 g, 0.02%), ethyl acetate (0.7 g, 0.05%) and methanol (2.1 g, 0.14%) as shown in (Table 1).
Table 1: Samples Weight for various Solvents

| Solvents     | Sonication (g/%) | Soxhlet (g/%) |
|--------------|-----------------|--------------|
| n-hexane     | 48.25 (3.22)    | 1.3 (0.09)   |
| Dichloromethane | 10.01 (0.80)    | 0.3 (0.02)   |
| Ethyl acetate | 20.91 (1.39)    | 0.7 (0.05)   |
| Methanol     | 73.92 (4.93)    | 2.1 (0.14)   |

The adequacy for predicted manual agita-sonication extraction process yielded crude extracts ranges from 20.91g to 73.90 g and Soxhlet method at 0.3 g to 1.39 g. The small quantity of crude extracts obtained from the latter showed the former method as an improved process of extraction. Analyses of crude extracts from *D. pinnata* were evaluated for their antioxidant ability to inhibit free radicals as shown in figure 1. The MeOH extracts showed high significant inhibition towards DPPH radical compared to the other extracts with IC$_{50}$ value of 22.07 µg/mL (91.70 % at 125 µg/mL).

![Figure 1: DPPH Inhibitory Activity of *D. pinnata* Leaves Extracts](image1)

Crude Extracts with Control Standards

The *n*-hexane, dichloromethane and ethyl acetate showed percentage scavenging at 84.24%, 51.38% and 74.39%. methanol. The *n*-hexane and ethyl acetate extract showed a percentage value greater than 70% and dichloromethane with less than 70% as weak inhibitors. The dichloromethane displayed inactive DPPH radical scavenging activity with the (IC$_{50}$) more than 1000 µg/mL. The standard control is a known compound used to evaluate crude extracts and isolates for their potency and activity. Seven standard control were used to evaluate both crude extracts and isolated compounds. The control standards were pyrogallol, quercetin, trolox, butylated hydroxyl anisole, butylated hydroxyl toluene, (+)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, gallic acid, and ascorbic acid. All control standards showed very strong inhibition towards DPPH radicals except ascorbic acid with lower IC$_{50}$ of 105.7 µg/mL (66.83% at 125 µg/mL) in Figure 2 along with the plant extracts. The inhibition scavenging activity are in ascending order of pyrogallol > quercetin > trolox > BHT > BHA > gallic acid (IC$_{50}$; 1.70>7.01>11.40>22.22>25.58 and 31.01) with percentage inhibition of 97.57%; PYR, 96.73%; QUE, 95.85%; TRX, 91.76%; BHT, 89.56%; BHA and 79.58%; GA.A respectively.

![Figure 2: DPPH Inhibitory Activity of Plant Extracts](image2)
The methanol extracts displayed close inhibition scavenging similarity with both BHT and BHA. The isolated compounds (1) and (2) showed significant DPPH inhibitory activity at 73.49% (IC\textsubscript{50}; 32.99) and 71.11% (IC\textsubscript{50}; 33.99) as displayed in Figure. (3). All percentage scavenging results were considered at concentration 125 µg/mL.

The MeOH extracts reacted with ABTS radicals with IC\textsubscript{50} value at 15.41 µg/mL higher than BHA, BHT, gallic acid, ascorbic acid and trolox. The ABTS radical scavenging activity of D. pinnata leave extracts with standard control were displayed in Figure 4 with DPME taken the lead. The two compounds demonstrated reasonable significant activity towards ABTS radicals. The high scavenging activity for isolated compounds (1) at SC\textsubscript{50} 48.81µg/mL; 87.98% and (2) at SC\textsubscript{50} 49.55µg/mL; 83.85% depicted in Figure 3 and Table 2. The comparison of FRAP Activities for Standards Control (BHA, Gallic acid, Ascorbic Acid, Pyrogallol and Quercetin) along with crude extracts is presented in Figure 5. The methanol extracts showed (SC\textsubscript{50} µg/mL) at 15.41 and lest for n-hexane which was interrupted. Reducing power of the methanol plant extracts was determined on the basis of formation of coloured complex with potassium ferricyanide, trichloroacetic acid (TCA) and Ferric chloride (Fe\textsubscript{3}Cl\textsubscript{3}). The correlation analysis of FRAP values for total antioxidant capacity were calculated using ferric sulfate (FeSO\textsubscript{4}.7H\textsubscript{2}O) solution as depicted in Figure 6 with R\textsuperscript{2} of 0.994. The MeOH extracts displayed high significant reducing potential with 3.45 ± 1.30 mM FeSO\textsubscript{4} equivalent as reported from cucurbit fruits [18], and EtOAc extracts (DPEA) with reducing potential value of 3.33 ± 0.09 mM FeSO\textsubscript{4} equivalent similar to the report from Marantochloa leucantha extracts and fractions [19]. Higher antioxidant capabilities were displayed by the MeOH (DPME) and EtOAc (DPEA) extract. The 1,4-Benzene dicarboxyl ester (1) with FRAP equivalent value at 0.05 ± 0.00 to 1.37 ± 0.02 and 1,2-Benzene
dicarboxyl ester (2) at 0.16 ± 0.04 to 1.29 ± 0.03 as shown in Table 3. The three assays (DPPH, ABTS and FRAP) results supported each other’s method for effective *in-vitro* antioxidant investigation.

Table 2: 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) and 2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulphonic acid (ABTS) of Extracts and Isolated Compounds from *D. pinnata* leaves

| Extracts     | DPPH (IC$_{50}$ µg/mL) | ABTS (SC$_{50}$ µg/mL) |
|--------------|-------------------------|-------------------------|
| n-Hexane (DPH) | 605.4                   | INT                     |
| Dichloromethane (DPCM) | -                     | 123.3                   |
| Ethyl Acetate (DPEA) | 125.                  | 54.38                   |
| Ethanol (DPME) | 22.07                   | 15.41                   |

**Compounds**

| Extracts | DPPH (IC$_{50}$ µg/mL) | ABTS (SC$_{50}$ µg/mL) |
|----------|-------------------------|-------------------------|
| (1)      | 32.99                   | 439.5                   |
| (2)      | 33.39                   | 448.9                   |

**Positive Standards**

| Extracts     | DPPH (IC$_{50}$ µg/mL) | ABTS (SC$_{50}$ µg/mL) |
|--------------|-------------------------|-------------------------|
| BHA          | 25.758                  | 47.28                   |
| BHT          | 22.22                   | 27.60                   |
| Trolox       | 11.40                   | 41.14                   |
| Gallic A.    | 31.01                   | 17.83                   |
| Ascorbic A   | 105.70                  | 40.25                   |
| Pyrogallol   | 1.70                    | 12.81                   |
| Quercetin    | 6.95                    | 32.81                   |

The inhibition capacity was graded as IC$_{50}$ (µg/mL) of antioxidant activity $<$ 50 (very strong), 51-100 (strong), 101-150 (moderate), 151-200 (weak), 201-250 (very weak and $>$ 251 (inactive). All values expressed as mean ± SD values for three replicates experiment; P $<$ 0.05. Isolated compounds were: 1,4-Benzene dicarboxyl ester (1) and 1,2-Benzene dicarboxyl ester (2). DP = Deinbollia pinnata; ME = Methanol. Standard Control: BHT = Butyl hydroxyl toluene; BHA = Butyl hydroxyl anisole; TRX = (+)-6-Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid; GA. A. = Gallic acid; AS. A. = Ascorbic acid; PYR = Pyrogallol; QUE = Quercetin. INT = Interrupted.

Table 3: Ferric Reducing Antioxidant Power (FRAP) of Extracts and Isolated Compounds

| FRAP (mM equivalent to FeSO$_4$.7H$_2$O) |
|-----------------------------------------|
| Extracts                               | 0.06 mM | 0.12 mM | 0.25 mM | 0.50 mM | 1.0 mM |
|-----------------------------------------|---------|---------|---------|---------|--------|
| DPHE                                    | 0.15 ± 0.01 | 0.31 ± 0.01 | 0.61 ± 0.02 | 1.99 ± 0.04 | 2.54 ± 0.08 |
| DPEA                                    | 0.53 ± 0.01 | 0.91 ± 0.04 | 1.79 ± 0.14 | 3.04 ± 0.16 | 3.33 ± 0.09 |
| DPME                                    | 0.83 ± 0.12 | 1.38 ± 0.18 | 2.26 ± 0.24 | 3.72 ± 0.82 | 3.45 ± 1.30 |

**Compounds**

| Extracts | 0.06 mM | 0.12 mM | 0.25 mM | 0.50 mM | 1.0 mM |
|----------|---------|---------|---------|---------|--------|
| (1)      | 0.05 ± 0.00 | 0.06 ± 0.00 | 0.13 ± 0.00 | 0.84 ± 0.01 | 1.37 ± 0.02 |
| (2)      | 0.16 ± 0.04 | 0.75 ± 0.04 | 0.77 ± 0.03 | 1.20 ± 0.03 | 1.29 ± 0.03 |

**Standard Control**

| Extracts | 0.06 mM | 0.12 mM | 0.25 mM | 0.50 mM | 1.0 mM |
|----------|---------|---------|---------|---------|--------|
| TRX      | 0.39 ± 0.06 | 0.65 ± 0.07 | 1.40 ± 0.11 | 2.15 ± 0.13 | 2.23 ± 0.12 |
| BHA      | 0.40 ± 0.12 | 0.74 ± 0.11 | 1.27 ± 0.07 | 2.36 ± 0.06 | 2.74 ± 0.11 |
| AS. A.   | 0.20 ± 0.03 | 0.89 ± 0.03 | 1.96 ± 0.12 | 2.98 ± 0.12 | 2.87 ± 0.03 |
| BHT      | 0.45 ± 0.02 | 0.83 ± 0.01 | 1.51 ± 0.02 | 2.50 ± 0.05 | 2.93 ± 0.18 |
| GA. A.   | 1.02 ± 0.03 | 1.88 ± 0.06 | 2.68 ± 0.12 | 2.70 ± 0.07 | 2.83 ± 0.02 |
| QUE      | 1.14 ± 0.05 | 2.10 ± 0.10 | 2.87 ± 0.06 | 2.95 ± 0.14 | 3.11 ± 0.05 |
| PYRO     | 0.84 ± 0.06 | 1.63 ± 0.12 | 2.26 ± 0.19 | 2.44 ± 0.21 | 3.07 ± 1.44 |
The ferric sulfate graph expressed as mM FeSO₄ equivalent with linearity over the calibration range with R² value of 0.9940. All values expressed as mean ± SD values for three replicates experiment; P<0.05. Isolated compounds are: 1,4-Benzene dicarboxyl ester (1) and 1,2-Benzene dicarboxyl ester (2). DP = Deinbollia pinnata; ME = Methanol. Standard Control: BHT = Butyl hydroxyl toluene; BHA = Butyl hydroxyl anisole; TRX = (+)-6-Hydroxy-2,5,7,8-tetramethylochromane-2-carboxylic acid; GA. A. = Gallic acid; AS. A. = Ascorbic acid; PYR = Pyrogallol; QUE = Quercetin.

The methanolic extracts afforded all isolated compounds. Compound (1) was obtained as a light-yellow oil; Rf = 0.50 in Hex: EtOAc (2:3) and its molecular formula was determined as C₂₄H₃₈O₄. The IR spectrum exhibit absorption bands for both compounds (1) and (2); carbonyl ester at 1726 cm⁻¹ (C=O), 2925 cm⁻¹ (C-H), 1121 cm⁻¹ (C-O) and aromatic (C-H) at 3010 cm⁻¹. ¹H NMR spectrum showed four aromatic protons at δH 8.11 (2H, s, H-11, H-11′ / 2H, s, H-12, H-12′). Two oxy-methylene protons at δH 4.26 (2H, d, J = 5.6 Hz, H-1′); four methylene proton at δH 1.33 (2H, m, H-2, H-4'), δH 1.33 (2H, m, H-5'), δH 1.33 (2H, m, H-7), δH 1.30 (2H, m, H-3'); six methyl proton at δH 0.99 (3H, t, J = 7.2 Hz, H-6) and δH 0.92 (3H, t, H-8').

The ¹³C NMR and DEPT spectra of (1) and (2) revealed signals of 12 carbons symmetrically depicted in Table 3 and 4. Two carbonyl carbons atom (δC 165.9), two non-protonated carbons at (δC 134.2) and four accumulated methine (δC 129.7) as showed in (Table 2). ¹H-H COSY and HMQC spectra enabled the identification of ¹H and ¹³C signals at various positions of methyl, methylene and methine. The gas chromatography-mass spectroscopy
(GC-MS) revealed \( m/z \) at 390.5 \([M^+]\) confirmed theirs as isomers as displayed in (C\(_{24}\)H\(_{38}\)O\(_4\)) (1) while at \( m/z \) 390.2 (2) Figure 7-14.

![Figure 9: FTIR Spectrum of 1,4-Benzene dicarboxyl ester (1)](image)

![Figure 10. GC-MS Spectrum of 1,4-Benzene dicarboxyl ester (1)](image)

| Table 4: NMR Spectra Data of Isomeric esters (1) and (2) (\( \delta \) in ppm) |
|-----------------|-----------------|-----------------|-----------------|
| No  | Type | Cpd (1) | Cpd (2) | Cpd (1) | Cpd (2) |
|-----|------|---------|---------|---------|---------|
| 1, 4 | C    | 134.2   | -       | -       | -       |
| 1, 6 | C    | -       | 130.8   | -       |         |
| 2, 5 | CH   | -       | 128.7   | 7.72    | (2H, dd, \( J = 3.2 \) Hz) |
| 2, 6 | CH   | 129.4   | 8.11 (2H, s) | -       |         |
| 3, 4 | CH   | -       | 132.4   | 7.55 (2H, m, \( J = 3.6 \) Hz) |
| 3, 5 | CH   | 129.4   | 8.11 (2H, s) | -       |         |
| 7, 8 | C    | 165.9   | -       | 167.7   | -       |
| 1'   | CH\(_2\) | 67.7 | 4.26 (2H, m) | 67.1 | 4.24 (2H, m) |
| 2'   | CH   | 38.9 | 1.78 (1H, m, \( J = 6.4 \) Hz) | 38.7 | 1.70 (1H, dd, \( J = 6.4 \) Hz) |
| 3'   | CH\(_2\) | 30.5 | 1.30 (4H, m) | 30.8 | 1.29 (4H, m) |
| 4'   | CH\(_2\) | 30.8 | 1.33 (4H, m) | 28.9 | 1.34 (4H, m) |
| 5'   | CH\(_2\) | 24.0 | 1.33 (4H, m) | 22.9 | 1.33 (4H, m) |
| 6'   | CH\(_3\) | 14.0 | 0.99 (6H, \( t, J = 7.2 \) Hz) | 14.0 | 0.96 (6H, \( t, J = 7.2 \) Hz) |
| 7'   | CH\(_2\) | 22.9 | 1.27 (4H, m) | 23.7 | 1.26 (4H, m) |
| 8'   | CH\(_3\) | 14.0 | 0.92 (6H, \( t \)) | 10.9 | 0.95 (6H, \( t \)) |

No = Carbon Numbering. \(^a\) Experimental Data Measured at 400 MHz in CDCl\(_3\)
Figure 11: $^1$H NMR Spectrum of 1,2-Benzene dicarboxyl ester (2)

Figure 12: $^{13}$C NMR Spectrum of 1,2-Benzene dicarboxyl ester (2)

Figure 13: FTIR Spectrum of 1,2-Benzene dicarboxyl ester (2)
Figure 14: GC-MS Spectrum of 1,2-Benzene dicarboxyl ester (2)

Table 5: NMR Spectra Data of Compound (2) and Di(2-ethylhexyl) phthalate[20] (δ in ppm)

| No | Type | δ_C-2 | δ_H(mult. J in Hz) | δ_C-Di | δ_H(mult. J in Hz) |
|----|------|-------|-------------------|--------|-------------------|
| 1, 6 | C | 130.8 | - | - | - |
| 2.5 | CH | 128.7 | 7.72 (2H, dd, J = 3.2 Hz) | 129.1 | 7.70 (2H, dd, 5.6 Hz, 3.3Hz, s) |
| 3, 4 | CH | 132.4 | 7.55 (2H, m, J = 3.6 Hz) | 131.3 | 7.52(2H, dd, J = 5.68, 3.3Hz) |
| 7, 8 | C | 167.7 | - | - | - |
| 1' | CH₂ | 67.1 | 4.24 (2H, dd, J = 5.6 Hz) | 68.5 | 4.20 (2H, dd, J = 6.12 Hz) |
| 2' | CH | 38.7 | 1.70 (1H, dd, J = 6.4 Hz) | 39.1 | 1.67 (2H, m) |
| 3' | CH₂ | 30.8 | 1.29 (4H, m) | 30.7 | 1.35 (4H, m) |
| 4' | CH₂ | 28.9 | 1.34 (4H, m) | 29.3 | 1.32 (4H, m) |
| 5' | CH₂ | 22.9 | 1.33 (4H, m) | 23.3 | 1.39 (4H, m) |
| 6' | CH₃ | 14.0 | 0.96 (6H, t, J = 7.2 Hz) | 14.4 | 0.89 (6H, t, J = 6.84 Hz) |
| 7' | CH₂ | 23.7 | 1.26 (4H, m) | 24.1 | 1.46 (4H, m) |
| 8' | CH₃ | 10.9 | 0.95 (6H, t, J = 7.48 Hz) | 11.3 | 0.92 (6H, t, J = 7.48 Hz) |

No = Carbon Numbering, Type of Carbons, a Experimental Data Measured at 400 MHz in CDCl₃, b Experimental Data Measured at 400 MHz in CDCl₃.

Conclusion

Plants are known to contain secondary metabolites for their survival, growth, development and protection from a broad spectrum of pathogens. The economic rationale behind extraction, antiradical activity, isolation and structural characterization of phytochemicals has led to the first report of 1,4-Benzene dicarboxyl ester (1) and 1,2-Benzene dicarboxyl ester (2) from D. pinnata leaves. Their antiradical evaluations also support traditional uses of the plant in herbal medicine for the management of numerous oxidant mediated diseases.

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References

[1] WHO, “Traditional Medicine – Growing Needs and Potential (2002),” WHO Policy Perspect. Med., 2, 1–6.
[2] K. Busia, “Medical Provision in Africa – Past and Present” (2005). Phyther. Res., 19, 919–923.
[3] A. A. Lasisi, M. O. Bamidele, S. Balogun, and S. A. Adebisi (2016), “Chemical Constituents and Antibacterial Evaluation of Deinbollia pinnata ( Schumand Thonn) Sapindaceae,” Pacific J. Sci. Technol.,
17(1), 183–199.

[4] Borokini, T. I., Ighere, D. A., Clement, M. T., Ajiboye, and Alowonle, A. (2013). “Ethnobiological Survey of Traditional Medicine Practice for The Treatment of Piles and Diabetes Mellitus in Oyo State,” *J. Med. Plants Stud.*, 1(5), 30–40.

[5] E. Ofor, M. Ogundesi, and W. Okiei, (2013). Analysis of Essential Oil from the Leaves of Deinbollia Pinnata by Combined Gas Chromatography/Mass Spectrometry. Proceedings; PITTCON Conference and Expo.

[6] S. E. Sotuboa, O. A, A. A. Lawalb, Osunsamia, and I. A. Ogunwande, (2016). “Constituents and Insecticidal Activity of Deinbollia pinnata Essential Oil,” *Nat. Prod. Commun.*, 11,(12), 1889–1890.

[7] Y. Rufai, N. Basar, and A. Sani, (2019). “Optimization and Isolation of 4,8,12,16-Tetramethylheptadecan-4-olide from Deinbollia pinnata,” *Asian J. Chem.*, 31(11),1-13.

[8] Y. Rufai, N. Basar, and A. Sani, (2020). “Deinbollia pinnata: Isolation and Characterization of Some Phytochemicals from Aerial Parts and their Antioxidant Potential,” *Nat. Prod. J.*, 10, 1–12.

[9] X. Wang, X. Yuan, Z. Hou, J. Miao, H. Zhu, and C. Song, (2009). Effect of di-(2-ethylhexyl) phthalate (DEHP) on Microbial Biomass C and Enzymatic Activities in Soil,” *Eur. J. Soil Biol.*, 45 (4), 370–376.

[10] F. Zhu, C. Zhu, E. Doyle, H. Liu, D. Zhou, and J. Gao, (2018). “Fate of di (2-ethylhexyl) phthalate in different soils and associated bacterial community changes Science of the Total Environment. *Sci. Total Environ.*, 2, 637–638.

[11] L. Narciso (2016). “The Response to Oxidative DNA Damage in Neurons: Mechanisms and Disease,” *Neural Plast.*, vol. 2016, 1–14.

[12] T. Hemnani and M. S. Parihar,(1998). “Reactive Oxygen Species and Oxidative DNA Damage,” *Indian J Physiol Pharmacol*, 42(4), 440–452.

[13] P. Lalitha, P. Jayanthi, and Thamaraiselvi, (2012). “Preliminary studies on phytochemicals and antimicrobial activity of solvent extracts of Eichhornia crassipes (Mart.) Solms,” *Asian J. Plant Sci. Res.*, 2(2), 115–122.

[14] N. M. Hashim (2012). “Antioxidant, Antimicrobial and Tyrosinase Inhibitory Activities of Xanthones Isolated from Artocarpus obtusus F.M. Jarrett,” *Molecules*, 17, 6071–6082.

[15] Y. Zou, S. K. C. Chang, Y. Gu, and S. Y. Qian, (2011). “Antioxidant activity and phenolic compositions of lentil (Lens culinaris var. Morton) extract and its fractions,” *J. Agric. Food Chem.*, 59(6), 2268–2276.

[16] D. E. Stevenson and R. D. Hurst, (2007). “Review Polyphenolic phytochemicals – just antioxidants or much more ?,” *Cell. Mol. Life Sci.*, 64, 2900–2916.

[17] J. Singh, V. Singh, S. Shukla, and A. K Rai, “Phenolic Content and Antioxidant Capacity of Selected Cucurbit Fruits Extracted with Different Solvents,” *J. Nutr. Food Sci.*, 6(6),1–8.

[18] W. O. Obonga, C. O. Nnadi, C. C. Chima, S. N. Okafor, and E. O. Omeje (2019). “In- vitro antioxidant and In vivo Anti-inflammatory Potentials of Marantocha leoantha (Marantaceae) Extracts and Fractions,” *Dhaka Univ. J. Pharm. Sci.*, 18(2), 233–240.

[19] J. J. Nair, A. R. Ndhlala, J. C. Chukwujekwu, and J. Van Staden (2012). “Isolation of di (2-ethylhexyl) phthalate from a Commercial South African Cognate Herbal Mixture,” *South African J. Bot.*, 80, 21–24.