Triterpenoids Characterization with Antioxidant activity from the Aerial Part of *Deinbollia pinnata*

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Abstract

*Deinbollia pinnata is a tropical and sub-tropical continental plant widely used by hunters for traditional medicine. It contains large constituents of triterpenoids from which seven **(7)** known triterpenes for the first time reported using combined chromatographic separation. These include squalene **(1)** stigmasterol **(2)**, stigmasta-5,22-diene-3-ol acetate **(3)**, γ-sitosterol **(4)**, lupeol **(5)**, taraxasterol **(6),** and betulinic acid **(7)** from aerial parts. Their structures were elucidated using HR-ESIMS, 1D NMR compared with existing data. Methanolic leave extracts showed a very high antioxidant potential of minimum inhibitory concentration for DPPH, 22.07 µg/mL; ABTS, 15.40 µg/mL; FRAP, 3.45 ± 1.30 mM. Stigmasta-5,22-diene-3-ol acetate **(3)** and betulinic acid **(7)** showed strong to moderate activity as an anti-oxidant reducing agent. These can be in the management of oxidative causative diseases.*

Keywords: *Deinbollia pinnata*, Triterpenoids, Antioxidant activity, oxidative causative diseases

Introduction

The term 'terpene' was originally used to describe mixtures of isomeric hydrocarbons ($C_{10}H_{16}$) occurring in essential oils obtained from sap in tissues of plants and trees (Connolly & Hill, 2010). Their broader meaning which is referred to as 'triterpenoid' encompasses secondary metabolites, found in the most variety of ethno-medicinal plant species with numerous pharmacological activities. The medicinal uses of triterpenoids are antioxidant sources for biological activities, ranging from anti-inflammatory, hepatoprotective, sedatives, cardiotonic, general tonic, ant-HIV-1 and anti-cancer (Hsu & Yen, 2014) are overwhelming. Plants have provided a source of inspiration for novel drug compounds as plant-derived (Walker, 2012), and medicines have made large contributions to human health and wellbeing. Herbal plants and their preparations have been reported for antimicrobial, antimalarial, anti-inflammatory, antidiabetic, anthelmintic, antiparasitic, anti-obesity, anticancer, and antiviral activities (Shakya, 2016). Phytochemicals have been recognized as the basis for traditional herbal medicine practised in the past and currently in all parts of the world (Lalitha et al., 2012). They have been in use and continue today as medicines. The *D. pinnata* roots and leaves are used in folkloric medicine as a remedy for numerous diseases (Lasisi, Bamidele, et al., 2016), (Borokini, 2018). Reports on their essential oil bioactivity and volatile constituents were documented (Sotubo et al., 2016). Isolated compounds range from flavonoids, coumarins, phenolics, esters and ethers (Lasisi, Akinhanmi, et al., 2016; Rufai et al., 2019, 2020) were identified. But up to date, comprehensive reports on terpenes as isolated phytochemicals from *D. pinnata* leaves are scanty talk-less of its antioxidant evaluation. This engrossed our interest in carryout extraction, isolation of their constituents and their antioxidants.

Materials and Method

Materials

The *D. pinnata* (Poir.) Schumach. & Thonn leaves were collected from Okehi Local Government Area of Kogi State, Nigeria during the dry season, early January, for one week (daytime temperature ranges from 28 °C in January to 32 °C). The plant 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 voucher specimen under the accession number of FHI 3251. The leaves were collected, washed, air-dried at room temperature for one month and grind to a powder.

Chemicals and Reagents

Folin-Ciocalteu phenol reagent, 5% sodium nitrite, acetate buffer, FRAP reagent, ABTS and potassium persulfate, 2, 2'-diphenyl-2-picrylhydrazyl (DPPH), 2, 2'-azino-bis-3-ethylbenzthiazolin-6-sulphonic acid (ABTS), (+) 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (trolox) and butylated hydroxyanisole (BHA) pyrogallol, quercetin, butylated hydroxyl toluene (BHT), gallic acid, and ascorbic acid were obtained from Sigma-Aldrich. tripyridyl-s-triazine (TPTZ) and potassium persulfate were purchased from Fluka. The absorbance for test samples and positive control solutions were determined against the reagent blank at 760 nm by microplate reader EPOCH (Bio Tek) ELISA. All determinations were carried out in triplicate and averaged. 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 ($CHCl_3$), ethyl acetate (EtOAc), acetone (ACE), methanol (MeOH), Deuterated acetone (D6) Deuterated chloroform ($CDCl_3$). TLC plates were sprayed with vanillin sulphuric acid reagent.

METHODS

Extraction and Isolation Method

Powdered plant material of *D. pinnata* (Poir.) Schumach. & Thonn was extracted using *the Ruf-*

azah method (Rufai et al., 2019). Briefly, the leaves were taken into several conical flasks (30 g each) and extracted with organic solvents (150 mL) such as *n*-hexane, ethyl acetate, and methanol in a sonicator using an ultrasonic-assisted extraction method at an interval of ten minutes' agitation with expert design software conditions; filtered into a bottle an allowed for 24hr; then filtered using Whatman No. 1 filter. The extraction was repeated for various solvents until complete extraction. The combined extracts were filtered and concentrated in *vacuo* at 40˚C using a rotary evaporator, the extractives were evaporated to dryness and residues were obtained in grams for all selected parts of *D. pinnata* (Poir.) Schumach. & Thonn as shown in

Table 1. Fractionation and purification of methanol crude extracts were carried out using vacuum liquid chromatography (VLC), column chromatography (CC), and guided by thin-layer chromatography (TLC). These purification processes using repeated chromatographic methods led to the isolation and identification of seven terpenes. The pure compounds isolated from *D. pinnata* leaves with the crude extract were evaluated for antioxidant activities.

Method of Antioxidant Assays

Evaluation of antioxidant potentials using different antioxidant assays was carried out for selected compounds. These include total antioxidant capacity using 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid (ABTS), Ferric reducing antioxidant potential (FRAP), and 2, 2'-diphenyl-2-picrylhydrazyl (DPPH).

2, 2'-diphenyl-2-picrylhydrazyl (DPPH) Assay

The free radical scavenging assay was conducted based on the method described in (Hashim et al., 2012). with minor modification. Briefly, 100 µM DPPH (1 mL) dissolved in MeOH was added to the MeOH solution (3 mL) of the tested samples at different concentrations. An equal volume of MeOH was added to the control test. The mixture was shaken vigorously and allowed to stand at room temperature for 30 mins. Then, the absorbance at 517 nm was measured with Epoch microplate reader. The percentage of scavenging of DPPH was calculated using Equation 1.

$$S\% = \frac{[\text{Ablank} - \text{Asample}]}{A\text{blank}} * 100 \qquad (1)$$

Where A_{blank} is the absorbance value of the control reaction (containing all reagents except the test compound) and A_{sample} is the absorbance value of the test compound. The sample concentration providing 50% scavenging/ inhibition (SC_{50}/IC_{50}) 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).

Ferric Reducing Antioxidant Potential (FRAP) Assay

The ferric reducing antioxidant potential (FRAP) assay was carried out according to (Jutiviboonsuk, 2012; Shahwar et al., 2012) with minor modification. FRAP reagent was freshly prepared, consisting of stock solution with a ratio of 10:1:1 of 300 mM acetate buffer, acetate buffer. 5 µL of the sample, 15 µL of MeOH and 150 µL of FRAP reagent were added to the 96-well plates. The absorbance at 573 nm was measured after 10 min of incubation at 37°C. $FeSO_4.7H_2O$ solution (0.06 mM – 1.0 mM) was used to build up calibration curves of standard antioxidants.

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

The ABTS assay was determined as described in (Zou et al., 2011) with minor modification. ABTS and potassium persulfate were dissolved with distilled water to obtain concentrations of 7 mM and 4.9 mM, respectively. The two solutions were mixed and incubated in 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 dark at room temperature. The percentage of antioxidant activity was calculated using Equation 2.

$$\text{Scavenging Percentage} = \frac{\text{Abs (ABTS)} - \text{Abs (ABTS + A Sample)}}{\text{Abs (ABTS)} * 100} \quad (2)$$

Where Abs (ABTS) is the absorbance of ABTS solution with methanol and A Sample is the absorbance of the tested samples with ABTS solution. All determinations were carried out in triplicate and averaged.

INSTRUMENTATION

Gravity column chromatography (CC) was carried out on Silica gel Merck silica 60 (70-230 mesh size) for VLC; Merck silica 60 (230-400 mesh size) for CC and 0.20 mm precoated gel aluminium plate (DC Kieselgel 60 F254) (TLC). The ^{1}H NMR (400 MHz) and ^{13}C NMR (400 MHz) spectra data were recorded on Bruker Avance AMX (400 MHz) instrument. UV spots were detected on UVITEC Cambridge CB4 IQB (light short and long waves (254 nm - 365 nm). Infrared (IR) were taken as KBr pellet on Perkin-Elmer series 1600 FT-IR spectrophotometer.

GC-MS analyses of isolates were performed on Agilent 7820A (G4350) instrument coupled with S9877E. The HP-5MS column with a dimension of 30 m × 0.25 µm × 0.25 µm was used. The initial temperature was 100°C, maintained for 10 min, while the final temperature was 300°C kept for 10 mins, with pressure at 10.686 psi, septum purge flow at 3.5 mL/min, split ratio (26.8: 0.1) and split the flow of 24.228 mL/min. Helium gas was used as a carrier gas. The ionization energy of 70eV was maintained for MS detection. Mass spectral data were obtained from Mass Spectrometry Laboratory, National Institute of Standards and Technology. The melting point was determined using a capillary tube on the electrothermal 9100, apparatus.

RESULTS AND DISCUSSION

The crude extracts from different solvents such as *n*-hexane, ethyl acetate and methanol were significant with various physical properties in terms of coloured. The manual agita-sonication extraction process yielded hexane (48.25 g, 3.22%), ethyl acetate (20.91 g, 1.39%) and ethanol (73.92 g, 4.93%) compared to the residual yield from the Soxhlet method; hexane (1.3 g, 0.09%), ethyl acetate (0.7 g, 0.05%) and methanol (2.1 g, 0.14%) respectively as shown in

Table 1.

Table 1: Yield from the Extraction of *D. pinnata* Leaves

	Extraction time mins	Extraction temperature 0°C	Solvents (g/%)		
	35	40	Hex	EtOAc	EtOH
Yield(g) (%)			48.25 (3.22)	20.91 (1.39)	73.92 (4.93)
The residue (marc)	Extraction time hrs 8	Extraction temperature 0°C 80	1.30 (0.09)	0.7 (0.05)	2.1 (0.14)

X mL/1.5kg proportion solvent to sample ratio was used in the experiment. Values are expressed in both grams/percentages compared to residual.

Compound **(1)** was obtained through elution of sub-fraction from the *D. pinnata* leaves *n*-hexane (DPLH) extracts over silica gel (70-230 mesh) using column chromatography afforded compound **(1)** as a colourless oil (35 mg, 0.91%), R_f value of 0.83 (*n*-hexane 100%). The IR

spectrum of **(1)** exhibited sp^3 C-H stretching absorption band at 2927, sp^2 C-H stretching at 3100 cm^{-1} and C=C stretching at 1602 cm^{-1}. The ^1H NMR spectrum displayed multiplet olefinic proton signals with different chemical shift values. The centre olefinic protons at H-11/H-14 both displayed signals at 5.15 (1H, *m*) while the outer olefinic protons H-3, H-7/H-18 showed signals at 5.10 (1H, *m*). Another multiplet signals range at δ_H 2.02 and δ_H 2.10 were observed in the upfield region and assigned to methylene (-CH_2-) protons H-4/H-21, H-5/H-20, H-8/H-7, H-9/H-16 and H-12/H-13. The singlet signals at δ_H 1.70 integrated for six protons corresponded to H-1/H-24. The other two singlet signals observed at 1.63 account for the remaining eighteen protons assigned to the methyl protons at H-25/H-30, H-26/H-29 and H-27/28. The ^{13}C NMR and DEPT spectra showed fifteen peaks from thirty carbons. The EIMS chromatogram displayed one peak at t_R 23.07 min and EIMS spectrum of a molecular ion, M+ at *m/z* 410 consistent with the molecular formula $C_{30}H_{50}$. Based on the spectral data obtained from *Hemigraphis hirta* (Alam et al., 2002). Compound **(1)** was characterized as squalene.

Compound **(2, 3 & 4)** was obtained by purification of *D. pinnata* ethyl acetate fraction (DPLEA). Elution through the column chromatography resulted in the isolation of compound **(2)** as white crystalline solid (8 mg, 0.24%), R_f value of 0.60 in pet. ether: EtOAc (19:1) with m.p. 167-169°C (Achika et al., 2016) 168-169°C) and compound **(3)** as white needles (15 mg, 0.43%), with m.p. 144-145°C (Osuntokun et al., 2018) 147-149°C). Compound **(4)** was obtained as colourless needles (10 mg, 0.33%), with m.p. 145°C (Pierre & Moses, 2015) 147-148°C). Their TLC profiles gave a purple spot after being sprayed with vanillin sulphuric acid reagent which suggested a terpene-type of compounds. The IR spectrum of **(2)** exhibited absorption bands at 2924 cm^{-1} attributed to C-H stretching (sp^3), a carbonyl (C=O) stretching at 1738 cm^{-1} and C-O stretching at 1170 cm^{-1}. Compound **(3)** showed an absorption band of sp^3 for C-H stretching at 2935 cm^{-1}, a typical hydroxyl group absorption band at 3406 cm^{-1}, C-O stretching at 1052 cm^{-1} and compound **(4)** displayed absorption bands at 2933 cm^{-1} for sp^3 C-H stretching. The presence of absorption bands of a hydroxyl group (OH) at 3391 cm^{-1} and C-O stretching was observed at 1051 cm^{-1}. The EIMS spectrum of **(2**) revealed a molecular ion, M+ at *m/z* 454.0 corresponded to the molecular formula $C_{31}H_{50}O_2$. Compound **(3)** revealed a molecular ion, M+ at *m/z* 412 corresponded to the molecular formula $C_{29}H_{48}O$ and that of **(4)** with a molecular ion, M+ at *m/z* 414 corresponded to the molecular formula $C_{29}H_{48}O$.

The ^1H NMR spectrum of **(3)** and **(4)** differed at C-3 with the presence of multiplet methine proton signals at δ_H 4.60 (1H, *m*) assigned to H-3 which bonded to an acetate group in compound **(2)** while multiplet signals at δ_H 3.54 (1H, *m*) assigned for methine proton at H-3 bonded to hydroxyl group. Another difference between compounds **(3)** and **(4)** is a C-22. Compound **(3)** revealed the presence of olefinic proton signals at δ_H 5.02 (1H, *m*) / 5.17 (1H, *m*) assigned to H-22 and H-23, while compound **(4)** showed multiplet methylene proton signals at δ_H 1.25 (4H, *m*) attributed to H-22/H-23. The major similarity was found in their methyl substituents. The spectrum of **(2)** showed a singlet methyl protons signal at δ_H 1.01 (3H, *s*) and δ_H 0.95 (3H, *s*) corresponded to H-18 and H-19. Another methyl in **(2)** at δ_H 0.91, 0.82, and 0.81 were corresponding to H-21, H-26 and H-27. In compound **(3)** revealed the presence of singlet methyl proton signals at 1.71 (3H, *s*) and 1.06 (3H, *s*) corresponded to H-18 and H-19 along with methyls proton signals at 0.92 (3H, *s*), 0.80 (3H, *m*), 0.88 (3H, *m*) corresponding to H-21, H-28 and H-29 while compound **(4)** afforded methyl proton signals at 0.69 (3H, *s*), 1.02 (3H, *s*) 0.93 (3H, *d, J* = 6.4 Hz), 1.29 (3H, *d, J* = 8.0 Hz) and 0.85 (3H, *d, J* = 7.6 Hz) corresponding to H-18, H-19, H-21, H-28 and H-29 respectively. The presence of triplet methine proton signals at δ_H 5.39 (1H, *t, J* = 6.0 Hz) **(2)**; 5.37 (1H, *t, J* = 6.0 Hz) **(3)**; 5.35 (1H, *t, J* = 2.16 Hz) **(4)** were corresponding to H-6. Analysis of the ^{13}C NMR spectrum of **(2)** showed signals at δ_C 139.7 (C-5) and δ_C 122.5 (C-6). The carbon peak value at δ_C 73.0 was assigned to C-3 which is linked to the carbonyl centre with a sigma bond. The carbon signals at δ_C 19.4 and δ_C 11.9 correspond to C-18 and C-19. The ^{13}C NMR

spectrum of compound **(3)** showed signals at the downfield region at δ_C 121.6, δ_C 140.7, δ_C 138.2, and δ_C 129.3 which were attributed to the unsaturated carbon (C=C) at C-6, C-5, C-22 and C-23 respectively. Another signal at δ_C 71.8 was assigned for C-3 which bonded to a hydroxyl group (OH). The remaining twenty-four carbons were observed in the upfield region at δ_C 11.8-56.8 ppm for **(3)**. Compound **(4)** showed signals at the downfield region at δ_C 121,7 and δ_C 140.7 which were attributed to the unsaturated carbon (C=C) C-6, respectively. Another signal at δ_C 71.7 was assigned for C-3 which bonded to a hydroxyl group (OH). The remaining twenty-four carbons were observed in the upfield region at δ_C 11.8-56.0 ppm. Based on the earlier report of ^{1}H and ^{13}C NMR data from the stem bark fraction of *Spondias mombin* (Linn.) (Osuntokun et al., 2018; Pierre & Moses, 2015; Yakubu et al., 2014) and from *Vitex Schiliebenii* extracts (Nyamoita et al., 2013). Compounds **(2), (3)** and **(4)** were characterized as stigmasterol, stigmasta-5,22-diene-3-ol acetate and γ- sitosterol.

Further elution of *n*-hexane resulted in the isolation of compound **(5)** as white needles crystal (5 mg, 0.29%), R_f value 0.59 (*n*-hexane: ethyl acetate, 7:1), m.p. 212-213°C (Alam et al., 2002) 215-216°C). IR spectrum of **(5)** showed hydroxyl group absorption band at 3421 cm^{-1}, C-O stretching at 1035 cm^{-1}, sp^3 C-H stretching at 2930 cm^{-1} and 2868 cm^{-1} and olefinic C=C stretching at 1637 cm^{-1}. The EIMS spectrum of **(5)** analysis showed a molecular ion, M+ at *m/z* 426 corresponded with a molecular formula of $C_{30}H_{50}O$. The ^{1}H NMR spectrum of **(5)** showed the characteristic of the triterpene skeleton, which showed overlapping signals at δ_H 0.76 - 1.68 corresponded to methyl groups, and the olefinic protons at (H-29) was assigned to two singlets at δ_H 4.70 and δ_H 4.57, a doublet at δ_H 3.22 was attributable to the oxymethine (H-3). Analysis of the ^{13}C NMR spectrum of **(5)** supported the triterpenoid skeleton by exhibiting thirty signals which were attributed to thirty carbons in the molecule. Based on the physical properties as well as spectroscopic data from the literature (Adeosun et al., 2019; Gandagule et al., 2018; No et al., 2018; Saratha et al., 2011), thus, compound **(5)** was identified as lupeol.

Elution of the *n*-hexane fraction afforded a white solid compound **(6)**, (18.0 mg, 0.60%), R_f value 0.59 (*n*-hexane: ethyl acetate, 8:2) with a purple colour when heated, m.p. 224-226°C (Sen et al., 2017) 221-222°C). The IR spectrum exhibited hydroxyl (O-H) group absorption band at 3386 cm^{-1}, C-H stretching at 1294 cm^{-1}, C-O stretching at 1072 cm^{-1}, C=C stretching at 1669 cm^{-1}and C-H stretching (sp^3) at 2917 cm^{-1}. The EIMS spectrum of **(6)** analysis molecular ion, M+ at *m/z* 426 consistent with the molecular formula $C_{30}H_{50}O$. The ^{1}H NMR spectrum of **(6)** revealed the presence of methyl groups resonated between δ_H 0.85-0.99. Other protons resonated at δ_H 5.53 (2H, *s*) corresponded to δ_C 116.8 (C-21) for olefinic protons. A methine proton showed mutiplet overlapped signals at δ_H 0.78 corresponded to δ_C 49.2 at C-9. Furthermore, doublet of doublet signal was observed at δ_H 3.20 (1H, *dd*, *J* = 10.6, 5.6 Hz) corresponded to δ_C 79.1 at C-3 bearing the O-H group. Analysis of the ^{13}C NMR spectrum of **(6)** showed thirty carbons signals. These include seven methyls, eleven methylenes, six methines and six quaternary carbons. The data obtained showed similarities with taraxasterol in medicinal plants isolated and identified from endemic *Centaurea kilaea* (Sen et al., 2017)(Mouffok et al., 2012; Sharma & Zafar, 2015). Based on the spectral data, compound **(6)** was elucidated as taraxasterol.

Compound **(7)** was obtained as a white solid (12 mg, 0.24%) from the CC of *n*-Hexane fraction after washing in cold *n*-hexane and the m.p. 314.0 – 314.7°C (lit. (Ahmed et al., 2013) 315 – 317°C) was determined. The EIMS spectrum revealed the presence of a molecular ion peak at *m/z* 456 which was consistent with the molecular formula $C_{30}H_{48}O_3$. The IR spectrum exhibited a broad peak typical of the hydroxyl group at 3224 - 2489 cm^{-1}, carbonyl absorption at 1747 cm^{-1} both for the carboxylic acid group and peaks due to double bond at 1641 and C-O stretching bands at 1230 cm^{-1}. The ^{1}H NMR spectrum revealed a pair of olefinic proton signals at δ_H 4.76 (1H, *d*, *J* = 2.0 Hz) and 4.63 (1H, s), a typical feature of the

exocyclic methylene group; six singlet methyl signals observed at δ_H 0.78, 0.85, 0.96, 0.99, 1.00, 1.71 and 1.96; along with a signal at δ_H 3.19 (*dd*, *J* = 11.6, 5.2 Hz) which was assigned to carbinolic proton (H-3). Other resonance at δ_H 2.99 (*dt*, *J* = 10.8, 4.4 Hz, H-19), 2.24 (*m*, H-16), 2.20 (*m*, H-13) and 1.96 (*dd*, *J* = 11.2, 5.2 Hz, H-21) were typical for lupane triterpene skeleton. The ^{13}C NMR spectrum exhibited the presence of thirty carbons. The DEPT spectra revealed six methines, eleven methylene, seven methyl and six quaternary carbons. The ^{13}C NMR data of compound (**7**) showed close similarities to betulinic acid (Ahmed et al., 2013). Based on the spectroscopic analyses above, compound (**7**) was identified as betulinic acid, a triterpene previous isolated from *Holoptelea integrifolia* (Ahmed et al., 2013).

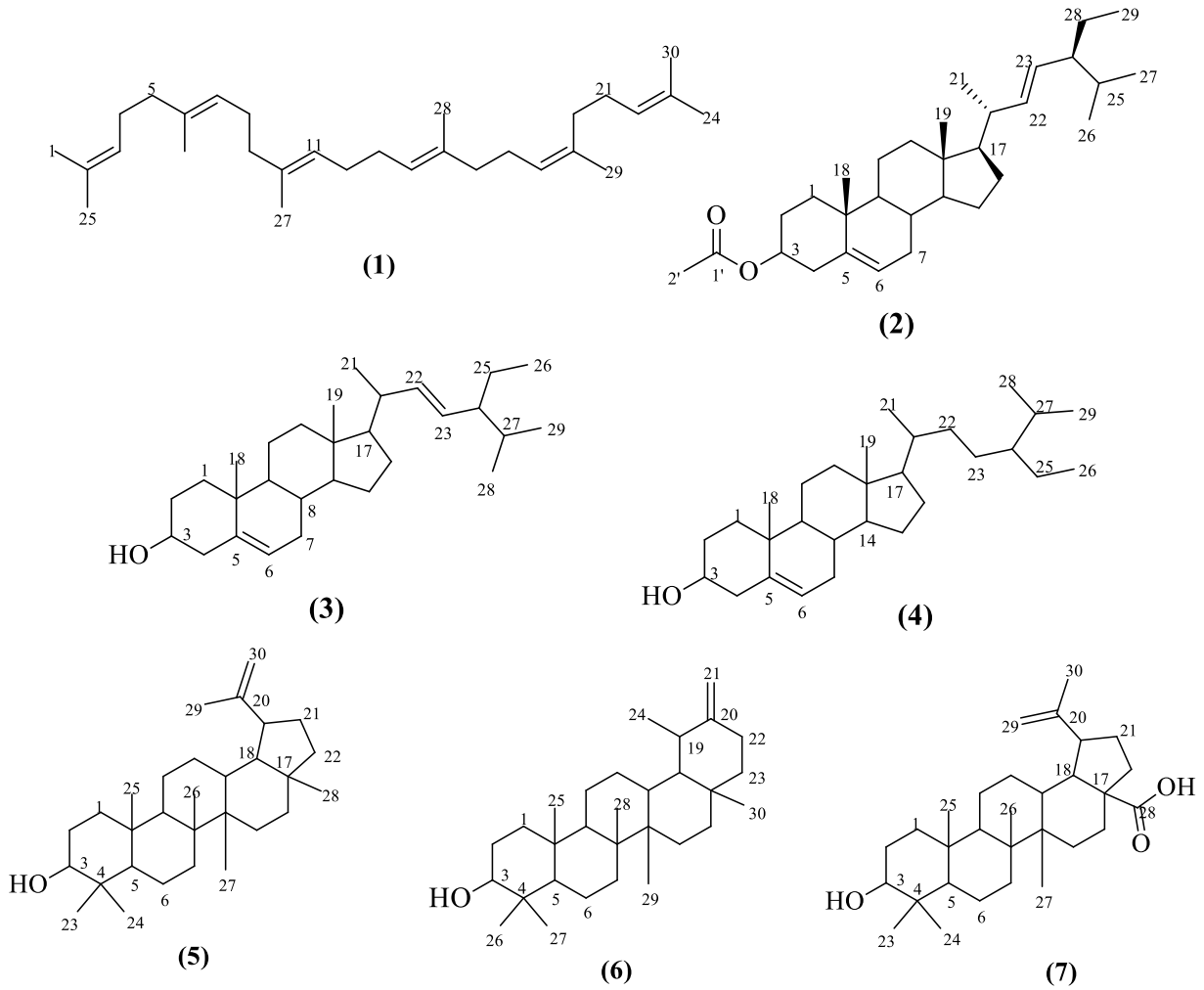

Figure 1: Structures of the Triterpenoids

Terpenoids Spectroscopic Data

Squalene (1): Elution of the same *n*-hexane fraction afforded a white amorphous solid as compound **(1)** (38 mg), R_f 0.59); m.p. 220-223°C; IR (Neat) ν_{max} cm^{-1}; δ_H 3386 (O-H), 1072 (C-O), 1669 (C=C), 2917 (C-H, 3011 (C-H). ^{1}H NMR (400 MHz, $CDCl_3$); 1.64 (2H, *m*, H-1), 1.91 (2H, *m*, H-2), 3.20 (1H, *dd*, *J* =10.6, 5.6 Hz, H-3), 0.78 (1H, *s*, H-5), 1.56 (2H, *m*, H-6), 1.63 (2H, *m*, H-7), 0.78 (1H, *m*, H-9), 1.57 (2H, *m*, H-11), 1.56 (2H, *m*, H-12), 1.58 (1H, *m*, H-13), 1.64 (2H, *m*,

H-15), 1.64 (2H, *m*, H-16), 2.34 (1H, *m*, H-18), 2.03 (1H, *m*, H-19), 5.53 (2H, *s*, H-21), 0.79 (3H, *m*, H-24), 0.85 (3H, *s*, H-25), 0.92 (3H, *s*, H-26), 0.96 (3H, *s*, H-27), 0.88 (3H, *s*, H-28), 0.79 (3H, *s*, H-29), 0.91 (3H, *s*, H-30). ^{13}C NMR (400 M Hz, $CDCl_3$): δ_C 16.0 (C-1), 134.8 (C-2), 17.6 (C-2'), 124.2 (C-3), 28.2 (C-4), 39.7 (C-5), 135.0 (C-6), 15.9 (C-6'), 124.2 (C-7), 28.2 (C-8), 39.7 (C-9), 135.0 (C-10), 15.9 (C-10'), 124.2 (C-11), 29.6 (C-12), 29.6 (C-13), 124.2 (C-18), 135.0 (C-19), 39.7 (C-20), 28.2 (C-21), 124.3 (C-22), 134.8 (C-23), 16.0 (C-23), 17.6 (C-24). EIMS; *m/z*: 410 $[M]^+$ ($C_{30}H_{50}$).

Stigmasterol (2): Compound **(2)** was obtained from the purification of *n*-hexane extracts as white needles (25 mg), m.p. 144-145°C; R*f* 0.70; IR (Neat) ν_{max} cm^{-1}; 2935 (C-H), 3406 (OH), 1052 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H1.27 (2H, *t*, *J* = 2.4 Hz, H-1), 1.47 (2H, *m*, H-2), 3.54 (1H, *m*, H-3), 2.00 (2H, *m*, H-4), 5.37 (1H, *d*, *J* = 6.0 Hz, H-6), 1.85 (2H, *m*, H-7), 1.47 (1H, *m*, H-8), 1.45 (1H, *m*, H-9), 1.30 (2H, *m*, H-11), 1.35 (2H, *m*, H-12), 1.30 (1H, *m*, H-14), 1.47 (2H, *m*, H-15), 1.48 (2H, *m*, H-16), 1.47 (1H, *m*, H-17), 1.71 (3H, *s*, H-18), 1.06 (3H, *s*, H-19), 1.47 (1H, *m*, H-20), 0.93 (3H, *m*, H-21), 5.02 (1H, *dd*, *J* = 15.2 Hz, 8.8 Hz, H-22), 5.17 (1H, *dd*, *J* = 15.2 Hz, 8.8 Hz, H-23), 0.92 (3H, *s*, H-24), 0.80 (2H, *m*, H-25), 0.84 (3H, *m*, H-26), 0.82 (3H, *d*, *J* = 7.2 Hz, H-27), 0.80 (3H, *m*, H-28), 0.88 (3H, *m*, H-29),); ^{13}C NMR (400 MHz, $CDCl_3$): δ_C 21.2 (C-19), 21.2 (C-18), 12.2 (C-29), 21.0 (C-27), 19.4 (C-26), 19.0 (C-21), 6.4 (C-2), 2.0 (C-1), 42.2 (C-4), 29.0 (C-7), 23.10 (C-11), 31.9 (C-12), 25.4 (C-15), 24.4 (C-16), 27.10 (C-28), 140.8 (C-6), 50.1 (C-9), 51.3 (C-8), 29.0 (C-14), 56.8 (C-17), 32.0 (C-20), 129.2 (C-22), 138.4 (C-23), 31.6 (C-25), 71.89 (C-3), 121.4 (C-5), 40.5 (C-13), 39.7 (C-10), 56.0 (C-24); EIMS; *m/z*: 412 $[M]^+$ ($C_{29}H_{48}O$).

Stigmasta-5,22-diene-3-ol acetate (3): The purification of DPLH 3-9 fraction through the column chromatography resulted to the isolation of compound **(3)** as white crystalline solid (18 mg), R_f 0.60; m.p. 167-169°C. IR (Neat) ν_{max} cm^{-1}: 2924 (C-H), 1738 (C=O), 1170 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.62 (2H, *t*, *J* = 8.0 Hz, H-1), 1.52 (2H, *m*, H-2), 4.60 (1H, *m*, H-3), 2.30 (2H, *m*, H-4), 5.39 (1H, *d*, *J* = 6.0 Hz, H-6), 1.62 (2H, *m*, H-7), 2.30 (1H, *m*, H-8), 2.06 (2H, *t*, *J* = 4.0 Hz, H-12), 1.06 (2H, *m*, H-15), 1.30 (2H, *m*, H-16), 1.01 (3H, *s*, H-18), 0.95 (3H, *s*, H-19), 0.91 (3H, *d*, *J* = 4.4 Hz, H-21), 5.34 (1H, *m*, H-22), 5.39 (1H, *m*, H-23), 0.97 (3H, *s*, H-23), 0.97 (3H, *s*, H-24), 0.71 (3H, *s*, H-25), 0.81 (3H, *d*, *J* = 4.0 Hz, H-27), 1.30 (3H, *s*, H-28), 0.89 (3H, *t*, *J* = 4.4 Hz, H-29), 2.01(3H, *s*, H-2'). ^{13}C NMR (400 MHz, $CDCl_3$): δ_C 37.0 (C-1), 31.5 (C-2), 73.0 (C-3), 39.7 (C-4), 139.7 (C-5), 122.5 (C-6), 31.5 (C-7), 31.9 (C-8), 51.2 (C-9), 36.6 (C-10), 21.2 (C-11), 39.7 (C-12), 56.9 (C-14), 25.0 (C-15), 29.0 (C-16), 56.9 (C-17), 11.8 (C-18), 21.2 (C-19), 39.7 (C-20), 23.4 (C-21), 139.7 (C-22), 129.3 (C-23), 50.2 (C-24), 31.9 (C-25), 21.0 (C-26), 19.0 (C-27), 25.3 (C-28), 12.0 (C-29), 173.2 (C-1'), 18.9 (C-2'). EIMS; *m/z:* 454.0 **(**$C_{31}H_{50}O_2$).

λ-sitosterol (4): Compound **(4)** was obtained from the purification of *n*-hexane extract as colourless needles (30 mg); m.p. 146-148°C; R_f 0.65; IR (Neat) ν_{max} cm^{-1}; 2933 (C-H), 3391 (OH), 1051 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 1.25 (2H, *m*, H-1), 1.47 (2H, *m*, H-2), 3.51 (1H, *m*, H-3), 2.00 (2H, *m*, H-4), 5.35 (1H, *t*, *J* = 2.16 Hz, H-6), 1.85 (2H, *m*, H-7), 1.45 (1H, *m*, H-8), 1.44 (1H, *m*, H-9), 1.42 (2H, *m*, H-11), 1.35 (2H, *m*, H-12), 1.42 (1H, *m*, H-14), 1.47 (2H, *m*, H-15), 1.48 (2H, *m*, H-16), 1.47 (1H, *m*, H-17), 0.69 (3H, *s*, H-18), 1.02 (3H, *s*, H-19), 1.53 (1H, *m*, H-20), 0.93 (3H, *d*, *J* = 6.4 Hz, H-21), 1.25 (2H, *m*, H-22), 1.25 (2H, *m*, H-23), 0.98 (1H, *m*, H-24). 1.86 ((2H, *m*, H-25), 0.82 (3H, *t*, *J* = 6.0 Hz, H-26), 0.83 (1H, *m*, H-27), 1.29 (3H, *d*, *J* = 8.0 Hz, H-28), 0.85 (3H, *d*, *J* = 7.6 Hz, H-29); ^{13}C NMR (400 MHz, $CDCl_3$); 37.2 (C-1), 32.4 (C-2), 71.8 (C-3), 42.2 (C-4), 140.7 (C-5), 121.7 (C-6), 31.9 (C-7), 31.6 (C-8), 50.2 (C-9), 33.7 (C-10), 21.0 (C-11), 39.7 (C-12), 42.3 (C-13), 56.0 (C-14), 26.1 (C-15), 28.2 (C-16), 56.7 (C-17), 11.8 (C-18), 19.3 (C-19), 36.5 (C-20), 33.9, 19.0 (C-21), (C-22), 26.1 (C-23), 45.8 (C-24), 29.1 (C-25), 18.2 (C-26), 19.8 (C-27), 23.0 (C-28), 12.2 (C-29). EIMS; *m/z*: 414 $[M]^+$ ($C_{29}H_{50}O$).

Lupeol (5): Further elution of *n*-hexane resulted in the isolation of white needles crystal (19 mg), m.p. 215-217°C; R_f 0.59; IR (Neat) ν_{max} cm^{-1};

3421 (OH), 1035 (C-O), 2930 (C-H), 1637 (C=C); ^{1}H NMR (400 MHz, $CDCl_3$): δ_H 0.91 (2H, *t, J* = 8.0 Hz, H-1), 1.54 (2H, *m*, H-2), 3.20 (1H, *m*, H-3), 1.39 (2H, *m*, H-6), 1.42 (2H, *m*, H-7), 1.26 (1H, *d, J* = 8.0 Hz, H-9), 1.24 (2H, *m*, H-11), 1.07 (2H, *m*, H-12), 1.66 (1H, *t, J* = 4.0 Hz, H-13), 1.68 (2H, *t, J* = 8.0 Hz, H-15), 1.39 (2H, *m*, H-16), 1.36 (1H, *m*, H-18), 2.37 (1H, *m*, H-19), 1.32 (2H, *m*, H-21), 1.24 (2H, *m*, H-22), 0.95 (3H, *s*, H-23), 0.79 (3H, *s*, H-24), 0.83 (3H, *s*, H-25), 1.03 (3H, *s*, H-26), 0.97 (3H, *s*, H-27), 0.80 (3H, *s*, H-28), 4.58 (1H, *s*, H-29), 1.68 (3H, *s*, H-30); ^{13}C NMR (400 MHz, $CDCl_3$): δc 19.2 (C-23),19.3 (C-24), 29.1 (C-25), 20.9 (C-26), 27.4 (C-27), 25.1 (C-28), 109.3 (C-30), 35.2 (C-1), 19.2(C-6), 35.5 (C-7), 50.4 (C-9), 27.4 (C-15), 40.0 (C-16), 34.2 (C-12), 40.0 (C-13), 29.83 (C-11), 42.81 (C-22), 43.0 (C-21), 34.2 (C-2), 29.1 (C-29), 50.4 (C-5), 48.2 (C-18), 151.0 (C-20), 55.25 (C-19), 79.0 (C-3); EIMS; *m/z*: 426 $[M]^+$ ($C_{30}H_{50}O$).

Taraxaterol (6): The unambiguous characterization of ursane skeleton for taraxasterol as a white solid, **(6)** (28 mg), with a purple color when heated, m.p. 224-226°C (lit. (Sen et al., 2017) 221-222°C). IR (Neat) ν_{max} cm^{-1}: 3486 (O-H), 2926 (C-H), 1159 (C-O), 1679 (C=C alkene). ^{1}H NMR (400 MHz, $CDCl_3$); δ_H1.64 (2H, *m*, H-1), 1.91 (2H, *m*, H-2), 3.20 (IH, *dd, J* = 10.6, 5.6 Hz, H-3), 0.78 (1H, *s*, H-5), 1.56 (2H, *m*, H-6), 1.63 (2H, *m*, H-7), 0.78 (1H, *m*, H-9), 1.57 (2H, *m*, H-11), 1.56 (2H, *m*, H-12), 1.58 (1H, *m*, H-13), 1.64 (2H, *m*, H-15), 1.64 (2H, *m*, H-17), 2.34 (1H, *m*, H-18), 2.03 (1H, *m*, H-19), 5.53 (2H, *s*, H-21), 1.35 (2H, *m*, H-22), 0.99 (3H, *s*, H-23), 0.79 (3H, *s*, H-24), 0.85 (3H, *s*, H-25), 0.92(3H, *s*, H-26), 0.96 (3H, *s*, H-27), 0.88 (3H, *s*, H-28), 0.79 (3H, *s*, H-29), 0.91 (3H, *s*, H-30) . ^{13}C NMR (400 MHz, $CDCl_3$); δ_C 38.7 (C-1), 27.3 (C-2), 79.1 (C-3), 38.7 (C-4), 55.5 (C-5), 18.2 (C-6), 34.0 (C-7), 41.3 (C-8), 49.2 (C-9), 57.3 (C-10), 21.4 (C-11), 26.0 (C-12), 38.9 (C-13), 41.3 (C-14), 26.6 (C-15), 38.7 (C-16), 34.0 (C-17), 48.7 (C-18), 38.9 (C-19), 158.0 (C-20), 116.8 (C-21), 38.9 (C-22), 28.1(C-23), 15.3 (C-24), 16.4 (C-25), 16.0 (C-26), 15.0 (C-27), 19.3 (C-28), 25.4 (C-29), 21.4 (C-30). EIMS; *m/z*: 426 $[M]^+$ ($C_{30}H_{50}O$).

Betulinic acid (7): Purification of *n*-hexane fraction using CC and then washing the solid obtained in cold *n*-hexane gave white solid of compound **(7),** (22 mg); R_f 0.60 (*n*-hexane-EtOAc, 3:2); m.p. 314.0 – 314.7°C (lit. (Ahmed et al., 2013) 315 – 317°C); IR (ATR) υ_{max} cm^{-1}: 3224 - 2489 (OH), 2938 (sp^3 CH), 1747 (C=O), 1641 (C=C), 1230 (C-O); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.78, 0.85, 0.96, 0.99, 1.00, 1.71, 1.96 (each, 3H, *s*, CH_3 ×7), 2.01 (1H, *m*, H-21), 2.20 (1H, *m*, H-13), 2.24 (1H, *m*, H-16), 2.99 (1H, *dt, J* = 10.8, 4.4, H-19), 3.19 (*dd, J* = 11.2, 5.2, H-3), 4.63 (1H, *s*, H-29), 4.76 (1H, *d, J* = 2.0, H-29); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.6 (C-27), 15.3 (24), 16.0 (C-25), 16.1 (C-26), 18.2 (C6), 19.3 (C-30), 20.8 (C-11), 25.4 (C-12), 27.3 (C-2),27.9 (C-23), 29.6 (C-21), 30.5 (C-15), 32.1 (C-16), 34.2 (C-7), 37.0 (C-22), 37.1 (C10), 38.3 (C-13), 38.6 (C-1), 38.8 (C-4), 40.6 (C-8), 42.4 (14), 46.8 (C18), 49.2 (C-19),50.4 (C-9), 55.3 (C-5), 56.3 (C-17), 79.0 (C-3), 109.7 (C-29), 150.4 (C-20), 180.7 (C-28); 248 (24), 203 (45), 189 (100). EIMS *m/z*; 456 $[M]^+$ ($C_{30}H_{48}O_3$).

The antioxidant properties for isolated terpenoids except for compounds **(3)** and **(7)** displayed inactive activity towards DPPH radicals with an IC_{50} value of more than 1000 µg/mL. Compound **(3)** and **(7)** showed IC_{50} valued 88.92 µg/mL and 143.7 µg/mL respectively. The potent •OH scavenging capacity present in compounds may be related to its protective power. The terpenoid least inhibitor percentage as calculated began at 30.38% **(1)**, 58.34% **(2)** 69.33 **(4),** 78.33 **(5)** and **(6)**, 60.34%. The isolated compounds were evaluated for ABTS radical scavenging activity but only a few compounds showed significant activity towards ABTS radical with IC_{50} value of 100.0-144.7 µg/mL for compounds **(1), (2), (3), (4) (5) (6)** and **(7)** as shown in **Table 2**. The terpenoids-related compounds showed a similar percentage of scavenging activity with least at 36.39%; **(1)** and others with 39.60%; **(6)**, 41.61%; **(2),** 43.95%, **(4)** and 62.95% **(5)**. The percentage of total ABTS radical scavenging activity of terpenoids level was moderately remarkable. Interestingly, only compound **(5)** showed a significant radical scavenging activity. The correlation analysis of FRAP values for total

antioxidant capacity was calculated using ferric sulfate ($FeSO_4.7H_2O$) solution. The ferric sulphate graph expressed as mM $FeSO_4$ equivalent with linearity over calibration range with an R^2 value of 0.994 shown in **Error! Reference source not found.**.

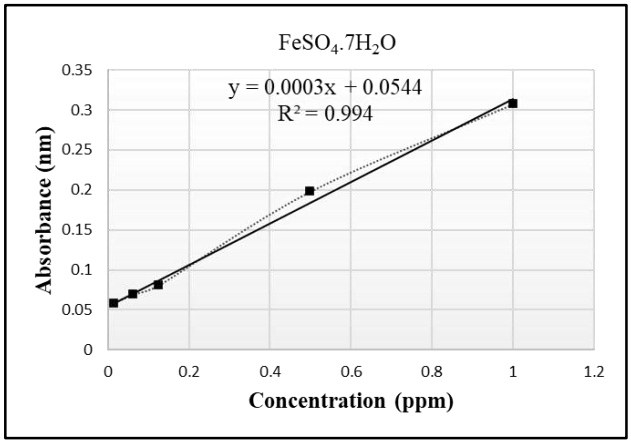

Figure 2: Calibration Curve of $FeSO_4.7H_2O$

The EtOAc extracts (DPLEA) with reducing potential value of 3.33 ± 0.09 mM $FeSO_4$ equivalent. This significant difference between MeOH extracts and EtOAc extracts compared to postive control in were Trolox (FRAP equivalent values = 2.23 ± 0.12 mM), BHA (FRAP equivalent values = 2.74 ± 0.11 mM), Gallic acid (FRAP equivalent values = 2.83 ± 0.02 mM), Ascorbic Acid (FRAP equivalent values 2.87 ± 0.03 mM), BHT (FRAP equivalent values = 2.93 ± 0.18 mM), Pyrogallol (FRAP equivalent values = 3.07 ± 1.44 mM), Quercetin (FRAP equivalent values = 3.11 ± 0.05 mM) at concentration of 1.0 Mm as indicated in Error! Reference source not found..

Table 2: DPPH Inhibitory Activity of Compounds from *D. pinnata* (Poir.) Schumach. & Thonn.

Fractions	**Compounds**	**IC_{50} (µg/mL)**
n-Hexane **(DPLH)**		105.0
Ethyl Acetate **(DPLEA)**		605.4
Methanol **(DPLMT)**		22.07
	(1)	>1000
	(2)	>1000
	(3)	88.92
	(4)	>1000
	(5)	>1000
	(6)	>1000
	(7)	143.7
Postive Control		**IC_{50} (µg/mL)**
BHA		25.758
BHT		22.22
Trolox		11.40
Gallic Acid		31.01

Ascorbic Acid	105.70
Pyrogallol	1.70
Quercetin	6.95

The IC_{50} value represented the concentration of the isolated compounds that caused 50% inhibition of DPPH●. 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. Positive Control: BHT = butyl hydroxyl toluene, BHA = butyl hydroxyl anisole, trolox = (+)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, gallic acid, ascorbic acid; pyrogallol and quercetin. Isolated compounds: Squalene **(1)** Stigmasterol **(2)**, Stigmasta-5,22-diene-3-ol acetate **(3)**, γ-sitosterol **(4)**, Lupeol **(5)**, Taraxasterol **(6),** and betulinic acid **(7).** DPL = *D. pinnata* leaves; **HE** = Hexane; **EA** = Ethyl acetate; M**T** = Methanol.

The higher antioxidant capability was displayed by MeOH (DPLMT) extracts as shown in

Table 3: ABTS Radical Scavenging Activity of Compounds from *D. pinnata* (Poir.) Schumach. & Thonn.

with 50% scavenging activity at 15.41 µg/mL. Ethyl acetate extract showed lower activity at 24.38 µg/mL and *n*-hexane showed interference. All the terpenoids under test showed moderate activity and compound **(3)** with 100.0 µg/mL haven greater activity. Compound **(6)** showed an inactive test. Among the positive controls, pyrogallol showed the highest activity with 12.81 µg/mL followed by quercetin at 15.81 µg/mL, gallic acid at 17.83 µg/mL, BHT at 27.60 µg/mL, ascorbic acid at 40.25 µg/mL, Trolox at 41.14 µg/mL and BHA at 47.28 µg/mL.

Table 3: ABTS Radical Scavenging Activity of Compounds from *D. pinnata* (Poir.) Schumach. & Thonn.

Fractions	**Compounds**	**SC_{50} (µg/mL)**
n-Hexane **(DPLHE)**		INT
Ethyl Acetate **(DPLEA)**		24.38
Methanol **(DPLMT)**		15.41
	(1)	125.0
	(2)	123.3
	(3)	100.0
	(4)	119.1
	(5)	144.7
	(6)	>1000
	(7)	114.4
Positive Control		**SC_{50} (µg/mL)**
BHA		47.28
BHT		27.60
Trolox		41.14

Gallic acid.	17.83
Ascorbic acid	40.25
Pyrogallol	12.81
Quercetin	15.81

The SC_{50} value represented the concentration of isolated compounds that caused 50% scavenging of ABTS radicals. SC_{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. Positive control: BHT = butyl hydroxyl toluene, BHA = butyl hydroxyl anisole, trolox = (+)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid, gallic acid, ascorbic acid, pyrogallol and Quercetin. Isolated compounds: Squalene **(1)** Stigmasterol **(2)**, Stigmasta-5,22-diene-3-ol acetate **(3)**, γ-sitosterol **(4)**, Lupeol **(5)**, Taraxasterol **(6),** and betulinic acid **(7).** DPL = *D. pinnata* leaves; HE = Hexane; EA = Ethyl acetate; MT = Methanol.

The evaluation of isolated compounds for FRAP scavenging activity presented in **Table 4** showed lower potency for only compounds **(3)** and **(7)** with FRAP equivalent values = 1.91± 0.02 mM and 1.11 ± 0.03. Methanol **(DPLMT)** showed higher activity at 3.45 ± 1.30 mM. The EtOAc extracts **(DPLEA)** with reducing potential value of 3.33 ± 0.09 mM $FeSO_4$ equivalent.

Table 4: Ferric Reducing Antioxidant Power (FRAP) of Compounds from *D. pinnata* (Poir.) Schumach. & Thonn. Leaves

FRAP (mM equivalent to $FeSO_4.7H_2O$)					
Conc.	**0.06 mM**	**0.12 mM**	**0. 25 mM**	**0.50 mM**	**1.0 mM**
Fractions/compds					
n-Hexane (**DPLHE)**	0.15 ± 0.01	0.31 ± 0.01	0.61 ± 0.02	1.99 ± 0.04	2.54 ± 0.08
Ethyl Acetate (**DPLEA)**	0.53 ± 0.01	0.91 ± 0.04	1.79 ± 0.14	3.04 ± 0.16	3.33 ± 0.09
Methanol **(DPLMT)**	0.83 ± 0.12	1.38 ± 0.18	2.26 ± 0.24	3.72 ± 0.82	3.45 ± 1.30
(1)	-	-	-	-	-
(2)	-	-	-	-	-
(3)	0.02 ± 0.03	0.10 ± 0.01	0.17 ± 0.05	0.71 ± 0.02	1.91± 0.02
(4)	-	-	-	-	-
(5)	-	-	-	-	-
(6)	-	-	-	-	-
(7)	0.12 ± 0.03	0.21 ± 0.06	0.60 ± 0.12	1.11 ± 0.02	1.21 ± 0.03

The ferric sulfate graph expressed as mM $FeSO_4$ equivalent with linearity over the calibration range with an R^2 value of 0.9940. All values are expressed as mean ± SD values for three replicates experiment; P<0.05. Squalene **(1)** Stigmasterol **(2)**, Stigmasta-5,22-diene-3-ol acetate **(3)**, γ-sitosterol **(4)**, Lupeol **(5)**, Taraxasterol **(6),** and betulinic acid **(7).** DPL = *D. pinnata* leaves; HE = Hexane; EA = Ethyl acetate; MT = Methanol.

This significant difference between MeOH extracts and EtOAc extracts compared to positive control were Trolox (FRAP equivalent values = 2.23 ± 0.12 mM), BHA (FRAP equivalent values = 2.74 ± 0.11 mM), Gallic acid (FRAP equivalent values = 2.83 ± 0.02 mM), Ascorbic Acid (FRAP equivalent values 2.87 ± 0.03 mM), BHT (FRAP equivalent values = 2.93 ± 0.18 mM), Pyrogallol (FRAP equivalent values = 3.07 ± 1.44 mM), Quercetin (FRAP equivalent values = 3.11 ± 0.05 mM) at concentration of 1.0 mM as indicated in Error! Reference source not found. which clearly approve of the plant as a promising antioxidant source to compact radical related disease.

Table 5: Ferric Reducing Antioxidant Power (FRAP) of Positive Control

	FRAP (mM equivalent to $FeSO_4.7H_2O$)				
Conc.	**0.06 mM**	**0.12 mM**	**0. 25 mM**	**0.50 mM**	**1.0 mM**
Positive Controls					
Trolox	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
VIT. C	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
GLA	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 $FeSO_4$ equivalent with linearity over the calibration range with R^2 value of 0.9940. All values expressed as mean ± SD values for three replicates experiment; $P<0.05$. Positive Control: BHT = butyl hydroxyl toluene, BHA = butyl hydroxyl anisole; Trolox = (+)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid; GLA. = Gallic acid; VIT. C. = Ascorbic acid; PYR = Pyrogallol; QUE = Quercetin.

CONCLUSION

It is rudimental information for the use of plant parts for research work to reveal phytochemicals and their activity. *D. pinnata* (Poir.) Schumach. & Thonn. leaves will have been more beneficial to our immediate society if their antioxidant potential was early studied and sourced. Based on the antioxidant activity profile found, methanolic extracts and their isolated constituents can be extensively investigated clinically for very common neurodegenerative diseases such as cancers, Alzheimer's, coronary artery disease, convulsion, epilepsy, Hallervorden-Spatz syndrome and other highly demanded use of antioxidant products in cosmetic and food industries.

AUTHOR CONTRIBUTIONS

All the experiments have been carried out by the authors listed.

CONFLICT OF INTEREST: Authors have none to declare.

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