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# Pentacyclic Triterpenoids Isolated from *Diospyros foxworthyi* Bakh. (Ebenaceae) with its Cytotoxic Activity Against HT-29 Human Colon Cancer Cell

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*Diospyros foxworthyi* is a tropical plant species of Ebenaceae family. This plant is native to Malaysia. This genus is rich with pentacyclic triterpenoids in the form of lupane-based skeletons, which subsequently intrigue to further its phytochemical findings. The ethyl acetate (EtOAc) extract from the bark of the plant was studied through the isolation, purification and structural elucidation processes resulting in the discovery of three known yet interesting lupane-based backbone compounds, namely betulin (**1**), lupeol (**2**) and lupenone (**3**). The isolated compounds were purified using the chromatographic technique (column chromatography and thin-layer chromatography). The structure of these compounds was elucidated and characterised by using spectroscopic methods consisting of 1D- and 2D-NMR in combination with FT-IR analysis and later compared with the literatures. These triterpenoids **1**-**3** were subjected to cytotoxic activity using MTT assay against HT-29 colorectal adenocarcinoma. The observed result showed that compounds **1** and **2** are able to suppress the growth of cancer cells in a dose-dependent manner with the half-maximal inhibitory concentration ($IC_{50}$) of 13.49 ± 0.53 and 37.57 ± 4.11 µM, respectively. This is the first report on chemical constituents isolated from *Diospyros foxworthyi* and their cytotoxic activity on HT-29 colorectal adenocarcinoma.

**Key words**: Ebenaceae; *Diospyros foxworthyi*; triterpenoids; cytotoxic activity; HT-29 colon cancer

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Cancer is one of the most complex and dynamic human disease. It is a condition that causes certain cells in the body to grow uncontrollably and spread to other parts of the body [1]. Colorectal cancer (CRC) or can be known as colon cancer or rectal cancer is one of the most common cancers worldwide [2]. The data from GLOBOCAN 2020 estimates that there are 1.93 million new CRC cases diagnosed with 0.94 million deaths, representing 10% of the global cancer incidence (total 19.29 million new cases) and 9.4% of all cancer caused deaths (total 9.96 million deaths) in 2020 [3]. The majority of the increase in the incidence of CRC can be attributed to the increased exposure to environmental risk factors as a result of the shift toward a westernised lifestyle and diet [4]. Hence, the conclusive treatment for this disease using novel drugs, therapies or natural products is very important. Natural products have been primarily used to treat human disease for thousands of years and are becoming more essential in medication research and discovery [5]. Indeed, most anticancer drugs are derived from nature [6]. The enormous interest in the development of cancer drugs candidates from natural resources has demonstrated its potential in decreasing the possibility of colon cancer and slowing its progression [7].

*Diospyros foxworthyi* Bakh., a tropical plant native to Malaysia, is a medium-sized tree with 20 m tall and 35 cm in diameter. It is locally known as *kayu arang* or *kayu malam* due to its outer bark which is hard and black in colour [8]. This plant belongs to *Diospyros* genus of Ebenaceae family and is classified as a 'least concern' species which prevailed further research of it [9]. Traditionally, ancient Chinese civilizations have opted for this plant genus to produce a cure for ischemia, high blood pressure, atherosclerosis and some infectious diseases [10]. The genus *Diospyros*

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is rich with triterpenoids [11] with variety of interesting biological activities such as antiviral [11], anti-inflammatory [12], antimicrobial [13], anti-proliferative [14], cytotoxic [15] and antitumor [16].

Therefore, this research is intended to isolate and structurally elucidate the chemical constituents of *Diospyros foxworthyi* and its cytotoxic activity towards HT-29 human colon cancer cell.

## MATERIALS AND METHOD

### Chemicals and Materials

All chemical reagents and solvents (AR grades) were acquired from QRëC (Asia) and Merck (Germany) and were used without further purification i.e *n*-hexane, dichloromethane, ethyl acetate, methanol, chloroform-D1, tetramethylsilane, vanillin and sulphuric acids. Column chromatography (CC) was performed using silica gel 60 of 70-230 and 230-400 mesh (Merck, Germany) as the stationary phase depending on the weight of the crude or fractions. Thin-layer chromatography (TLC) was performed on alumina plates pre-coated with silica gel 60 $F_{254}$ plates (Merck, Germany) to distinguish the presence of compounds in the samples. Vanillin-sulphuric acid vapour was used as a detector reagent to examine the spots of compounds on TLC plates.

### Instrumentations

The TLC plates were visualized under UV radiation lamp ($\lambda_{max}$ = 254 and 365 nm). Melting points were determined on open capillary tubes and are uncorrected using Stuart SMP-10 apparatus (Barloworld Scientific, Stafforshire, United Kingdom). All spectral data were procured using the following instruments: The Fourier-transform Infrared (FTIR) spectra were taken by employing the Bruker Alpha II Compact FT-IR spectrometer (Bruker Bioscience, Billerica, Massachusetts, United States) and Perkin Elmer Universal ATR FT-IR spectrometer (PerkinElmer, Waltham, Massachusetts, United States) with chloroform as solvent. In addition, 1D- and 2D-nuclear magnetic resonance (NMR) spectra were recorded on FT-NMR Bruker Advance 500 spectrometer (Bruker Bioscience, Billerica, Massachusetts, United States) in $CDCl_3$ and tetramethylsilane (TMS) as internal standard. Data were analysed using Mnova 14.2.2 and TopSpin 3.6.2 software package. Chemical shifts are reported in part per million ($\delta$-scale) and the coupling constants, *J* are reported in Hertz (Hz).

### Plant Material

The bark of *Diospyros foxworthyi* Bakh. with a code number of KL5262B was collected at Hutan Simpan Bubu, Beruas, Perak, Malaysia on 22nd June 2006. It was notified by Teo L.E., the botanist from University of Malaya. A voucher specimen then has been deposited at the Herbarium of the Department of Chemistry, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia.

### Extraction and Isolation

The air-dried, grounded barks of *Diospyros foxworthyi* (1.0 kg) were extracted with 3.0 L ethyl acetate (EtOAc) using maceration method at room temperature for 4 days and then filtered. The extract was decanted and then concentrated at 40°C using the rotary evaporator. The extraction was then repeated for 2 times and later using 3.0 L of methanol (MeOH). The yield of crude extracts for EtOAc-soluble (185.0 g; 18.50%) and MeOH-soluble (121.5 g; 12.15%) were obtained. The MeOH-soluble crude extract was kept for future use. The EtOAc-soluble crude extract (175.0 g) was fractionated using CC over silica gel 60 (70-230 mesh) by employing hexane/EtOAc/dichloromethane (DCM)/ MeOH step gradient solvent system to give eight major fractions (Fr1 – Fr8). Subsequently, Fr2 to Fr8 was further purified by CC using silica gel 60 (230-400 mesh) with a stepwise elution gradient of hexane/ EtOAc (0-100 % EtOAc in hexane) to later obtain three interesting compounds, which were betulin (**1**), lupeol (**2**) and lupenone (**3**).

Betulin (**1**): Yellowish-white powder; Yield: 11.10 g (6.34%); M.p.: 242–246 °C; FT-IR(ATR) $\nu_{max}$ $cm^{-1}$: 3357 (O-H), 2937 ($C_{sp}^2$-H), 2867 ($C_{sp}^3$-H), 1689 (C=C), 1031 (C-O); $^1$H-NMR and $^{13}$C-NMR data see Table 1.

Lupeol (**2**): White powder; Yield: 3859.9 mg (2.20%); M.p.: 219–221 °C; FT-IR(ATR) $\nu_{max}$ $cm^{-1}$: 3349 (O-H), 2942 ($C_{sp}^2$-H), 2871 ($C_{sp}^3$-H), 1638 (C=C), 1035 (C-O); $^1$H-NMR and $^{13}$C-NMR data see Table 1.

Lupenone (**3**): Red-brownish oil; Yield: 568.6 mg (0.33%); FT-IR(ATR) $\nu_{max}$ $cm^{-1}$: 2924 ($C_{sp}^2$-H), 2854 ($C_{sp}^3$-H), 1703 (C=O), 1643 (C=C); $^1$H-NMR and $^{13}$C-NMR data see Table 1.

### Cell Viability Assay (MTT assay)

Cytotoxicity test was carried out using the Calbiochem® (Germany) 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay to measure the metabolic activity of the cell. HT-29 colon cancer cell was used as a targeted human cancer cell and Calbiochem® (Germany) cisplatin as the positive control standard. The cell line was cultured in Minimum Essential Medium Eagle (EMEM) (Sigma-Aldrich, USA) with 10% foetal bovine serum (FBS) (Sigma-Aldrich, USA) as a supplement. Then, the cell was allowed to grow as a monolayer in a humidified 5% $CO_2$ incubator at 37°C. The cell has seeded a total of 10000 cells/well in a 96-well plate and treated with different concentrations of extract: 0–100 µg/mL for crude and 0–100 µM for triterpenoids. After 24 hrs of incubation, a volume of 20.0 µL of MTT reagent (5.0 mg/mL) was added into each well and further incubated for 1.5 hrs. Spent media were discarded and 200.0 µL of dimethyl sulfoxide (DMSO, Merck, Germany) was added to dissolve the purple formazan precipitates. The result was obtained by measuring the absorbance at the test wavelength, 570 nm, and reference wavelength, 650 nm

using a microtiter plate reader (Tecan Sunrise™, Männedorf, Switzerland). The half maximal inhibitory concentration ($IC_{50}$) was calculated from the graph of percentage cell viability versus the concentration of compounds. The percentage of cell viability was calculated as follows [17]:

$$\text{Viable cell (\%)} = \frac{(\text{Absorbance sample} - \text{absorbance blank})}{(\text{Absorbance control} - \text{absorbance blank})} \times 100$$

**Statistical Analysis**

Result was expressed as mean values ± standard deviation (SD). All data collected from the experiments were performed in three replicates and analysed using the one-way analysis of variance (ANOVA) at a significance level of $p<0.05$ indicated by * and $p<0.005$ indicated by **.

**Table 1.** $^{1}$H-NMR (in $CDCl_3$, 500 MHz) and $^{13}$C-NMR (in $CDCl_3$, 125 MHz) data of compounds **1–3**

| Position | Compound | | | | | |
|---|---|---|---|---|---|---|
| | **1** | | **2** | | **3** | |
| | **$\delta_H$ (*J* in Hz)** | **$\delta_C$** | **$\delta_H$ (*J* in Hz)** | **$\delta_C$** | **$\delta_H$ (*J* in Hz)** | **$\delta_C$** |
| 1 | 0.88 dd (4.7, 12.6)<br>1.65 m | 38.7 | 0.90 m<br>1.65 m | 38.9 | 1.39 m<br>1.90 m | 39.8 |
| 2 | 1.60 m | 27.4 | 1.59 m | 27.5 | 2.47 m | 34.3 |
| 3 | 3.18 dd (4.8, 11.4) | 79.0 | 3.18 dd (4.8, 11.4) | 79.1 | - | 218.2 |
| 4 | - | 38.9 | - | 39.0 | - | 47.4 |
| 5 | 0.68 d (9.9) | 55.3 | 0.67 d (9.4) | 55.5 | 0.77 m | 55.1 |
| 6 | 1.39 m, 1.52 m | 18.3 | 1.38 m, 1.51 m | 18.5 | 1.48 m, 1.70 m | 19.5 |
| 7 | 1.39 m | 34.2 | 1.39 m | 34.5 | 1.43 m | 33.7 |
| 8 | - | 40.9 | - | 41.0 | - | 40.9 |
| 9 | 1.29 m | 50.4 | 1.26 m | 50.6 | 1.29 m | 50.0 |
| 10 | - | 37.2 | - | 37.3 | - | 37.0 |
| 11 | 1.21 m, 1.40 m | 20.8 | 1.41 m | 21.1 | 1.41 m | 21.6 |
| 12 | 1.05 m, 1.65 m | 25.2 | 1.64 m | 25.3 | 1.65 m | 25.3 |
| 13 | 1.60 m | 37.3 | 1.65 m | 38.2 | 1.66 m | 38.3 |
| 14 | - | 42.7 | - | 43.0 | - | 43.1 |
| 15 | 1.64 m, 1.70 m | 27.0 | 1.66 m | 27.6 | 1.71 m | 27.6 |
| 16 | 1.28 m, 1.90 m | 29.2 | 1.37 m, 1.48 m | 35.8 | 1.48 m | 35.7 |
| 17 | - | 47.7 | - | 43.2 | - | 43.0 |
| 18 | 1.58 m | 48.8 | 1.36 m | 48.5 | 1.41 m | 48.4 |
| 19 | 2.37 m | 47.8 | 2.37 m | 48.1 | 2.38 m | 48.1 |
| 20 | - | 150.5 | - | 151.1 | - | 150.9 |
| 21 | 1.26 m, 1.97 m | 29.7 | 1.29 m, 1.91 m | 30.0 | 1.28 m, 1.93 m | 29.9 |
| 22 | 1.03 m, 1.85 m | 33.9 | 1.20 m, 1.37 m | 40.2 | 1.20 m, 1.39 m | 40.1 |
| 23 | 0.97 s | 27.9 | 0.97 s | 28.2 | 1.25 s | 26.8 |
| 24 | 0.76 s | 15.3 | 0.76 s | 15.5 | 1.02 s | 21.2 |
| 25 | 0.83 s | 16.1 | 0.83 s | 16.3 | 1.07 s | 15.9 |
| 26 | 1.02 s | 15.9 | 1.03 s | 16.2 | 0.93 s | 16.1 |
| 27 | 0.98 s | 14.8 | 0.94 s | 14.7 | 0.96 s | 14.6 |
| 28 | 3.32 d (10.7)<br>3.75 d (11.0) | 60.6 | 0.79 s | 18.2 | 0.80 s | 18.2 |
| 29 | 4.58 s, 4.68 s | 109.7 | 4.56 s, 4.69 s | 109.5 | 4.57 s, 4.69 s | 109.6 |
| 30 | 1.68 s | 19.1 | 1.68 s | 19.5 | 1.68 s | 19.8 |

(s=singlet, d=doublet, dd=doublet of doublet, m=multiplet)

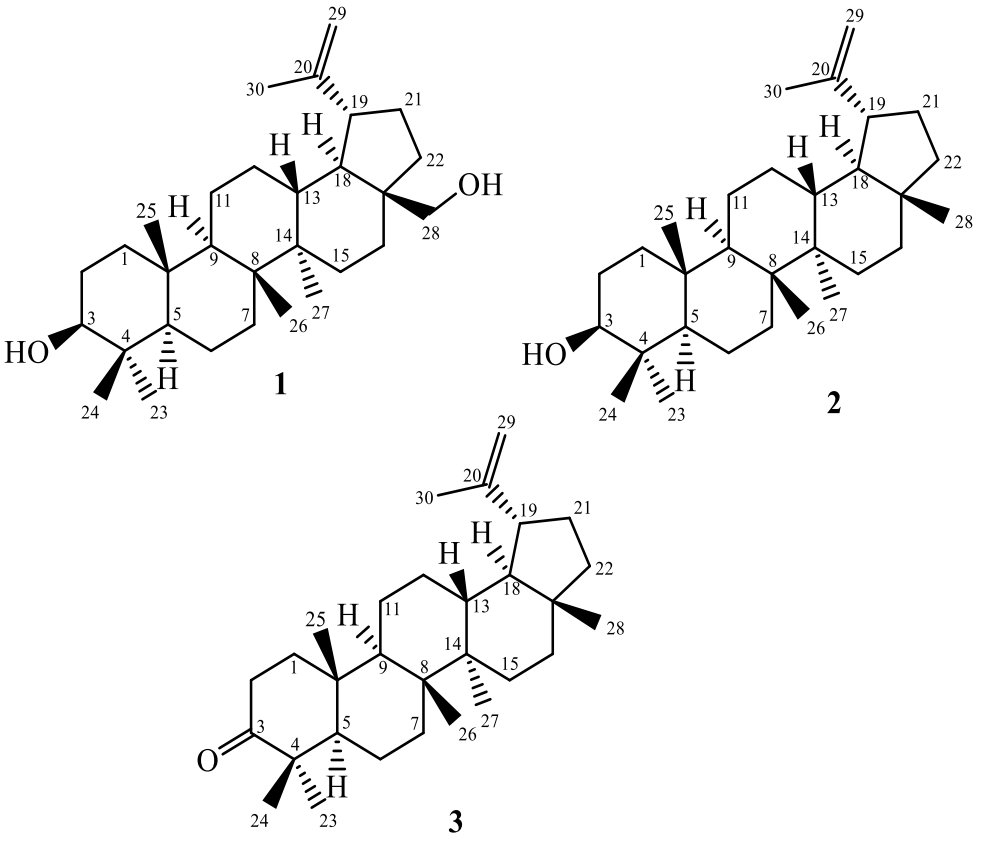


**Figure 1.** Chemical constituents isolated from *Diospyros foxworthyi*.

## RESULTS AND DISCUSSION

### Isolation and Structural Elucidation

The EtOAc-soluble crude extract was subjected to CC to afford eight fractions (Fr1 – Fr8). Consequently, further purifications using CC were performed on seven fractions (Fr2 – Fr8) led to the discovery of betulin (**1**), lupeol (**2**) and lupenone (**3**). All assignments of $^{1}$H- and $^{13}$C-NMR data were established through detailed analysis of 2D-NMR namely COSY, HSQC and HMBC paired with FT-IR analysis. This spectroscopic information was compared with the literatures data for confirmation. In general, the spectroscopic data suggested that compounds **1**–**3** is likely to be a lupane type triterpenes with seven methyl group and germinal double bond at ($\Delta_{20,29}$).

Compound **1** (Betulin) was isolated as a yellowish-white powder in 6.34% yield with the melting point of 242–246 °C. The important absorption bands in IR spectrum of **1** were observed at 3357 (O-H absorption), 2937 ($C_{sp}{}^{2}$-H stretching), 2867 ($C_{sp}{}^{3}$-H stretching), 1689 (C=C stretching) and 1031 cm$^{-1}$ (C-O bond). The $^{1}$H-NMR spectrum of **1** as tabulated in Table 1 showed important characteristics signals within the range of $\delta_H$ 0.68 – 4.68. Six singlet signals at $\delta_H$ 0.76, 0.83, 0.97, 0.98, 1.02 and 1.68 indicates the presence of six methyl protons of H-24, H-25, H-23, H-27, H-26 and H-30, respectively. The appearance of doublet of doublets at $\delta_H$ 3.18 of H-3 ($J$=4.8 and 11.4 Hz) signified the $\alpha$-oriented hydrogen of 3$\beta$-hydroxy triterpene. Meanwhile, the existence of isopropenyl group were confirmed with the presence of diastereotopic protons for methylene groups attached to hydroxyl at $\delta_H$ 3.32 (J=10.7 Hz, H-28a) and 3.75 (J=11.0 Hz, H-28b) together with exocyclic methylene protons at $\delta_H$ 4.58 (H-29a) and 4.68 (H-29b) in addition with methyl proton signal of $\delta_H$ 1.68 (H-30) suggested the lupane-type backbone for **1**. The $^{13}$C-NMR together with DEPT-135 spectrum of **1** as tabulated in Table 1 illustrated thirty carbons signals consisting of six methines, twelve methylenes, six methyls and six quaternary carbons which further affirmed the pentacyclic triterpene skeleton. At $\delta_C$ 79.0 and 60.6, the presence of oxygen-bearing methine and methylene carbons at C-3 and C-28 can be notified. A set of exocyclic olefinic carbons at $\delta_C$ 109.7 and 150.5 was identified to be methylene carbon (C-29) and quaternary carbon (C-20) respectively. The resonances of this olefinic carbons with $\delta_C$ 109.7 of methyl carbon (C-30) together was later assigned to the isopropenyl moiety. According to $^{1}$H-$^{1}$H COSY spectrum, the cross peaks correlations between H-3/H-2, H-6a/H-5, H-19/H-18, H-19/H-21b, H-28b/H-28a, H-29b/H-29a, H-29a/H-30 and H-29b/H-30 demonstrated the inter-connection between the core of pentacyclic and the isopropenyl structure. These observations were further confirmed by the correlations shown in the HMBC experiment between H-18/C-13,C-19, C-28 and H-

19/C-13,C-18,C-21 (pentacyclic core) while between H-19/C-29, H-29b/C-30,C-19 and H-30/C-20,C-29 (isopropenyl group). Besides, at C-28 that was attached with methylene protons and hydroxyl group, the correlations of H-28a/C-16 and H-28b/C-22 suggested the connections of this substituent to the core group. The spectral data of **1** were compared with the literature [18].

Compound **2** (Lupeol) was obtained as white powder in 2.20% yield with the melting point range of 219–221 °C. The IR spectrum of **2** showed distinctive absorption at 3349 (O-H bond stretching), 2942 ($C_{sp}^2$-H stretching), 2871 ($C_{sp}^3$-H stretching), 1638 (C=C bond) and 1035 $cm^{-1}$ (C-O bond). By referring to the $^1$H and $^{13}$C-NMR spectra of **1**, **2** and **3** (Table 1), it can be observed that all three of these compounds possessed the similarity in chemical shifts which affirmed the lupane-type backbone. The $^1$H-NMR spectrum of **2** revealed seven singlet signals at $\delta_H$ 0.76, 0.79, 0.83, 0.94, 0.97, 1.03 and 1.68 showcase the presence of seven angular methyl protons corresponding to H-24, H-28, H-25, H-27, H-23, H-26, and H-30. Another finding show that proton H-3 appears to be a doublet of doublet at $\delta_H$ 3.18 (J=4.8 and 11.4 Hz). Further extracting from the spectrum, the presence of olefinic protons which represents the exocyclic double bond also can be found within the readings of $\delta_H$ 4.56 (H-29a) and 4.69 (H-29b). These two signals with the addition of a methyl proton signal at $\delta_H$ 1.68 (H-30) indicate the presence of an isopropenyl group in the structure of **2** which confirmed the main characteristics the lupane-type triterpenes. From the data displayed by the $^{13}$C-NMR spectrum and DEPT spectrum (Table 1), there are thirty carbon signals which consists of six methines, eleven methylenes, seven methyls and six quaternary carbons which established the pentacyclic triterpenoid backbone. Besides, the involvement of deshielded O-H group appeared at only one carbon of $\delta_C$ 79.1 (C-3) in comparison with **1** that attached with two hydroxyl moieties. The olefinic carbon of the exocyclic double bond was identified at the position of $\delta_C$ 151.1 and 109.5 which assigned to the quaternary carbon (C-20) and methylene carbon (C-29) that later resonates together at $\delta_C$ 19.5 (C-30) ascribed the isopropenyl group, which similar to **1**. By studying the COSY spectrum, it exhibited some cross peaks such as between $\delta_H$ 2.37 (H-19) and methylene proton signal of $\delta_H$ 1.91 (H-21) and also between oxygenated methine proton signal of $\delta_H$ 3.18 (H-3) and methylene proton of $\delta_H$ 1.59 (H-2). By further assignments of HMBC spectrum, the methine proton signal at $\delta_H$ 3.18 (H-3) revealed cross peaks with a methyl carbon signal of $\delta_C$ 15.5 (C-24) while with a methylene carbon of $\delta_C$ 27.5 (C-2) and a quaternary carbon of $\delta_C$ 39.0 (C-4). The multiplet methyl signal at $\delta_H$ 2.37 (H-19) indicated cross peaks with two methylene carbon signals of $\delta$c 30.0 (C-21) and $\delta$c 109.5 (C-29). Besides, this methyl signal cross peaks as well with a quaternary carbon signal of $\delta$c 151.1 (C-20) and a methyl carbon signal of $\delta$c 19.5 (C-30). The pair of broad singlets of olefinic proton at $\delta_H$ 4.56 and 4.69 revealed the cross peaks with a methylene carbon signal $\delta$c 48.1 (C-19) and $\delta$c 19.5 (C-30). After comparison with the spectroscopic data from literature [19], the structure of this compound is confirmed.

Compound **3** was isolated in red-brownish oil condition with the yield of 0.33%. The IR spectrum of **3** demonstrated the absorption bands at 2924 ($C_{sp}^2$-H stretching), 2854 ($C_{sp}^3$-H stretching), 1703 (C=O bond), and 1643 $cm^{-1}$ (C=C stretching). As mentioned before, the 1D- and 2D-NMR data of **3** will retained similar value and explanations to **1** and **2** with some variation. At position C-3, the $\alpha$-oriented hydrogen of 3$\beta$-hydroxy triterpene was replaced by the C=O which shift the neighboring protons to more downfield region of $\delta_H$ 1.39 (H-1a), 1.90 (H-1b), 2.47 (H-2), 0.77 (H-5), 1.25 (H-23), 1.02 (H-24) and 1.07 (H-25). Besides, the furthest downfield signals at $\delta_C$ 218.2 signified the carbonyl carbon of C-3. Upon conclusion, the data from $^1$H and $^{13}$C-NMR spectra of **3** were identical with the literature [20].

**Cytotoxic Activity**

In order to examine the cytotoxicity effects of the isolated compounds from *Diospyros foxworthyi*, MTT cell viability assay was conducted by measuring the metabolic activity on HT-29 colon cancer cells with cisplatin as standard reference. Figure 2 highlighted the concentration of tested compounds towards cancer cell viability percentage. The results showed that two of the compounds treatment were able to suppress the growth of cancer cell in dose-dependent manner. The percentage of viable cells decreasing as the concentration of the compounds increasing (0, 20, 40, 60, 80 and100 µM). The cytotoxic activity were categorized based on $IC_{50}$ value and categorized as very strong (<5 µg/mL), strong (<5–10 µg/mL), moderate (10–20 µg/mL), weak (20–100 µg/mL) and not active (>100 µg/mL) [21]. The $IC_{50}$ of **2** is 37.57 ± 4.11 µM (equivalent to 16.03 ± 1.76 µg/mL), whereas the value of $IC_{50}$ for **1** is 13.49 ± 0.53 µM (equivalent to 5.98 ± 0.23 µg/mL). In contrast, there are no $IC_{50}$ value recorded for **3** and cisplatin treated cells (>100 µM) indicating the profound effect of **1** and **2** on colon cancer cell lines. This data suggested that the treatment of **1** is more effective than **2** against HT-29 colon cancer cells and could be a potential antiproliferative agent [22-23].

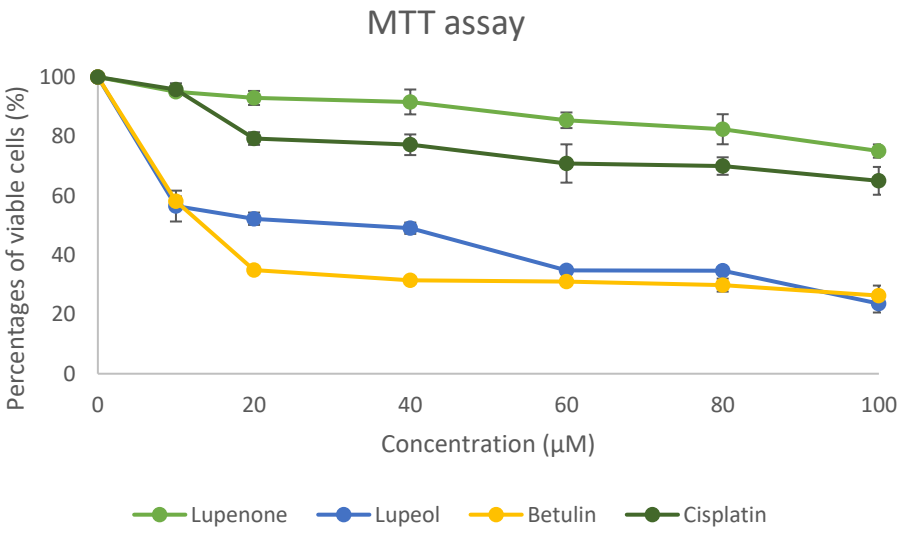


**Figure 2.** Dose-response curve of compounds **1**–**3** on HT-29 colon cancer cell lines.

**Table 2.** Cytotoxic activity of compounds **1**–**3**

| Compound | $IC_{50}$ (µM) | Viable cells (%) at 100 µM |
|---|---|---|
| Betulin (**1**) | 13.49 ± 0.53 (Moderate) | 26.34 ± 3.37** |
| Lupeol (**2**) | 37.57 ± 4.11 (Moderate) | 23.63 ± 3.00** |
| Lupenone (**3**) | >100 (Weak) | 75.06 ± 2.23 |
| Cisplatin | >100 | 65.01 ± 4.69 |

P-value ANOVA between the compound and cisplatin,
*P<0.05, **P<0.05, Mean ± Standard Deviation

## CONCLUSION

The chemical composition of the EtOAc extract of *Diospyros foxworthyi* had led to the isolation of three interesting pentacyclic triterpenes of lupane-backbone namely betulin (**1**), lupeol (**2**) and lupenone (**3**). The characterisation of these compounds was performed with the aid of the various spectroscopic methods. All of these compounds were tested against HT-29 human colorectal adenocarcinoma cell lines for the cytotoxicity analysis. Two compounds, which were betulin (**1**) and lupeol (**2**) showed moderate cytotoxic effect with $IC_{50}$ value of 13.49 ± 0.53 and 37.57 ± 4.11 µM, respectively. By comparison, the treatment of betulin was more effective than cisplatin (control) against the colon cancer cells thus providing a better prospects as antiproliferative agent.

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