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Sesquiterpenoids from the Stem Bark of *Dysoxylum excelsum* and Their Cytotoxic Activities against HeLa Cancer Cell Lines

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ABSTRACT. Sesquiterpenoids belong to a group of terpenoid compounds with interesting structures that are abundant in natural products especially in higher plants. Sesquiterpenoids have a wide variety of bioactivities with great potential cytotoxic activity. The species *Dysoxylum excelsum* belongs to the Meliaceae family known as higher plant, but only a few sesquiterpenoids have been reported particularly for their cytotoxic activity. Therefore, this research aims to isolate and elucidate the sesquiterpenoids structure from *D. excelsum* stem bark and examines their cytotoxicity against HeLa cervical cancer cells. Through various column chromatography separations, four known sesquiterpenes namely *β*-caryophyllene oxide (**1**), caryophyllenol II (**2**), humulene dioxide A (**3**), and guai-6-en-10*β*-ol (**4**) were acquired from the *n*-hexane extract. Compounds **1-4** were isolated for the first time from *D. excelsum* species. The sesquiterpenoid structures were elucidated according to Nuclear Magnetic Resonance, Infrared, and HR-TOF-MS analysis. The cytotoxicity compounds **1-4** was determined against HeLa cervical cancer cells by examination with the PrestoBlue method and compound **3** exhibited the most potent cytotoxicity with an IC_{50} value of 160.74 μM.

Keywords: Cytotoxic activity, *Dysoxylum excelsum*, HeLa, Meliaceae, sesquiterpenoid.

INTRODUCTION

Sesquiterpenoids are a wildly fascinating group of terpenoids with more than 10.000 types up till now, on account of the presence of FPP (farnesyl pyrophosphate) as a precursor, which bears a triple bond and pyrophosphate that gives flexibility of carbon chain (Kouloura et al., 2014; Ludwiczuk *et al.*, 2017). Their structure diversity are classified based on their skeleton, the number and formation of cyclic, alkyl positions, and functional groups (Fraga, 2013). Some of the main sources of sesquiterpenoids were produced from natural materials, with the most abundant being found in higher plants (Lorigooini *et al.*, 2020). In addition, sesquiterpenoids have a variety of pharmacological effects such as antiviral (Yarovaya & Salakhutdinov, 2021), antibacterial (Dharmayani *et al.*, 2016), anti-inflammatory (Mora-Ramiro *et al.*, 2020), antimalaria (White *et al.*, 2015), antifeedant (Inocente *et al.,* 2019), and cytotoxic activities (Fidyt *et al.*, 2016; Saavedra *et al.*, 2020). Dhyani *et al.* (2022) reported, cytotoxic activity is the most potential bioactivity of the sesquiterpenoid group.

The *Dysoxylum* genus belongs to the Meliaceae family, with a tree height characteristic of ± 36 m. Around 110 species of the genus *Dysoxylum* have been found worldwide, scattering in tropical and subtropical areas that possess rain forests, such as Asia-China, India, Malaysia, Solomon Islands, Northeast Australia and other Southeast Asian countries (Heads, 2019), including 80 species have been found in Indonesia (Parcha *et al.*, 2004). The genus *Dysoxylum* plants are commonly used as traditional medicinal for various diseases, such as the leaves of *D. richii*, which are made into a tea drink to provide pain relief (Lakshmi *et al.*, 2009) as well as *D. binectariferum* is used by the Tharu people in India as a medicine for cancer, bone infections and skin diseases (Arya *et al.*, 2017). As of now, approximately fifty-three sesquiterpenoids have been successfully isolated and reported from this genus (Riyadi *et al.*, 2023). The manifoldness and enchanting structures of sesquiterpenoid that has been discovered makes it noteworhty from this genus. As found in *D. parasiticum* (osbeck) kosterm stem bark, forming a

dimeric structure namely dysotican A and B with their moderate cytotoxic activities against HeLa and MCF cancer cells (Naini *et al.*, 2023a). Along with trimeric sesquiterpene phenols that are tridysoxyphenols A and B (Sofian *et al.,* 2022). Furthermore, a new compound such a dysosesquiflorins A from *D. densiflorum* was shown as active cytotoxicity against HL-60 leukemia cancer cells with IC_{50} value of 3.1 μM (Nugroho *et al.*, 2015).

Subtle reports have been comprised on isolating sesquiterpenoid from *D. excelsum*. Solely thirteen sesquiterpenoids have been isolated from this species, consisting of eudesmane, guaiane, isodaucane, cadinene, hidroazulene, oppositane, and maaliane types (Liu *et al.*, 2012; Mayanti *et al.*, 2019). Even to date, there has not been reported their bioactivity, specifically cytotoxic activity. Hence, this study describes the isolation, identification, and examination of four known sesquiterpenoid compounds (**1-4**) from *D. excelsum* stem bark with their cytotoxicity against HeLa cervical cancer cells as a first report of this species bioactivity.

EXPERIMENTAL SECTIONS

Material and Methods

Evaporation process using a rotary evaporator type R-215 Buchi with a V-700 Buchi vacuum system, B-491 Buchi water bath, and F-100 cooling circulator. Compound isolation was led by TLC (Thin Layer Chromatography) analysis, conducted on SiO_2 60 F_{254} (Merck) and RP (Reversed Phase) -18 F_{254s} plates (Merck) using various solvent systems with spraying 10% sulfuric acid in ethanol through detection by Vilbert Luomart UV detector lamps (λ at 254 and 365 nm). Fractionation was performed with column chromatography in normal phase (SiO_2 60, 230-400 Mesh and 70-230 Mesh, Merck) and reversed-phase (ODS, 100–200 mesh, Chromatorex® C_{18} DM1020T, Fuji Sylisia Chemical, LTD.), including vacuum liquid chromatography (VLC). The infrared spectra were obtained with Shimadzu 8400 FTIR using the KBr plate. Mass measurement using a Waters Q-TOF-HR-TOF-MS-XEV^{otm} mass spectrometer. Characterization compound uses an NMR instrument with a JEOL Delta type ECA 500 spectrometer (^{1}H-NMR, ^{13}C-NMR, and NMR-2D). Cytotoxic activity testing using PrestoBlue® reagent, 96 plate wells, incubators, and microplate readers at λ at 570 nm.

Plant Collection

The stem bark from *Dysoxylum excelsum* (Spreng.) Blume ex G.Don (https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:578122-1) plant was acquired in January 2021 from alongside of the Manggar River area in Balikpapan, Indonesia. Specimen were determined by Herbarium Wanariset (WAN) in East Kalimantan with specimen number FF12.21.

Extraction and Isolation

Whole specimen of the dried *Dysoxylum excelsum* stem bark (600 g) was macerated with 70% ethanol at room temperature overnight (4 × 4L). The crude ethanol extract (49.59 g) was obtained by solvent removal. Moreover, ethanol crude extract was solvated in water and extracted excessively, evaporated, and yielded crude as much *n*-hexane (49.59 g), EtOAc (4.03 g), and *n*-BuOH (6.30 g). The concentrated extract *n*-hexane was fractionated with VLC (*n*-hexane/EtOAc/MeOH, 100:0:0-0:50:50, 10% gradient) to provided seven fractions (A-G).

Subsequently, fraction B (3.71 g) was separated in normal-phase column chromatography (*n*-hexane /$CHCl_3$/EtOAc, 100:0-60:40, gradient 10%) and yielded nine fractions (B1-B9). Fraction B5 (501.1 mg) was chromatographed further in normal-phase (*n*-hexane/$CHCl_3$/EtOAc, 10:5:0.5, isocratic) to afford seven fractions (B5A-B5G). Furthermore, fraction B5D was parted by normal-phase column chromatography (*n*-hexane/$CHCl_3$, 9:1, isocratic) and obtained four fractions (B5D1-B5D4). In fraction B5D4, compound **1** was obtained by reversed-phase column chromatography (MeOH/ H_2O, 8:2, isocratic).

The C fraction (4.98 g) was further fractionated with column chromatography in normal phase (*n*-hexane/ EtOAc, 100:0-60:40, 2.5% gradient) to give eight fractions (C1-C8). Fraction C4 (168 mg) was chromatographed in normal-phase (*n*-hexane/$CHCl_3$/EtOAc, 10:3:1, isocratic) to yield four fractions (C4A-C4D). A fraction of C4C was purified using reversed-phase column chromatography (MeCN/MeOH/H_2O, 1:6:3, isocratic) to produce compound **4** (6.8 mg). The C5 fraction (691.2 mg) was fractionated using chromatography in normal phase column (*n*-hexane/$CHCl_3$/EtOAc, 30:3:1, isocratic) to obtain seven fractions (C5A-C5G). Moreover, C5D fraction (313.2 mg) was separated using a normal phase chromatography column (*n*-hexane/EtOAc, 75:1, isocratic) with the addition of formic acid to create an acidic environment and yielded nine fractions (C5D1-C5D9). Fraction C5D1 was purified by column chromatography in reverse-phase (MeCN/ H_2O, 5:5, isocratic) to give compound **2** (4.1 mg). A C5G fraction (61.3 mg) was refined by reversed-phase chromatography (MeOH: H_2O, 8:2, isocratic) to yield compound **3** (4.7 mg).

β-Caryophyllene oxide (1)

Isolated as a colorless oil. $C_{15}H_{24}O$. IR (KBr) ν_{max} cm^{-1} 3050, 2950, 1650, 1350, 1100. ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 1.74 (1H, t, *J*=9.1 Hz, H-1), 1.65 (1H, m, H-2a), 1.61 (1H, m, H-2b), 2.06 (1H, m, H-3a), 2.09 (1H, m, H-3b), 2.86 (1H, dd, *J*= 4.3, 10.6 Hz, H-5), 2.07 (1H, m, H-6a), 2.11 (1H, m, H-6b), 2.23 (1H, m, H-7a), 2.32 (1H, m, H-7b), 2.60 (1H, q, *J*=9.1 Hz, H-9), 1.67 (1H, m, H-10a), 1.59 (1H, m, H-10b), 1.18 (3H, s, H-12), 4.95 (1H, d, *J*=1.7 Hz, H-13a), 4.84 (1H, s, H-13b), 0.98 (3H, s, CH_3-14),

0.96 (3H, s, CH_3-15). ^{13}C-NMR data (Table 1). HR-TOF-MS: peak at *m/z* 219.2551 $[M-H]^+$ (calculated as 219.1827).

Caryophyllenol II (2)

Isolated as a colorless oil. $C_{15}H_{24}O$. IR (KBr) ν_{max} cm^{-1} 3400, 2951, 1710, 1631, 1451, 1367, 1016, 888. ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 1.54 (1H, m, H-1), 2.08 (1H, m, H-2a), 1.91 (1H, m, H-2b), 5.55 (1H, t, *J*= 8.1, 1.7 Hz, H-3), 4.77 (1H, dd, *J*=11.4, 5.7 Hz, H-5), 1.70 (1H, m, H-6a), 1.60 (1H, m, H-6b), 1.84 (1H, br t, *J*= 13.1 Hz, H-7a), 2.17 (1H, qd, *J*= 13.9, 6.7, 1.9 Hz, H-7b), 2.66 (1H, q, *J*= 9.2, 2.4 Hz, H-9), 1.95 (1H, m, H-10a), 1.56 (1H, m, H-10b), 1.62 (3H, br s, H-12), 4.48 (1H, t, *J*= 2.4, H-13a), 4.73 (1H, q, *J*= 2.4, H-13b), 1.00 (3H, s, CH_3-14), 0.95 (3H, s, CH_3-15). ^{13}C-NMR data (Table 1). HR-TOF-MS: peak at *m/z* 221.1733 $[M+H]^+$ (calculated as 221.1702).

Humulene diepoxide A (3).

Isolated as a colorless oil. $C_{15}H_{24}O_2$ IR (KBr) ν_{max} cm^{-1} 2960, 1711, 1684, 1468, 1389, 1188, 980. ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 1.37 (1H, m, H-1a), 1.61 (1H, d, *J*= 14.3 Hz, H-1b), 2.48 (1H, d, *J*=9.8 Hz, H-3), 1.10 (1H, m, H-4a), 2.13 (1H, m, H-4b), 1.40 (1H, m, H-5a), 2.21 (1H, m, H-5b), 2.72 (1H, dd, *J*= 10.3, 5.1 Hz, H-6), 1.65 (1H, m, H-8a), 2.64 (1H, dd, *J*= 10.2, 5.1 Hz, H-8b), 5.47 (1H, ddd, *J*=15.6, 10.2, 4.5 Hz, H-9), 5.30 (1H, d, *J*=15.6, H-10), 1.30 (3H, s, CH_3-12), 1.30 (3H, s, CH_3-13), 1.19 (3H, s, CH_3-14), 1.07 (3H, s, CH_3-15). ^{13}C-NMR data (Table 2). HR-TOF-MS: peak at *m/z* 237.1777 $[M+H]^+$ (calculated as 237.1775).

Guai-6-en-10*β*-ol (4).

Isolated as a colorless oil. $C_{15}H_{26}O$. IR (KBr) ν_{max} cm^{-1} 3376, 2956, 1713, 1647, 1463, 1378, 1126, 880. ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 1.92 (1H, m, H-1), 1.68 (1H, m, H-2a), 1.77 (1H, m, H-2b), 1.36 (1H, m, H-3a), 1.68 (1H, m, H-3b), 2.19 (1H, m, H-4), 2.21 (1H, m, H-5), 5.46 (1H, d, *J*= 3.5 Hz, H-6), 1.92 (1H, m, H-8a), 2.17 (1H, m, H-8b), 1.42 (1H, m, H-9), 5.30 (1H, d, *J*=15.6, H-10), 2.13 (1H, m, H-11), 0.96 (3H, d, *J*= 6.9 Hz, CH_3-12), 0.97 (3H, d, *J*= 6.9 Hz, CH_3-13), 1.21 (3H, s, CH_3-14), 0.97 (3H, d, *J*= 6.3 Hz, CH_3-15). ^{13}C-NMR data (Table 2). HR-TOF-MS: peak at *m/z* 223.2056 $[M+H]^+$ (calculated as 223.1905).

Cytotoxic Assay In Vitro

The cytotoxic activity **1-4** against HeLa cervical cancer cells was determined by the PrestoBlue® method based on observing cell viability with the presence of a resazurin base. Initiates with the cell culture stage by maintaining the cells in 96 wells with Roswell Park Memorial Institute Medium (RPMI), consist Fetal Bovine Serum (FBS) 10% and 1 µL/mL of antibiotic (1% of penicillin). Maintained for 48 h at 37°C with 5% CO_2 gas until a density attained to 17.000 cells/well. Thereafter, the medium was disposed and the sample in the new medium was added in increasing concentrations of 10% along with cisplatin as a positive control and incubated for 48 h. Subsequently, substituted the medium by 10% PrestoBlue® reagent and maintained for 1-2 h. Observation was conducted with a multimode reader for the absorption analysis at 570 nm. Acquired absorption results are processed into percent cell viability to achieve the IC_{50} value of compounds **1-4** against HeLa cancer cells.

RESULTS AND DISCUSSION

After being subjected to various chromatography fractionation, the crude *n*-hexane extract from the *Dysoxylum excelsum* stem bark affords four known sesquiterpenoids compound **1-4** (**Figure 1**).

Structure Elucidation

Compound **1,** colorless oil, was assigned as $C_{15}H_{24}O$ by its HR-TOF-MS ion peak *m/z* 219.2551 $[M+H]^+$ and corresponding to four unsaturation degrees. The IR spectrum indicated absorptions of existence C-H sp^2 (2950 cm^{-1}), C=C (1650 cm^{-1}), *gem*-dimethyl (1462 & 1350 cm^{-1}), and C-O (1100 cm^{-1}). Analysis data of ^{1}H-NMR implied the existence of three tertiary methyls at δ_H 1.18 (3H, s, CH_3-12), 0.98 (3H, s, CH_3-14), and 0.96 ppm (3H, s, CH_3-15) along with two olefinic *gemінal*-protons at δ_H 4.84 (1H, d, *J*=1.8, CH_2-13a) and 4.95 ppm (1H, d, *J*=1.8, CH_2-13b). Moreover, analysis ^{13}C-NMR with DEPT 135° implied the existence of fifteen carbons with three methyls sp^3 [δ_C 17.1 (C-12), 21.7 (C-14), 30.0 (C-15)], one methylene sp^2 [δ_C 112.9 (C-13)], five methylenes sp^3 [δ_C 27.3 (C-2), 39.2 (C-3), 29.8 (C-6), 30.3 (C-7), 39.8 (C-10)], one oxygenated methine sp^3 [δ_C 63.9 (C-5)], two methines sp^3 [δ_C 50.7 (C-1), 48.8 (C-9)], one oxygenated quaternary carbon sp^3 [δ_C 59.9 (C-4)], one quaternary sp^2 [δ_C 151.9 (C-8)], and one quaternary carbon sp^3 [δ_C 34.1 (C-11)]. A pair of olefinic carbons at δ_C 151.9 (C-8) and δ_C 112.9 (C-13) form one terminal olefinic. The non-existence of OH groups in the IR spectrum indicates that the oxygenated carbon

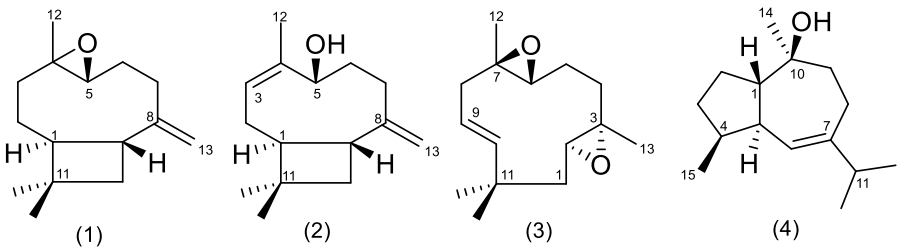

Figure 1. Structures of compounds **1-4**

forms an ether bond by forming an epoxide ring with the presence of two oxygenated carbons. Based on 1D-NMR and IR analysis provides two out of four degrees of unsaturation, indicating **1** is a sesquiterpenoid with a bicyclic core. From the obtained data analysis with the biogenesis literature approach, known the caryophyllene-type is one of the bicyclic sesquiterpenoids found in Meliaceae. Compound **1** has likeness data features with caryophyllene-type sesquiterpenoid by comparing the number of carbon types present. Presuming the position of functional groups built upon the biosynthesis pathway for caryophyllene-type formation, with the epoxide ring position at C-4/5 and terminal olefin at C-8/13. It is known that compound **1** has data similarity to the isolated caryophyllene oxide compound by Milawati *et al.* (2019). Accordingly, compound **1** was defined as caryophyllene oxide.

Compound **2** was procured as a colorless oil. Given a $C_{15}H_{24}O$ as molecular formula with four-degree unsaturation by analysis HR-TOF-MS with ion peak at *m/z* 221.1733 $[M+H]^+$. The IR spectrum **2** resembles spectrum compound **1** with an addition of strong absorption of OH (3400 cm^{-1}). The ^{1}H-NMR spectrum showed the signal of three methyls in tertiary carbon at δ_H 1.62 ppm (3H, s, CH_3-12), 1.00 ppm (3H, s, CH_3-14), and 0.95 ppm (3H, s, CH_3-15). The spectrum of ^{13}C-NMR demonstrated the occurrence of fifteen carbons signals, classified guided by the DEPT 135° spectrum, which resulted in three methyls sp^3 [δ_C 22.8 (C-12), 30.0 (C-13), 15.7 (C-15)], one methylene sp^2 [δ_C 109.8 (C-13)], four methylenes sp^3 [δ_C 28.6 (C-2), 34.3 (C-6), 32.6 (C-7), 39.7 (C-10)], one methine sp^2 [δ_C 126.1 (C-4)], four methines sp^3 [δ_c 50.3 (C-1), 42.6 (C-9), 126.1 (C-12)] along with one oxygenated carbon [δ_c 69.7 (C-5)], two quaternary carbons sp^2 [δ_C 137.7 (C-4), 154.8 (C-8)], and one quaternary carbon sp^3 [δ_C 33.2 (C-11)]. Compound **2** requires two olefinic bonds based on ^{13}C-NMR data, hence completing two out of the four degrees of unsaturation to indicate bicyclic as a sesquiterpenoid core structure. Comparing the NMR data of compounds **2** and **1** shows similarities. Distinctness is on the existence of hydroxyl signal in **2** replacing epoxide ring (C-5) and addition of olefinic (C-3/4). Has been reported by Ninh *et al.*, (2022), a compound with identical data to compound **2** as caryophyllenol-II. Therefore, compound **2** was assigned as caryophyllenol-II.

Compound **3** was acquired as a colorless oil with given molecular formula of $C_{15}H_{24}O_2$ based on analysis HR-TOF-MS showed a molecular ion peak at *m/z* 237.1777 $[M+H]^+$ and requiring four-degree unsaturation. IR absorption has a spectrum similarity with **1**, showing the presence of an olefinic, *gem*-dimethyl, and ether bond. The ^{1}H-NMR identified the occurrence of four tertiary methyls at δ_H 1.07 (3H, s, CH_3-15), 1.19 (3H, s, CH_3-14), and 1.30 ppm (6H, s, CH_3-14,15). The signal at δ_H 1.30 ppm with six carbons indicates two methyl groups with identical environments. The ^{13}C-NMR and DEPT 135° spectra confirmed fifteen carbons containing four methyls sp^3 [δ_c 16.5 (C-12), 16.5 (C-13), 23.4 (C-14), 30.8 (C-15)], four methylenes sp^3 [δ_c 38.4 (C-1), 34.9 (C-4), 25.2 (C-5), 43.4 (C-8)], two methines sp^2 [δ_c 122.6 (C-9), 142.9 (C-10)], two methines sp^3 [δ_c 64.8 (C-2), 63.5 (C-6)], and three quaternary carbons sp^2 [δ_c 60.2 (C-3), 60.4 (C-7), 35.7 (C-11)]. The presence of oxygenated carbons was assumed to form an ether bond with the inexistence of OH moiety in the IR spectrum. Confirmed by comparing the NMR chemical shift data of compounds **3** to **1**, giving presumptions that the ether bonds in compound **3** forms two epoxide rings from four oxygenated carbons. It is known **3** has four-degree unsaturation, one fulfilled by the existence of one olefinic bond and two by the epoxide rings. Therefore, **3** has one cyclic core in its sesquiterpenoid structure. Based on the comparison of analysis data with **1** and **2**, there is a share of similarity features and chemical shift data in between. Thus, a literature approach to the biogenesis and biosynthesis of compounds **3** with **1**-**2** is carried out, acknowledging the monocyclic sesquiterpenoid structure that qualifies it as humulene. Known the structures of caryophyllene and humulene share the same analogs, namely humulyl cation (Dewick, 2009). A similar approach is carried out in the placement of functional groups by a biosynthesis pathway for the arrangement of humulene, assuming an epoxide ring at C-2/3 and C-6/7 along with an olefinic bond at C-9/10. Comparing NMR data compound **3** with a known compound that is humulene dioxide A, reported by Heymann *et al.* (1994), both showed indistinguishable signals. Hence, compound **3** was determined as a humulene dioxide A.

Compound **4** was acquired as a colorless oil. Assigned the molecular formula as $C_{15}H_{27}O$ with three-degree unsaturation based on HR-TOF-MS ion peak at *m/z* 223.2056 $[M+H]^+$. The IR spectrum of compound **4** exhibited a strong absorption band for OH (3376 cm^{-1}), C-H sp^2 (2956 cm^{-1}), C=C (1713 & 1647 cm^{-1}), *gem*-dimethyl (1463 & 1378 cm^{-1}), and C-O (1126 cm^{-1}). According to ^{1}H-NMR analysis, compound **4** has four methyls with one methyl in tertiary position at δ_H 1.21 ppm (3H, s, CH_3-14) along with three secondary methyls at δ_H 0.96 (3H, d, *J*= 6.9 Hz, CH_3-14a), 0.97 (3H, d, *J*= 6.9 Hz, CH_3-14b), and 0.87 ppm (3H, d, *J*= 6.3 Hz, CH_3-15), including one signal olefinic proton at δ_H 5.46 ppm (1H, d, *J*=3.5 Hz, H-6). The presence of a *gem*-dimethyl bonded to a methine is indicated by the appearance of two methyls protons at δ_H 0.96 and 0.97 ppm with a doublet multiplicity, thereby both mutually coupled by one proton and providing identical coupling values. Spectra of ^{13}C-NMR and DEPT 135° showed resonance for four methyls [δ_c 21.3

(C-12), 21.4 (C-13), 21.6 (C-14), 15.3 (C-15)], four methylenes sp^3 [δ_c 24.0 (C-2), 33.2 (C-3), 25.2 (C-8), 42.6 (C-9)], one methines sp^2 [δ_c 124.1 (C-6)], four methines sp^3 [δ_c 51.3 (C-1), 37.2 (C-4), 43.8 (C-5), 37.6 (C-11)], one quaternary sp^2 [δ_c 148.3 (C-7)], with one quaternary carbon sp^3 [δ_c 75.7 (C-10)]. Whereabouts of one olefin in the structure leaves two degrees of unsaturation, concluded compound **4** is a bicyclic sesquiterpenoid. It has been investigated that the *gem*-dimethyl moiety bonded to methine provides other possible structures, with biogenesis literature approaches in *Dysoxylum*, namely guaiane as the main structure for compound **4**. The possible olefinic moiety position is at C-6/7, supported by the appearance of a single olefinic proton with a multiplicity of doublet at δ_H 5.46 ppm and biosynthesis pathways in an arrangement of olefinic bond in guaiane. The similarity of the NMR data regarding the position of their functional groups with their chemical shift signals between compound **4** and guai-6-en-10*β*-ol (as reported by Lago *et al.* (2000)) gives a closure. As a result, compound **4** was reported as guai-6-en-10*β*-ol.

Table 1. Comparison of ^{13}C-NMR data of compound **1-2** ($CDCl_3$, 125 MHz) and literatures

Carbon Position	Compounds			
	1	Caryophyllene Oxide *	2	Caryophyllenol-II **
	δ_C (mult.)	δ_C (mult.)	δ_C (mult.)	δ_C (mult.)
1	50.7 (d)	50.7 (d)	50.3 (d)	50.3 (d)
2	27.3(t)	27.3(t)	28.6 (t)	28.5 (t)
3	39.2 (t)	39.2 (t)	126.1 (d)	125.9 (d)
4	59.9 (s)	59.7 (s)	137.7 (s)	137.7 (s)
5	63.9 (d)	63.9 (d)	69.7 (d)	69.7 (d)
6	29.8 (t)	29.8 (t)	34.3 (t)	34.3 (t)
7	30.3 (t)	30.3 (t)	32.6 (t)	32.5 (t)
8	151.9 (s)	151.9 (s)	154.8 (s)	154.7 (s)
9	48.8 (d)	48.8 (d)	42.6 (d)	42.5 (d)
10	39.8 (t)	39.8 (t)	39.7 (t)	39.6 (t)
11	34.1 (s)	34.1 (s)	33.2 (s)	33.1 (s)
12	17.1 (q)	17.1 (q)	15.7 (q)	15.6 (q)
13	112.9 (t)	112.9 (t)	109.8 (t)	109.7 (t)
14	21.7 (q)	21.7 (q)	22.8 (q)	22.7 (q)
15	30.0 (q)	30.0 (q)	30.0 (q)	29.9 (q)

*($CDCl_3$, ^{13}C-NMR 100 MHz)
**($CDCl_3$, ^{13}C-NMR 125 MHz)

Table 2. Comparison of ^{13}C-NMR data of compound **3-4** ($CDCl_3$, 125 MHz) and literatures

Carbon Position	Compounds			
	3	Humulene dioxide A *	4	Guai-6-en-10*β*-ol **
	δ_C (mult.)	δ_C (mult.)	δ_C (mult.)	δ_C (mult.)
1	38.4 (t)	38.4 (t)	51.3 (d)	51.2 (d)
2	64.8 (d)	64.7 (d)	24.0 (t)	23.9 (t)
3	60.2 (s)	60.1 (s)	33.2 (t)	33.1 (t)
4	34.9 (t)	34.9 (t)	37.2 (d)	37.2 (d)
5	25.2 (t)	25.2 (t)	43.8 (d)	43.8 (d)
6	63.5 (d)	63.5 (d)	124.1 (d)	124.0 (d)
7	60.4 (s)	60.4 (s)	148.3 (s)	148.2 (s)
8	43.4 (t)	43.4 (t)	25.2 (t)	25.1 (t)
9	122.6 (d)	122.6 (d)	42.6 (t)	42.6 (t)
10	142.9 (d)	142.9 (d)	75.7 (s)	76.0 (s)
11	35.7 (s)	35.7 (s)	37.6 (d)	37.6 (d)
12	16.5 (q)	16.6 (q)	21.3 (q)	21.2 (q)
13	16.5 (q)	16.6 (q)	21.4 (q)	21.3 (q)
14	23.4 (q)	23.4 (q)	21.6 (q)	21.4 (q)
15	30.8 (q)	30.8 (q)	15.3 (q)	15.2 (q)

*($CDCl_3$, ^{13}C-NMR 100 MHz)
**($CDCl_3$, ^{13}C-NMR 125 MHz)

Table 3. The cytotoxic activity against HeLa cells of compounds **1-4** (IC_{50} /μM)

Compounds	IC_{50} (μM)
1	290.67
2	160.74
3	>1000
4	182.94
Cisplatin (+)	19.00

Cytotoxic Activity

All four sesquiterpenoid were examined for their cytotoxicity against HeLa cervical cancer cells using the PrestoBlue assay by comparing cisplatin as a positive control, as described previously (Boncler *et al.*, 2014; Naini *et al.*, 2023b). Notably, the HeLa cell line is one of the most abnormally fast-growing cervical cancer cells and has been reported to be resistant to current drugs (Muniandy *et al.*, 2021; Zhu *et al.*, 2016). In **Table 3**, all compounds **1-4** showed the cytotoxic activity results against HeLa cell line, which are classified as inactive due to their IC_{50} values were higher than 100 μM (Boncler *et al.*, 2014; Naini *et al.*, 2023b). These findings were consistent with previous results, such as guai-6-en-10*β*-ol (**4**) that also showed no activity against HeLa cell (Naini *et al.*, 2023a). Furthermore, the evaluation showed that caryophyllenol II (**2**) exhibited the strongest cytotoxic activity compared to the rest of compound with IC_{50} value of 160.74 μM. Their activity indicated to be affected by the presence of hydroxyl groups present in compound **2** and even in compound **4**. Whereas the presence of an epoxide group in the structure can reduce the activity of compounds against HeLa cancer cells such as in compounds **1** and **3**. The cyclic number also contributes to its cytotoxic activity as bicyclic formation in compound **1** showed stronger activity rather than monocyclic in compound **3**, which both are derivatives of humulyl cation.

CONCLUSIONS

Four known sesquiterpenoids acquired from *n*-hexane extract of the *Dysoxylum excelsum* stem bark were obtained namely, *β*-caryophyllene oxide (**1**), caryophyllenol II (**2**), humulene dioxide A (**3**), and guai-6-en-10*β*-ol (**4**). For the first time, compound **1-3** were found in genus *Dysoxylum* and compound **4** first time in this species. Compound **2** showed the most potent cytotoxicity against HeLa cancer cells with an IC_{50} value of 160.74 μM through the existence of hydroxyl moiety in structure. Moreover, the occurrence of epoxide ring and lack of cyclic in humulyl cation derivative can decreasing the cytotoxic activity against HeLa cancer cell.

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