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Terpenoids from The Stem bark of *Aglaia elaeagnoidea* and Their Cytotoxic Activity against HeLa and DU145 Cancer Cell lines

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ABSTRACT. *Aglaia* is the largest genus of the Meliaceae family which contains terpenoid compounds. This type of compounds showed a diverse structures and biological activities that can be found in natural resources. *Aglaia elaeagnoidea* is a species from *Aglaia* genus that only has a few previous research. This study was aimed to isolate and determine the chemical structure of terpenoid compounds from the ethyl acetate extract of *A. elaeagnoidea* stem bark. Ethyl acetate extracts were separated and purified by various chromatographic techniques to obtain compounds **1-5**. Compounds **1-5** were identified their chemical structures by spectroscopic methods (IR, MS, and NMR) and comparison with previous reported spectral data. Compounds **1** and **2** were identified as eudesmane-type sesquiterpenoids, 5-*epi*-eudesm-4(15)-ene-1β,6β-diol (**1**) and 6*α*-Hydroxy-eudesm-4(15)-en-1-one (**2**). Compounds **3-5** were identified as dammarane-type triterpenoids, 20*S*,24*S*-epoxy-25-hydroxydammarane-3-one (**3**), 20*S*,24*S*-epoxydammarane-3*α*,25-diol **(4)**, and 3*α*-epi-cabraleahydroxy lactone (**5**). These compounds are first time reported from this plant. Compounds **1-5** were tested for cytotoxicity against HeLa cervical cancer cell and DU145 prostate cancer cell and as a result, compound **4** (20*S*,24*S*-epoxydammarane-3*α*,25-diol) showed the stronger activity compared to other compounds.

Keywords: *Aglaia elaeagnoidea*, DU145 prostate cancer cell, HeLa cervical cancer cell, Meliaceae, terpenoids.

INTRODUCTION

Aglaia is the largest genus of the Meliaceae family (Pannell, 2018), consisted of over 150 species of woody plants mainly distributed in tropical and subtropical regions such as Sri Lanka, India, south China, Taiwan, Vietnam, Malaysia, the Philippines, and Australia including the pacific islands (Ebada, Lajkiewicz, Porco, Li-Weber, & Proksch, 2011; Grudinski, Pannell, Chase, Ahmad, & Muellner-Riehl, 2014), and more than 65 species only grow in Indonesia (Hidayat et al., 2017; Farabi et al., 2018). Since 1965, studies on the investigation of chemical constituents from *Aglaia* plant have been isolated of various types of compounds, such as sesquiterpenoids of caryophyllene-type (Kurniasih et al., 2019; Milawati et al., 2019), aromadendrane-type (Milawati et al., 2020), cadinene-type (Pointinger et al., 2008), isodaucane-type (Pan et al., 2013), eudesmane-type (Pan et al., 2010), guaiane-type (Liu et al., 2014), and dolabellane-type diterpenoids (Cai, Wang, Zhao, Li, & Luo, 2010), triterpenoids of dammarane-type (Joycharat, Plodpai, Panthong, Yingyongnarongkul, & Voravuthikunchai, 2010; Hidayat et al., 2018; Harneti et al., 2019), tirucallane-type (Benosman et al., 1995), apotirucallane-type (Xie et al., 2007), Cycloartane-type (Inada et al., 2001; Awang et al., 2012), glabretal-type (Su et al., 2006), protostane-type (Yodsaoue et al., 2012), lupane-type, and ursane-type (Zhang, Xu, Song, Chen, & Wen, 2012), steroids (Mohammad et al., 1999; Hutagaol et al., 2020), protolimonoids (Farabi et al., 2017), lignans (Sianturi et al., 2016), alkaloids (Sianturi et al., 2015), and flavaglins (Kim et al., 2006; An et al., 2016). Terpenoid compounds were also found in the *Aglaia* species as the major constituents (Harneti & Supratman, 2021).

Terpenoids have been found to be useful for the treatment of various types of diseases and have biological activities such as antimicrobial, antifungal, antiparasitic, antiviral, antihyperglycemic, antihypoglycemic, anti-inflammatory, immunomodulatory (Singh & Sharma, 2014), and anticancer (Awang et al., 2012). Although terpenoids of *Aglaia* species have been previously reported, the terpenoid compounds of the stem bark of *A. elaeagnoidea* are yet to be reported. The chemical constituents that have been reported previously from the bark of *A. elaeagnoidea* are dammarane-type triterpenoids, lignans (Fuzzati, Dyatmiko, Rahman, Achmad, & Hostettmann, 1996), benzofuran

derivatives (Seger, Hofer, & Greger, 2000), and rocaglamide (Ngo et al., 2021). In this paper, the isolation and structure identification of an eudesmane-type sesquiterpenoid and dammarane-type triterpenoids along with their cytotoxic activity are described.

EXPERIMENTAL SECTION

General Experiment Procedure

IR spectra were recorded on Nicolet Summit FTIR Spectrophotometer with DTGS KBr detector. Mass spectra were determined by Waters QTOF-HRTOFMS-XEV^{otm} mass spectrophotometer. Furthermore, NMR spectra of compounds **1**, **2**, and **5** were measured by JEOL JNM-ECZ500R/S1 spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C, whereas compounds **3** and **4** were obtained with a JEOL spectrometer at 400 MHz for ^{1}H and 100 MHz for ^{13}C, with $CDCl_3$ as a solvent, chemical shifts were given on a δ (ppm) scale and tetramethyl silane (TMS) as the internal standard. Column chromatography was performed on silica gel 60 (70–230 mesh and 230–400 mesh; Merck, Darmstadt, Germany) and Octa Decyl Silane (ODS, Fuji Sylisia, Japan). Thin layer chromatography were precoated with silica gel GF_{254} (Merck, 0.25 mm) and detection was achieved by spraying with 10% H_2SO_4 in EtOH, followed by heating and analyzed under UV light at wavelength 254 and 365 nm.

Plant material

The stem bark of *A. elaeagnoidea* was collected in Bogor Botanical Garden, Bogor, West Java, Indonesia on August 2017. The plant was identified by Center for Plant Conservation Botanic Gardens, Bogor, Indonesia and the voucher specimen is Vak.III.C.93.

Extraction and isolation

The dried stem bark of *A. elaeagnoidea* (2.5 kg) was extracted exhaustively with methanol at room temperature, filtered, and concentrated. The methanol extract (486.4 g) was suspended in a mixture of water and methanol (9:1), then partitioned with *n*-hexane, ethyl acetate, and *n*-butanol successively. Each extract was evaporated under a reduce pressure to give *n*-hexane extract (115.9 g, 4.5%), ethyl acetate extract (90.9 g, 3.6%), and *n*-butanol extract (62.1 g, 2.5%).

The ethyl acetate extract (90.9 g) was separated by vacuum liquid chromatography (VLC) packed with silica gel G60 by gradient elution of *n*-hexane-EtOAc-MeOH (100:0:0 – 0:0:100) to give eight combined fractions (A-H). Fraction D (1.93 g) was subjected to silica gel column chromatography *n*-hexane-EtOAc gradient system, yielding 11 fractions (D1-D11). Fraction D4 (103.5 mg) was further separated by column chromatography on ODS using water : methanol (2:8) to give eight fractions (D4a-D4h). Purification of the fraction D4b (23.9 mg) on ODS column chromatography eluted with water : methanol (3:7) give **1** (7.6 mg) and **2** (5.8 mg). Fraction D5 (489 mg) was separated using *n*-hexane-EtOAc gradient system to afford eight fractions (D5a-D5h). Purification of the fraction D5c (21.6 mg) on ODS column chromatography eluted with water : acetonitrile (0.5:9.5) afforded **3** (8.9 mg). Purification of the fraction D5d (108 mg) on ODS column chromatography eluted with water : acetonitrile (1:9) afforded **4** (13.7 mg). Fraction **D7** (120.3 mg) was separated by column chromatography on ODS using water : methanol (3:7) to give **5** (22 mg).

Bioassays for Cytotoxic Activity

The first stage is media preparation, positive control, and samples. The second stage is cell preparation. The cell to be used has a confluent of min 70%. The third stage is cell culture. Determined the number and viability of cells and then suspend with the appropriate cell density. Seeding / culture cells into 96 well plates, then incubated (temperature 37 °C and 5% CO_2 gas) for 24 hours or until the cells reach a minimum confluence of 70%. The fourth stage is the treatment of cells with samples, positive control, and negative control. Using micropipettes, 100 µL of each sample and positive control of cisplatin from the microtube were transferred into each of the corresponding microplates on 96 well plates that already contained cells. Then incubate again for 24 hours. The last stage is the provision of PrestoBlue reagents and absorbance measurements. Discarded media at each well. 9 mL of media were prepared in the tube to which 1 mL of "PrestoBlue ™ Cell Viability Reagent" was added (10 µL of reagent for 90 µL of media), then put 100 µL of the solution mixture into each well microplate then incubated for 1-2 hours until color changes were seen. In the living cells, the PrestoBlue® reagent is reduced from a blue resazurin compound with no intrinsic fluorescent value, to a resorufin compound which is red in color and highly luminous. The conversion value is proportional to the number of metabolically active cells and can therefore be measured quantitatively. To measure the absorbance, the absorbance spectrum for resazurin and resorufin was used. Then the absorbance was measured at a wavelength of 595 nm using an ELISA reader.

RESULTS AND DISCUSSIONS

The ethyl acetate extract of the stem bark of *A. elaeagnoidea* was chromatographed over vacuum-liquid chromatography (VLC) column packed with silica gel 60 by gradient elution. The VLC fractions were repeatedly subjected to silica gel 60 and ODS column chromatography to give **1**-**5** (**Figure 1**).

Compound **1** was obtained as a colourless oil. The molecular formula of **1** was identified as $C_{15}H_{26}O_2$ based on the HR-TOFMS *m/z* 239.1996 $[M+H]^+$, calculated for $C_{15}H_{27}O_2$, *m/z* 239.2011, thus required three degree of unsaturation. The IR spectra showed

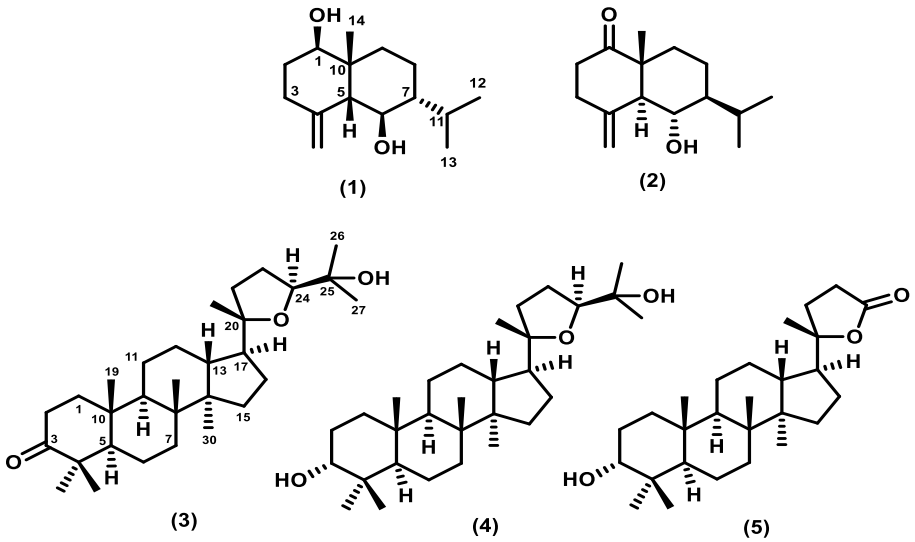

Figure 1. Chemical Structures of **1 – 5**

the presences of hydroxyl (3422 cm^{-1}), an aliphatic (2872 cm^{-1}), an isolated double bond (1704 cm^{-1}), *gem*-dimethyl (1368 cm^{-1}), and an ether group (1049 cm^{-1}). ^{1}H-NMR spectrum of **1**, displayed the presence of a tertiary methyl signal at δ_H 0.85 and two doublet methyl signals at δ_H 0.83 and 0.93, respectively. Then, the presence of a typical signal for olefinic protons at δ_H 4.96 and 4.82. In addition, the signal for the oxygenated protons at δ_H 3.93 and 3.50 was also observed in the ^{1}H-NMR spectrum. ^{13}C-NMR spectrum with detailed analysis of DEPT showed the presence of fifteen carbons consisting of three methyls which resonance at δ_C 21.4 (Me-1), 21.0 (Me-12), and 16.3 (Me-13), four sp^3 methylenes, two oxygenated methines, one quaternary carbon, one sp^2 quaternary carbon, and one sp^2 methylene carbon at δ_C 114.3 (C-15). These functionalities accounted for one out of the total three degree of unsaturation. Two remaining hydrogen deficiency indexes has corresponded to the bicyclic sesquiterpenoid structure (Kurniasih et al., 2018; Milawati et al., 2019).

Based on the number of methyl and methylene in the ^{1}H-NMR, ^{13}C-NMR, and DEPT, **1** considered as an eudesmane-type sesquiterpenoid structure (Liu et al., 2014). ^{1}H-NMR spectrum of **1** showed a proton sp^2 signal at δ_H 4.96 (H-15α, J=1.5 Hz) and 4.82 (H-15β, J=2.0 Hz) and based on the value of the coupling constant, the signal indicates the geminal proton of terminal alkene (C=CH_2) (Newsoroff & Sternhell, 1972; Silverstein, Webster, & Kiemle, 2005). The position of the terminal double bond in **1** is C-4/C-15 based on biosynthetic approached. While, the hydroxyl groups at C-1 and C-6 (Dewick, 2009).

Regarding to biosynthesis approached, the position of methyl in C-10 is in β-orientation (Tong et al., 2018, Christianson & Blank, 2019). Compound **1** showed the chemical shift for C-5 (δ_C 61.7), thus, the configuration for C-5 is in β-orientation (Zhang et al., 2003). A methine proton at C-6 has a ^3J 10.5 Hz, indicating that H-5 and H-6 have axial-axial orientation (Silverstein et al., 2005), consequently, 6-OH has β-orientation. A methine proton at C-1 has a double doublet (*dd*) split pattern with a ^3J 11.5 Hz, indicating that H-1 has an axial position, consequently, 1-OH has β-orientation. Meanwhile, a methine proton at C-7 has a multiplet split pattern, indicating that H-1 has an equatorial position, so, the isopropyl group has α-orientation (Sun, Zhang, & Hu, 2004).

A detailed comparison of **1** to those 5-*epi*-eudesm-4(15)-ene-1β,6β-diol isolated from the leaves and twigs *Litsea verticillata* (Lauraceae) (Zhang et al., 2003) (**Table 1**), revealed that both compounds were very similar, consequently **1** was identified as 5-*epi*-eudesm-4(15)-ene-1β,6β-diol. Compound **1** was isolated from *Aglaia* species for the first time an consider as a rare natural *cis*-eudesmane.

Compound 2 was obtained as a yellow oil. The molecular formula of **2** was identified as $C_{15}H_{24}O_2$ based on the HR-TOFMS *m/z* 237.1850 $[M+H]^+$, calculated for $C_{15}H_{25}O_2$, *m/z* 237.1855, thus required four degree of unsaturation. The IR spectra showed the presences of hydroxyl (3462 cm^{-1}), an aliphatic (2932 cm^{-1}), an isolated double bond (1705 cm^{-1}), a carbonyl (1739 cm^{-1}), *gem*-dimethyl (1369 cm^{-1}), and an ether group (1054 cm^{-1}). The NMR data (**Table 2**) for **2** is similar to **1**. The main differences are the absence of one of the hydroxyl group instead of a carbonyl group at δ_C 213.5 (C-1). Compound **2** showed the chemical shift for C-5 δ_C 55.6 thus, C-5 is in α orientation. Based on the splitting pattern, a methine proton at C-6 has a ^3J 10.5 Hz, indicating that H-5 and H-6 have axial-axial orientation consequently, 6-OH has α-orientation. Meanwhile, a methine proton at C-7 has a multiplet split pattern, indicating that H-1 has an equatorial position consequently, the isopropyl group has β-orientation (Silverstein et al., 2005). A detailed comparison of the

Table 1. Comparison of NMR data between **1** and 5-*epi*-eudesm-4(15)-ene-1*β*,6*β*-diol

Position of Carbon	**1***		5-*epi*-eudesm-4(15)-ene-1*β*,6*β*-diol*	
	d_H (∑H, mult., *J*= Hz)	d_C (mult.)	d_H (∑H, mult., *J*= Hz)	d_C (mult.)
1	3.93 (1H, dd, 11.5, 4.5)	68.2 (d)	3.92 (1H, dd, 11.6, 4.8)	68.1 (d)
2	1.87 (1H, m)	31.1 (t)	1.82 (1H, dtd, 12.5, 5.2, 2.7)	31.0 (t)
	1.59 (1H, m)		1.58 (qd (12.2, 5.7)	
3	2.27 (1H, m)	29.8 (t)	2.27 (1H, m)	29.7 (t)
	2.44 (1H, m)		2.27 (1H, m)	
4	-	145.3 (s)	-	145.4 (s)
5	1.83 (1H, d, 10.5)	61.7 (d)	1.82 (1H, d, 10.2)	61.6 (d)
6	3.50 (1H, t, 10.5)	67.2 (d)	3.49 (1H, t, 10.0)	67.1 (d)
7	1.23 (1H, m)	49.1 (d)	1.22 (1H, 12.5, 2.6)	49.0 (d)
8	1.45 (1H, m)	18.0 (t)	1.45 (1H, dq, 13.4, 3.4)	18.0 (t)
	1.27 (1H, d, 3.5)		1.26 (1H, qd, 12.9, 3.2)	
9	2.04 (1H, dt, 14.0, 3.0)	34.4 (t)	2.04 (1H, dt, 14.0, 3.2)	34.3 (t)
	1.03 (1H, dt, 14.0, 4.0)		1.02 (1H, td, 13.7, 3.9)	
10	-	40.2 (s)	-	40.1 (s)
11	2.21 (1H, m)	26.5 (d)	2.19 (1H, dd, 7.1, 2.5)	26.4 (d)
12	0.93 (3H, d, 7.5)	21.0 (q)	0.92 (3H, d, 7.1)	20.9 (q)
13	0.83 (3H, d, 6.5)	16.3 (q)	0.82 (3H, d, 7.0)	16.2 (q)
14	0.85 (3H, s)	21,4 (q)	0.84 (3H, s)	21.3 (q)
15	4.96 (1H, t, 1.5)	114.3 (t)	4.95 (1H, brt, 2.1)	114.2 (t)
	4.82 (1H, t, 2.0)		4.81 (1H, brt, 2.0)	

*Measured in $CDCl_3$ (500 MHz for ^1H and 125 MHz for ^{13}C)

NMR spectra of **2** to those of 6*α*-Hydroxy-eudesm-4(15)-en-1-one, isolated from the twigs *A. grandis* (Inada et al., 2002), revealed that the structures of the two compounds were very similar, consequently **2** was identified as 6*α*-hydroxy-eudesm-4(15)-en-1-one, which found from this plant for the first time.

Table 2. Comparison of NMR data between **2** and 6*α*-Hydroxy-eudesm-4(15)-en-1-one

Position Carbon	**2***		6*α*-Hydroxy-eudesm-4(15)-en-1-one**	
	d_H (∑H, mult., *J*= Hz)	d_C (mult.)	d_H (∑H, mult., *J*= Hz)	d_C (mult.)
1	-	213.5 (s)	-	213.2 (s)
2	2.40 (1H, m)	38.4 (t)	2.39-2.42 (1H, m)	38.3 (t)
	2.67 (1H, dd, 13.0, 5.0)		2.67 (1H, ddd, 14.4, 12.4, 5.3)	
3	2.37 (1H, m)	35.5 (t)	2.35-2.38 (1H, m)	35.4 (t)
	2.59 (1H, m)		2.58-2.63 (1H, m)	
4	-	144.1 (s)	-	144.4 (s)
5	2.14 (1H, d, 7.0)	55.6 (d)	2.12 (1H, d, 9.8)	55.5 (d)
6	3.81 (1H, t, 10.0)	67.1 (d)	3.83 (1H, dd, 9.8, 9.8)	67.1 (d)
7	1.26 (1H, m)	49.1 (d)	1.25-1.30 (1H, m)	49.1 (d)
8	1.18 (1H, m)	17.9 (t)	1.16-1.24 (1H, m)	17.9 (t)
	1.59 (1H, m)		1.59-1.62 (1H, m)	
9	1.56 (1H, m)	31.5 (t)	1.55-1.58 (1H, m)	31.5 (t)
	1.80 (1H, m)		1.78-1.82 (1H, m)	
10	-	50.2 (s)	-	50.1 (s)
11	2.23 (1H, m)	26.0 (d)	2.21-2.26 (1H, m)	26.0 (d)
12	0.95 (3H, d, 7.0)	21.1 (q)	0.88 (3H, d, 7.0)	16.2 (q)
13	0.86 (3H, d, 7.0)	16.2 (q)	0.96 (3H, d, 7.0)	21.0 (q)
14	0.98 (3H, s)	18.0 (q)	1.00 (3H, s)	17.9 (q)
15	4.99 (1H, s)	110.2 (t)	5.00 (1H, d, 0.8)	110.1 (t)
	5.25 (1H, s)		5.26 (1H, d, 0.8)	

*Measured in $CDCl_3$ (500 MHz for ^1H and 125 MHz for ^{13}C)
**Measured in $CDCl_3$ (500 MHz for ^1H and 125 MHz for ^{13}C)

Table 3. NMR spectral data for compounds **3-5**

Position Carbon	**3***		**4***		**5****	
	d_H [∑H, mult., *J* (Hz)]	d_C (mult.)	d_H [∑H, mult., *J* (Hz)]	d_C (mult.)	d_H [∑H, mult., *J* (Hz)]	d_C (mult.)
1	1.89 (1H, m) 1.46 (1H, m)	40.0 (t)	1.43 (1H, m) 1.30 (1H, m)	33.7 (t)	1.30 (1H, m) 1.37 (1H, m)	33.7 (t)
2	2.47 (1H, m) 1.80 (1H, m)	34.2 (t)	1.96 (1H, m) 1.55 (1H, m)	25.4 (t)	1.56 (1H, m) 1.93 (1H, m)	25.4 (t)
3	-	218.4 (s)	3.38 (1H, t, 2.8)	76.4 (d)	3.39 (1H, br.s)	76.3 (d)
4	-	47.5 (s)	-	37.7 (s)	-	37.7 (s)
5	1.22 (1H, m)	55.4 (d)	1.25 (1H, m)	49.6 (d)	1.24 (1H, s)	49.4 (d)
6	1.38 (1H, m) 1.54 (1H, m)	19.7 (d)	1.42 (1H, m) 1.62 (1H, m)	18.3 (d)	1.39 (1H, m) 1.60 (1H, m)	18.3 (t)
7	1.66 (1H, m) 1.26 (1H, m)	49.1 (t)	1.59 (1H, m) 1.25 (1H, m)	35.2 (t)	1.26 (1H, m) 1.58 (1H, m)	35.2 (t)
8	-	40.4 (s)	-	40.7 (s)	-	50.3 (s)
9	1.47 (1H, m)	50.2 (d)	1.46 (1H, m)	50.7 (d)	1.44 (1H, m)	50.4 (d)
10	-	36.9 (s)	-	37.3 (s)	-	37.3 (s)
11	1.52 (1H, m) 1.80 (1H, m)	26.0 (t)	1.54 (1H, m) 1.19 (1H, m)	21.7 (t)	1.20 (1H, m) 1.74 (1H, m)	26.9 (t)
12	1.72 (1H, m) 2.04 (1H, m)	27.1 (t)	1.76 (1H, m) 1.80 (1H, m)	27.1 (t)	1.22 (1H, m) 1.53 (1H, m)	21.3 (t)
13	1.64 (1H, m)	43.0 (d)	1.65 (1H, m)	42.9 (d)	1.55 (1H, m)	43.2 (d)
14	-	50.1 (s)	-	50.2 (s)	-	40.6 (s)
15	1.06 (1H, m) 1.80 (1H, m)	31.5 (t)	1.46 (1H, m) 1.10 (1H, m)	31.5 (t)	1.09 (1H, m) 1.50 (1H, m)	31.2 (t)
16	1.58 (1H, m) 2.02 (1H, m)	25.9 (t)	1.78 (1H, m) 1.29 (1H, m)	25.9 (t)	1.29 (1H, m) 1.80 (1H, m)	25.1 (t)
17	1.47 (1H, m)	49.8 (d)	1.47 (1H, s)	49.8 (d)	1.97 (1H, td, 11.0, 6.5)	49.6 (d)
18	0.93 (3H, s)	15.3 (q)	0.96 (3H, s)	15.6 (q)	0.93 (3H, s)	15.6 (q)
19	1.03 (3H, s)	16.2 (q)	0.87 (3H, s)	16.2 (q)	0.84 (3H, s)	16.1 (q)
20	-	86.6 (s)	-	86.7 (s)	-	90.3 (s)
21	1.14 (3H, s)	27.3 (q)	1.13 (3H, s)	27.3 (q)	1.34 (3H, s)	25.4 (q)
22	1.31 (1H, m) 1.80 (1H, m)	34.8 (t)	1.59 (1H, m) 1.23 (1H, m)	34.8 (t)	1.92 (1H, m) 2.10 (1H, ddd, 12.5, 10.0, 9.5)	31.2 (t)
23	1.92 (1H, m) 2.42 (1H, m)	26.4 (t)	1.86 (1H, m) 1.76 (1H, m)	26.4 (t)	2.52 (1H, ddd, 18.0, 10.0, 4.5) 2.65 (1H, 18.0, 10.0, 9.0)	29.2 (t)
24	3.63 (1H, dd, 9.6, 5.2)	86.4 (d)	3.62 (1H, dd, 10.0, 5.6)	86.3 (d)	-	176.9 (s)
25	-	70.3 (s)	-	70.3 (s)		-
26	1.18 (3H, s)	27.9 (q)	1.17 (3H, s)	27.9 (q)		-
27	1.10 (3H, s)	24.1 (q)	1.09 (3H, s)	24.1 (q)		-
28	1.07 (3H, s)	26.8 (q)	0.95 (3H, s)	28.4 (q)	0.91 (3H, s)	28.4 (q)
29	1.00 (3H, s)	21.1 (q)	0.85 (3H, s)	22.2 (q)	0.82 (3H, s)	22.2 (q)
30	0.87 (3H, s)	16.4 (q)	0.87 (3H, s)	16.6 (q)	0.87 (3H, s)	16.4 (q)

*400 MHz for ^{1}H and 100 MHz for ^{13}C in $CDCl_3$, **500 MHz for ^{1}H and 125 MHz for ^{13}C in $CDCl_3$.

Compound **3** was obtained as a white crystal. The molecular formula of **3** was identified as $C_{30}H_{50}O_3$ based on the HR-TOFMS *m/z* 481.3632 $[M+Na]^+$, calculated for $C_{30}H_{50}O_3Na$, *m/z* 481.3658, thus required six degree of unsaturation. The IR spectra showed the presences of hydroxyl (3384 cm^{-1}), aliphatic (2945 cm^{-1}), carbonyl (1698 cm^{-1}), and *gem*-dimethyl (1374 cm^{-1}). ^{1}H-NMR spectrum showed the presenc e of eight tertiary methyl signals at δ_H 0.87 (3H, s, Me-30), 1.03 (3H, s, Me -19), 0.93 (3H, s, Me -18), 1.00 (3H, s, Me-29), 1.07 (3H, s, Me-28), 1.10 (3H, s, Me-27), 1.14 (3H, s, Me -21) and 1.18 (3H, s, Me-26), which indicate the characteristic for dammarane-type triterpenoid (Harneti et al., 2014). An oxygenated sp^3 methine in part of tetrahydrofuran ring at δ_H 3.63 (1H, *dd*, *J*=5.2, 9.6 Hz) was also revealed in the ^{1}H-NMR spectra, supporting the presence of dammarane-type triterpenoid structure in **3**. ^{13}C-NMR spectrum with DEPT measurements showed the presence of thirty carbon signals consisting of eight methyls, ten methylenes, six methines, and six quaternary carbons, indicating the presence of dammarane-type triterpenoid (Harneti *et al.,* 2014).

Table 4. Cytotoxic activity of compounds **1-5**

Compounds	HeLa Cells IC_{50} (µg/mL)	DU145 Cells IC_{50} (µg/mL)
1	3544.00	971.69
2	9010.62	16883.7
3	712.75	991.56
4	105.08	168.59
5	6913.00	591.06
Cisplatin	19.00	53.00

The presence of eight methyl resonances at δ_C 15.3 (C-18), 16.2 (C-19), 27.3 (C-21), 27.9 (C-26), 24.1 (C-27), 26.8 (C-28), 21.1 (C-29) and 16.4 (C-30), a carbonyl group at δ_C 218.4 (C-3), as well as two oxygenated quaternary carbon at δ_C 86.4 (C-24) and 86.6 (C-20), supporting the presence of dammarane-type triterpenoid with the addition of tetrahydrofuran ring (Roux *et al.,* 1998). The position of the carbonyl group is at C-3 considered from its biosynthetic approached. Compound **3** showed δ_C 86.4 and δ_H 3.63 (1H, *dd*, *J*=5.2, 9.6 Hz), as well as δ_C 86.6 for C-20, consequently configuration for C-20 and C-24 are *S* orientation (Aalbersberg & Singh, 1991; Roux et al., 1998; Harneti et al., 2012). Comparison of the NMR data of **3** with those of the 20*S*,24*S*-epoxy-25-hydroxydammarane-3-one isolated from *Dysoxylum malabaricum* (Hisham, Bai, Fuzimoto, Hara, & Shimada, 1996) revealed that the structures of the two compounds are closely related, consequently **3** was identified as the 20*S*,24*S*-epoxy-25-hydroxydammarane-3-one, which found from this plant for the first time. Compound **4** was obtained as a white crystal.

The NMR data of **4** had a fairly identical pattern to those of **3**. The main differences are the absence of one of the carbonyl group and the presence of a hydroxyl group at δ_C 76.4 (C-3). A methine proton at C-3 has a ^3J 2.8Hz, indicating that H-2 and H-3 have axial-equatorial orientation consequently, 3-OH has *α* orientation (Hidayat et al., 2018). Thus, **4** was identified as the 20*S*,24*S*-epoxydammarane-3*α*,25-diol (Hisham et al., 1996).

Compound **5** was obtained as a white crystal. The ^{13}C-NMR spectra showed 27 carbons and were classified by the DEPT 135° experiment as six methyl groups, one carbonyl lactone at δ_C 176.9 (C-24), an oxymethine group at δ_C 76.3 (C-3), and an oxygenated quaternary carbon at δ_C 90.3 (C-20). These suggested that the loss of two methyl groups from the side chain by oxidative degradation leading to the formation of the *γ*-lactone (Rao, Meshulam, Zeln, & Lavie, 1975), exhibiting the characteristics of tris nor-triterpenoid compounds. This compound was found to be identically obtained by the oxidation of **4** (**Figure 2**). Thus, **5** was identified as 3*α*-*epi*-cabraleahydroxy lactone (Phongmaykin, Kumamoto, Ishikawa, Suttisri, & Saifah, 2008).

The cytotoxic activity of **1-5** isolated from the stem bark of *A. elaeagnoidea* against cervical cancer cells HeLa and prostate cancer DU145 has been tested (**Table 4**). Among the triterpenoid compounds, **4** showed moderately activity (Widiyastuti, Sholikhah, & Haryanti, 2019) with IC_{50} values of 105.08 and 168.59 *µ*g/mL, whereas **3** and **5** showed no activity, indicated the presence of hydroxyl groups at C-3 and C-25 play important role for cytotoxic activity in the triterpenoid structure. Furthermore, sesquiterpenoids, **1** and **2** showed no activity against both cancer cell lines.

CONCLUSIONS

Five terpenoids namely, two eudesmane-type sesquiterpenoids, 5-*epi*-eudesm-4(15)-ene-1β,6β-diol (**1**), and 6*α*-Hydroxy-eudesm-4(15)-en-1-one (**2**), as well as three dammarane-type triterpenoids, 20*S*,24*S*-epoxy-25-hydroxydammarane-3-one (**3**), 20*S*,24*S*-epoxydammarane-3*α*,25-diol (**4**), 3*α*-*epi*-cabraleahydroxy lactone (**5**) has been isolated from ethyl acetate extract of the stem bark of *A. elaeagnoidea* for the first time. 20*S*,24*S*-epoxydammarane-3*α*,25-diol (**4**), showed the stronger cytotoxic activity than other compounds against HeLa cervical cancer and DU145 prostate cancer lines, indicated that the cytotoxic activity are influenced by the presence of hydroxyl group.

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