Sesquiterpenoids from the Stem Bark of *Aglaia grandis*

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Abstract – Five sesquiterpenoids, 7-epi-eudesm-4(15)-ene,1β,6α-diol (**1**), 7-epi-eudesm-4(15)-ene,1β,6α-diol (**2**), saniculamoid D (**3**), aphanamol I (**4**), and 4β,10α-dihydroxyaromadendrane (**5**), were isolated from the stem bark of *Aglaia grandis*. The compounds' (**1-5**) chemical structures were identified by spectroscopic data including, IR, NMR (^{1}H, ^{13}C, DEPT 135°, HMQC, HMBC, ^{1}H-^{1}H COSY), and HRTOFMS, as well as by comparing with the previously reported spectral data. Therefore, this study described the structural elucidation of compounds **1-5** and evaluated their cytotoxic effects against Hela cervical and B16F10 melanoma cells for the first time, but no significant result was discovered.
Keywords – *Aglaia grandis*, HeLa cell lines, B16F10 cell lines, Meliaceae, sesquiterpenoids

Introduction

Meliaceae or the Mahogany family of flowering plants consist of trees and shrubs, grouped as 575 species in 50 genus[1,2] among which *Aglaia* is the largest comprised of more than 150 species[3,4] that are distributed in tropical and subtropical regions, ranging from Sri Lanka, Indomalaysia, to Australia, and other Pacific Islands.[3] The stem bark of these species has cytotoxic, insecticidal, antiviral, antituberculosis, anti-inflammatory, antifungal, and molluscicide effects.[4] Furthermore, the *Aglaia* genus is a rich source of natural compounds which often perform remarkable biological activities, such as the triterpenes, sesquiterpenes, flavaglines, bisamides, lignans, and flavonoids.[4] During the course of the continual search for anticancer substances from Indonesian *Aglaia* plants, some cytotoxic substances were isolated and described from *A. smithii*,[5] *A. eximia*,[6,7,8] *A. argentea*,[9,10] *A. elliptica*,[11,12] *A. angustifolia*,[13,14] *A. harmsiana*,[15,16] *A. simplicifolia*,[17] and *A. minahassae*.[18]

A. grandis can be found in Sulawesi Island, and from it, triterpenoids, sesquiterpenoids, steroids, and bisamides have been previously isolated.[19,20,21] In the further search for novel sesquiterpenoid constituents from this plant, the bark's ethyl acetate extract was investigated and the five sesquiterpenoids obtained (Fig. 1) were then evaluated for cytotoxic effects against HeLa cervical and B16F10 melanoma cells. Therefore, this study described the structural elucidation of isolated compounds **1-5** and their cytotoxic activity.

Experimental

General experimental procedures – The IR spectra were obtained in Nicolet Summit FTIR spectrometer with DTGS KBr detector, while mass spectra were measured using a Water QTOF-HR-TOFMS-XEV^otm mass spectrometer. NMR spectra were recorded with a JEOL ECZ-400 and ECZ-500 spectrometer using tetramethylsilane (TMS) as an internal standard. Furthermore, chromatographic separations were carried out on silica gel 60 (70-230 Mesh and 230-400 mesh, Merck). The preparative thin-layer chromatography (PTLC) glass plates were precoated with GF$_{254}$ silica gel (Merck, 0.25 mm) as well as the TLC plates which the detection was carried out

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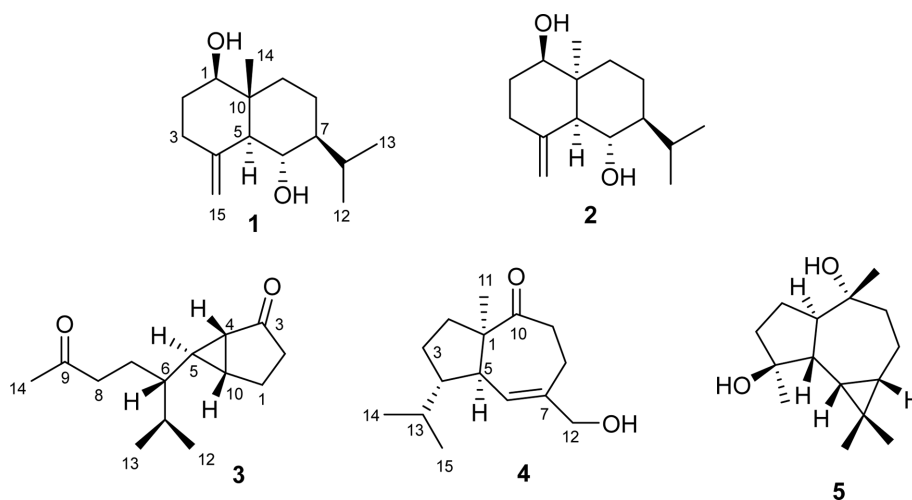
Fig. 1. Chemical structures of compounds **1-5**.

using 10% vanillin in sulfuric acid followed by heating. The vanillin/ sulfuric acid spray reagent was prepared by dissolving 1.0 g of vanillin in 100 mL of concentrated sulfuric acid.

Plant materials – Stem bark of *A. grandis* was collected at Bogor Botanical Garden, West Java Province, Indonesia in 2019. An exhibition specimen (No. Bo-104) was deposited after the plant was identified by the staff of the Herbarium Bogoriense, Bogor.

Extraction and isolation – Dried powder of *A. grandis* stem bark (1.2 kg) was ground and extracted with methanol at room temperature. The extract generated was dissolved with methanol-water (4:1), then successively extracted with *n*-hexane, ethyl acetate, and methanol. The evaporation performed led to the crude extracts of *n*-hexane (9.30 g), ethyl acetate (18.0 g), and methanol (45.0 g), respectively. Afterward, they were detected for the presence of sesquiterpenoid constituent in TLC plates by spraying of 10% vanillin in sulfuric acid followed by heating and the ethyl acetate showed the green to blue spot color indicated the presence of sesquiterpenoid. The phytochemical analysis, therefore, focused on ethyl acetate extract due to showing the sesquiterpenoid.

The ethyl acetate extract (18.06 g) was subjected to vacuum liquid chromatography (VLC) over silica gel using a gradient elution mixture of *n*-hexane-ethyl acetate (10:0-0:10) to isolate 18 fractions (A-R). Fraction B (2.07 g) was subjected to column chromatography (CC) over silica gel (70-230 mesh) using a 5% gradient mixture of *n*-hexane-ethyl acetate (10:0-7:3) to produce 15 sub-fractions (B1-B15). Then, B7 (463 mg) was subjected to CC over silica gel (230-400 mesh) using an isocratic mixture of *n*-hexane-methylene chloride-ethyl acetate (5:

3:3) as an eluent to produce five sub-fractions (B7A – B7E). Afterward, B7D (87.8 mg) was subjected to CC reverse phase on octadesylsilane (ODS) using an isocratic mixture of methanol-water (4:1) as an eluent to isolate compound **1** (9.1 mg) and sub-fraction B7D2. The B7D2 (22.0 mg) was subjected to CC reverse phase on ODS using an isocratic mixture of methanol-water (4:1) as an eluent to isolate compound **2** (3.6 mg).

Sub-fraction B9 (520.0 mg) was subjected to CC over silica gel (230-400 mesh) using an isocratic mixture of methylene chloride-ethyl acetate (9.5:0.5) as an eluent to produce five sub-fractions (B9A – B9E). Then, B9A (116.7 mg) was subjected to CC over silica gel (230-400 mesh) using an isocratic mixture of *n*-hexane-ethyl acetate (9:1) as an eluent to generate four sub-fractions (B9A1-B9A4). The B9A4 (30.2 mg) was subjected to CC reverse phase on ODS using an isocratic mixture of methanol-water (4:1) as an eluent to isolate compound **3** (8.5 mg).

Fraction C (1.76 g) was subjected to CC over silica gel (70-230 mesh) using a 5% gradient mixture of *n*-hexane-ethyl acetate (10:0-7:3) to produce ten combined sub-fractions (C1-C10). The C8 (45.0 mg) was subjected to CC reverse phase on ODS using an isocratic mixture of methanol-water (1:1) as an eluent to isolate compound **4** (3.5 mg).

Fraction E (1.20 g) was subjected to CC over silica gel (230-400 mesh) using an isocratic mixture of methylene chloride-ethyl acetate (4:1) to generate six combined sub-fractions (E1-E6). Afterward, E4 (255.2 mg) was subjected to CC over silica gel (230-400 mesh) using an isocratic mixture of chloroform-ethyl acetate (9:1) to produce nine combined sub-fractions (E4A-E4I). Further-more, E4H (31.8 mg) was subjected to CC reverse phase

on ODS using an isocratic mixture of methanol-water (4:1) as an eluent to isolate compound **5** (4.5 mg).

7-epi-eudesm-4(15)-ene,1β,6α-diol (1) – yellow oil; IR ν_{max} cm^{-1}: 3377, 2955, 1376, 1373, 1059; ^{1}H-NMR (CDCl$_3$, 400 MHz): δ_H 3.37 (1H, dd, J = 4.4,11.2 Hz, H-1), 1.95 (2H, m, H-2), 2.23 (2H, ddd, J = 1.6, 4.7, 11.8 Hz, H-3), 2.10 (1H, m, H-5), 3.65 (1H, t, J = 10.0 Hz, H-6), 1.77 (1H, m, H-7), 1.05 (2H, m, H-8), 2.11 (2H, m, H-9), 2.00 (1H, m, H-11), 0.80 (3H, d, J = 6.8 Hz, CH$_3$-12), 0.88 (3H, d, J = 7.2 Hz, CH$_3$-13), 0.64 (3H, s, CH$_3$-14), 5.01 (2H, d, J = 1.0 Hz, H-15a), 4.72 (2H, d, J = 1.0 Hz, H-15b); ^{13}C-NMR (CDCl$_3$, 100 MHz), Table 1; HR-TOFMS, m/z 239.1999 [M+H]$^+$ (calcd. C$_{15}$H$_{27}$O$_2$ m/z = 239.2011).

7-epi-eudesm-4(15)-ene,1β,6α-diol (2) – colorless smooth crystals; IR ν_{max} cm^{-1}: 3458, 2950, 1378, 1374, 1059; ^{1}H-NMR (CDCl$_3$, 500 MHz): δ_H 3.39 (1H, br.s, H-1), 1.96 (2H, m, H-2), 2.25 (2H, ddd, J = 4.8, 5.2, 7.2 Hz, H-3), 2.35 (1H, m, H-5), 3.71 (1H, t, J = 10.0 Hz, H-6), 1.39 (1H, m, H-7), 1.05 (2H, m, H-8), 2.11 (2H, m, H-9), 2.24 (1H, m, H-11), 0.87 (3H, d, J = 6.8 Hz, CH$_3$-12), 0.95 (3H, d, J = 7.2 Hz, CH$_3$-13), 0.75 (3H, s, CH$_3$-14), 5.01 (2H, d, J = 1.0 Hz, H-15a), 4.72 (2H, d, J = 1.0 Hz, H-15b); ^{13}C-NMR (CDCl$_3$, 125 MHz), Table 1; HR-TOFMS, m/z 239.2010 [M+H]$^+$ (calcd. C$_{15}$H$_{27}$O$_2$ m/z = 239.2011).

Saniculamoid (3) – Colorless oil; IR ν_{max} cm^{-1}: 2928, 1713; ^{1}H-NMR (CDCl$_3$, 500 MHz): δ_H 2.06 (2H, m, H-1), 1.98 (2H, m, H-2), 1.63 (1H, m, H-4), 1.07 (1H, m, H-5), 0.70 (1H, m, H-6), 1.66 (2H, m, H-7), 2.55 (2H, m, H-8), 1.86 (1H, m, H-10), 1.79 (1H, m, H-11), 0.91 (3H, d, J = 7.0 Hz, CH$_3$-12), 0.91 (3H, d, J = 7.0 Hz, CH$_3$-13), 2.18 (3H, s, CH$_3$-14); ^{13}C-NMR (CDCl$_3$, 125 MHz), Table 1; HR-TOFMS, m/z = 223.1695 [M+H]$^+$, (calcd. C$_{14}$H$_{23}$O$_2$ m/z = 223.1698).

Aphanamol I (4) – Colorless oil; IR ν_{max} cm^{-1}: 3319, 2956, 1380, 1372, 1045; ^{1}H-NMR (CDCl$_3$, 500 MHz): δ_H 1.72 (2H, m, H-2), 1.91 (2H, m, H-3), 1.36 (1H, m, H-4), 2.29 (1H, m, H-5), 5.52 (1H, d, J = 4.5 Hz, H-6), 2.24 (2H, m, H-8), 2.55 (2H, m, H-9), 1.27 (3H, s, CH$_3$-11), 4.02 (2H, s, H-12), 1.39 (1H, m, H-13), 0.90 (3H, d, J = 5.5 Hz, CH$_3$-14), 0.89 (3H, d, J = 7.0 Hz, CH$_3$-15); ^{13}C-NMR (CDCl$_3$, 125 MHz), Table 1; HR-TOFMS, m/z = 237.1968 [M+H]$^+$ (calcd. C$_{15}$H$_{25}$O$_2$ m/z = 237.1969).

4β,10α-dihydroxyaromadendrane (5) – Colorless oil; IR ν_{max} cm^{-1}: 3359, 2969, 1380, 1375, 1105; ^{1}H-NMR (CDCl$_3$, 500 MHz): δ_H 1.85 (1H, m, H-1), 1.53 (2H, m, H-2), 1.66 (2H, m, H-3), 1.13 (1H, m, H-5), 0.44 (1H, t, J = 9.5 Hz, H-6), 0.66 (1H, m, H-7), 0.91 (1H, m, H-8), 1.51 (1H, m, H-9), 1.02 (3H, s, CH$_3$-12), 1.02 (3H, s, CH$_3$-13), 1.15 (3H, s, CH$_3$-14), 1.23 (3H, s, CH$_3$-15); ^{13}C-NMR (CD$_3$OD, 125 MHz), Table 1; HR-TOFMS, m/z = 237.1858 [M-H]$^+$ (calcd. C$_{15}$H$_{25}$O$_2$ m/z = 237.1855).

Cytotoxicity assay – The cytotoxicity of the compounds against HeLa cervical and B16-F10 melanoma cancer cells was measured using the PrestoBlue cell viability assay. The cells were maintained in a Roswell

Table 1. ^{13}C-NMR data for compounds **1-5**

Position carbon	Compounds				
	1* δ_C (mult.)	**2**** δ_C (mult.)	**3**** δ_C (mult.)	**4**** δ_C (mult.)	**5**** δ_C (mult.)
1	79.1 (d)	74.7 (d)	23.0 (t)	59.0 (s)	56.4 (d)
2	32.0 (t)	32.0 (t)	32.7 (t)	34.6 (t)	23.8 (t)
3	35.2 (t)	33.2 (t)	214.4 (s)	27.2 (t)	41.1 (t)
4	146.3 (s)	147.9 (s)	34.5 (d)	56.1 (d)	80.5 (s)
5	55.9 (d)	50.7 (d)	30.1 (d)	52.0 (d)	48.4 (d)
6	67.1 (d)	67.5 (d)	46.4 (d)	133.1 (d)	28.2 (d)
7	49.4 (d)	49.5 (d)	24.8 (t)	141.8 (s)	26.6 (d)
8	18.2 (t)	18.1 (t)	41.8 (t)	25.0 (t)	20.1 (d)
9	36.3 (t)	31.2 (t)	208.9 (s)	40.0 (t)	44.5 (d)
10	41.8 (s)	42.1 (s)	30.1 (d)	213.8 (s)	75.1 (s)
11	26.0 (d)	26.0 (d)	31.1 (d)	24.8 (q)	19.7 (s)
12	21.2 (q)	21.2 (q)	19.3 (q)	67.3 (t)	16.5 (q)
13	16.2 (q)	16.2 (q)	19.3 (q)	33.2 (d)	28.7 (q)
14	11.7 (q)	18.0 (q)	28.1 (q)	22.2 (q)	20.3 (q)
15	107.9 (t)	107.0 (t)		20.1 (q)	24.5 (q)

*Measured in 400 MHz for ^{1}H and 100 MHz for ^{13}C
**Measured in 500 MHz for ^{1}H and 125 MHz for ^{13}C

Park Memorial Institute (RPMI) medium supplemented with 10% (v/v) Fetal Bovine Serum (FBS) and 1 μL/1 mL antibiotics, and then cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2. Tumor cells were seeded in 96-well microliter plates at 1.7×10^4 cells per well. After 24 h, the compounds were added to the wells. Following 96 h, viability was determined by measuring the metabolic conversion of resazurin substrate into pink fluorescent resofurin product yielded from the reduction in viable cells. The PrestoBlue assay results were read using a multimode reader at 570 nm. All compounds were tested at eight concentrations 7.81, 15.63, 31.25, 62.50, 125.00, 250.00, 500.00, and 1000.00 μg/mL for B16F10 cell and 2.34, 4.69, 9.38, 18.75, 37.50, 75.00, 150.00, and 300.00 μg/mL for HeLa cell in 100% DMSO and with a final concentration of 2.7% in each well. Each concentration of the compounds was tested in two parallel experiments, while IC_{50} values were calculated by linear regression method using Microsoft Excel software.

Results and Discussion

The phytochemical test for the ethyl acetate extract showed the presence of sesquiterpenoids with the green to blue spot colors in TLC plate after spraying with 10% vanillin in sulphuric acid followed by heating. Then by phytochemical test to guide separations, the ethyl acetate fraction was separated by column chromatography over silica gel by gradient elution. The fractions were repeatedly subjected to normal-phase and reverse phase column chromatography on silica gel GF_{254} and octa desylsilane to yield five sesquiterpenoids **1–5** (Fig. 1).

Compound **1** was obtained as a yellow oil, where the HR-TOFMS spectrum showed $[M+H]^+$ *m/z* 239.1999 (calcd. *m/z* 239.2011), which corresponded to the molecular formula $C_{15}H_{27}O_2$ and hence required three degrees of unsaturation, originating from one pair of sp^2 carbon. Then, the remaining bicyclic eudesmane-type sesquiterpenoid was established from NMR data (Table 1). The IR spectra indicated absorption peaks at $3377\,cm^{-1}$ (OH), $2955\,cm^{-1}$ (C-H sp^3), $1464\,cm^{-1}$ (C=C), 1376 and $1373\,cm^{-1}$ (*gem*-dimethyl groups), and $1056\,cm^{-1}$ (C-O). The ^{1}H-NMR ($CDCl_3$ 400 MHz) spectrum showed three methyl groups consisting of two secondary members resonating at δ_H 0.80 (3H, d, $J = 6.8$ Hz, CH_3-12) and 0.88 (3H, d, $J = 7.2$ Hz, CH_3-13), and one tertiary methyl group resonating at δ_H 0.64 (3H, s, CH_3-14), which indicated the presence of eudesmane-type sesquiterpenoid skeleton. One olefinic methylene group, resonating at δ_H 5.01 (2H, d, $J = 1.0$ Hz, H-15α) and δ_H 4.72 (1H, d, $J = 1.0$ Hz, H-

15β) plus two oxymethine groups resonating at δ_H 3.65 (1H, t, $J = 10.0$ Hz, H-6) and δ_H 3.37 (1H, dd, $J = 4.4$, 11.2 Hz, H-1) were also observed. The ^{13}C-NMR ($CDCl_3$, 100 MHz) and DEPT 135 spectra showed the presence of three methyl groups, which indicated the characteristic of eudesmane-type sesquiterpenoids, one olefinic methylene, one olefinic quaternary carbon, and two oxygenated methine group, resonating at δ_C 79.1 (C-1) and δ_C 67.1 (C-6). These functionalities accounted for one of the total three degrees of unsaturation, while remaining two were consistent with the eudesmane-type sesquiterpenoid. A comparison of the NMR data of compound **1** with the data for 7-*epi*-eudesm-4(15)-ene,1β,6α-diol,[22] indicated the structures of both were very similar, consequently **1** was identified as 7-epi-eudesm-4(15)-ene,1β,6α-diol.

Compound **2** was obtained as a colorless smooth crystal where the HR-TOFMS spectrum showed $[M+H]^+$ *m/z* 239.2010 (calcd. *m/z* = 239.2011), which corresponded to the molecular formula $C_{15}H_{27}O_2$ and hence required three degrees of unsaturation. The compound **1** has a similar ^{1}H and ^{13}C-NMR spectrum with **2**, but the main disparity was that the ^{13}C-NMR of **2** at C-1, C-3, C-5, C-9, and C-14 and different multiplicity of ^{1}H-NMR in the oxymethine group in **2** was obtained as a broad singlet. Therefore, a stereochemical difference is assumed to exist between **1** and **2**. The stereochemical structure of compound **2** was determined by NOESY correlation (Fig. 3). NOESY correlation of compound **2** was compared with absolute proton H-5 which is α-oriented, then, methyl H-14 and H-5 were correlated thereby causing the stereochemical difference between **1** and **2** to be at methyl H-14. In compound **1,** the methyl H-14 has a β-configuration while in **2** it possesses an α-configuration and notably, **2** has just been isolated for the first time from natural compounds.

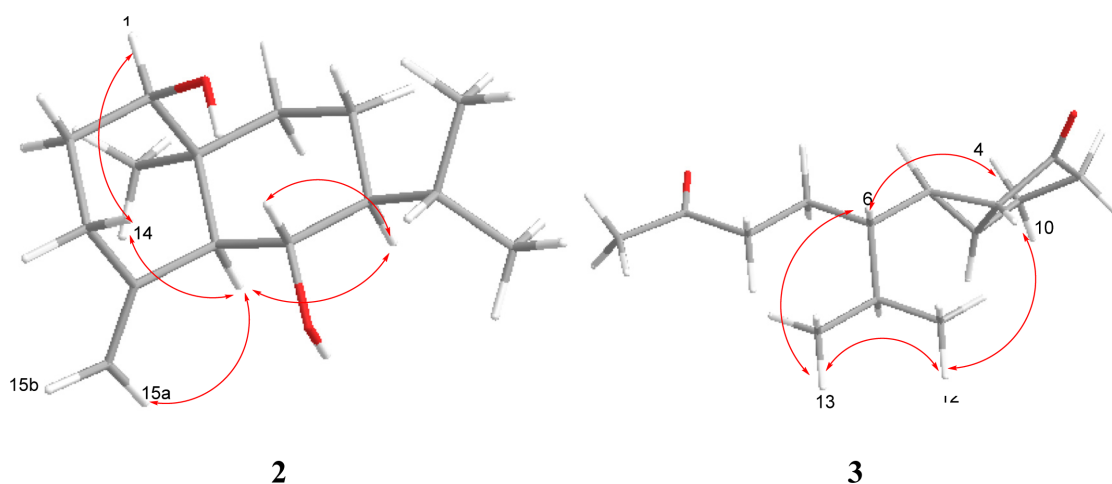Compound **3** was obtained as a colorless oil, where the HR-TOFMS spectrum showed $[M+H]^+$ *m/z* 223.1695 (calcd. *m/z* = 223.1698), which corresponded to the molecular formula $C_{14}H_{23}O_2$ and hence required four degrees of unsaturation, originating from two pairs of carbonyl ketone (C=O). Then, the remaining bicyclic-type *nor*-sesquiterpenoid was established from NMR data (Table 1). The IR spectrum suggested the presence of 2955 and $2870\,cm^{-1}$ (C-H sp^3), and $1713\,cm^{-1}$ (C=O). The ^{1}H-NMR ($CDCl_3$, 500 MHz) spectrum showed three methyl groups, namely two secondary which resonates at δ_H 0.91 (3H, d, $J = 7.0$ Hz, CH_3-12) and 0.91 (3H, d, $J = 7.0$ Hz, CH_3-13), plus one tertiary which resonates at δ_H 2.18 (3H, s, CH_3-14), which indicated the presence of sesquiterpenoid skeleton. The ^{13}C-NMR ($CDCl_3$, 125 MHz)

and DEPT 135 spectra showed the presence of three methyl groups and fourteen carbon that indicate the characteristic of *nor*-sesquiterpenoid-type, as well as two quaternary carbon ketones, resonating at δ_C 214.4 (C-3) and δ_C 208.9 (C-9). These functionalities accounted for two of the total four degrees of unsaturation, while the remaining two were consistent with the bicyclic-type *nor*-sesquiterpenoid. The HMBC spectrum showed correlations between H-12 and H-13 (δ_H 0.91) to C-11 (δ_C 31.1) and C-6 (δ_C 46.4), indicating *gem*-dimethyl was linked at C-11 and C-6 positions. The presence of tertiary methyl groups was established by the correlation between H-14 (δ_H 2.18) to C-9 (δ_C 208.9) which was a ketone. Another ketone group's presence was established by the correlation between H-2 (δ_H 1.98) to C-3 (δ_C 214.4). The proton pairing was also confirmed with the ^{1}H-^{1}H COSY spectrum, such that ^{1}H-^{1}H COSY cross peak was observed at H-4/H-10, while the HMBC correlations of compound **3** can be seen in Fig. 2. In the comparison of compound **3** with literature data,[23] **3** was identified as saniculamoid D, which is even isolated for the first time in *Aglaia* genus.

Compound **4** was obtained as colorless oil where the
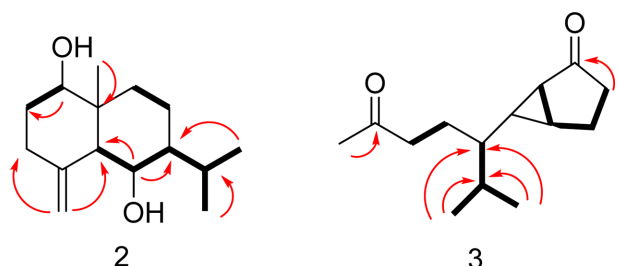
HR-TOFMS spectrum showed [M+H]$^+$ *m/z* 237.1968 (calcd. *m/z* = 237.1969), which corresponded to the molecular formula $C_{15}H_{25}O_2$ and hence required four degrees of unsaturation, originating from one pair of carbon sp^2, one carbonyl ketone, and the remaining being bicyclic isodaucane-type sesquiterpenoid. Its molecular composition $C_{15}H_{25}O_2$, was established from NMR data (Table 2). The IR spectra showed absorption peaks at 3319 cm^{-1} (OH), 2956 and 2880 cm^{-1} (C-H sp^3), 1464 cm^{-1} (C=C), 1380 and 1372 cm^{-1} (*gem*-dimethyl groups), and 1045 cm^{-1} (C-O). Furthermore, the ^{1}H-NMR (CDCl$_3$ 500 MHz) spectrum indicated three methyl groups, namely two secondary resonating at δ_H 0.90 (3H, d, *J* = 5.5 Hz, CH$_3$-14) and 0.89 (3H, d, *J* = 7.0 Hz, CH$_3$-15), plus one tertiary resonating at δ_H 1.27 (3H, s, CH$_3$-11), which indicated the presence of an isodaucane-type sesquiterpenoid skeleton. One olefinic methine group, resonating at δ_H 5.52 (1H, d, *J* = 4.5 Hz, H-6), and an oxymethylene group resonating at δ_H 4.02 (2H, H-12), were also observed. The ^{13}C-NMR (CDCl$_3$, 125 MHz) and DEPT 135 spectra showed the presence of three methyl groups, which indicated the characteristic of isodaucane-type sesquiterpenoids, one olefinic methine, one olefinic quaternary carbon, and one oxygenated methylene group resonating at δ_C 67.3 (C-12) as well as one carbonyl ketone resonating at δ_C 213.8 (C-10). These functionalities accounted for two of the total four degrees of unsaturation, while the remaining two were consistent with the isodaucane-type sesquiterpenoid. A comparison of the NMR data of **4** and aphanamol I,[24,25] showed the structures of the two compounds were very similar, consequently **4** was identified as an aphanamol I, which has just been isolated for the first time in *Aglaia* genus.

Fig. 2. Selected ^{1}H-^{1}H COSY and HMBC correlations of compounds **2** and **3**.

Fig. 3. Selected NOESY correlations for compounds **2** and **3**.

Table 2. IC$_{50}$ inhibition values of compounds **1-5** against HeLa cervical and B16F10 melanoma cancer cell line

Compounds	IC$_{50}$ (µM) HeLa	IC$_{50}$ (µM) B16F10
7-epi-eudesm-4(15)-ene,1β,6α-diol (**1**)	749.25	945.03
7-epi-eudesm-4(15)-ene,1β,6α-diol (**2**)	1030.69	1180.73
Saniculamoid (**3**)	6086.83	3375.63
Aphanamol I (**4**)	380.35	440.77
4β,10α-dihydroxyaromadendrane (**5**)	18043.84	17210.98
Cisplatin*	19.00	43.00

*positive control

Compound **5** was obtained as colorless oil where the HR-TOFMS spectrum showed [M-H]$^+$ m/z 237.1858 (calcd. m/z = 237.1855), which corresponded to the molecular formula $C_{15}H_{25}O_2$ and hence required three degrees of unsaturation, originating from tricyclic aromadendrane-type sesquiterpenoid, which was established from NMR data (Table 2). The IR spectra showed absorption peaks at 3359 cm^{-1} (OH), 2969 and 2865 cm^{-1} (C-H sp^3), 1464 cm^{-1} (C=C), 1380 and 1375 cm^{-1} (gem-dimethyl groups), and 1105 cm^{-1} (C-O). The ^{1}H-NMR (CDCl$_3$ 500 MHz) indicated four tertiary methyl groups resonating at δ$_H$ 1.02 (3H, s, CH$_3$-12), 1.02 (3H, s, CH$_3$-13), 1.15 (3H, s, CH$_3$-14) and 1.23 (3H, s, CH$_3$-15), which indicated the presence of aromadendrane-type sesquiterpenoid skeleton. Furthermore, the ^{13}C-NMR (CDCl$_3$ 125 MHz) and DEPT 135 spectra showed the presence of four methyl groups, which indicated the characteristic of aromadendrane-type sesquiterpenoids, and two oxygenated quaternary groups, resonating at δ$_C$ 80.5 (C-4) and δ$_C$ 75.1 (C-10). These functionalities accounted for zero of the total three degrees of unsaturation, while the remaining three were consistent with the aromadendrane-type sesquiterpenoid. A comparison of the NMR data of **5** and 4β,10α-dihydroxyaromadendrane,[16] indicated that the structures of the two compounds were very similar, consequently **5** was identified as 4β,10α-dihydroxyaromadendrane.

The isolated compounds, **1-5**, were evaluated for cytotoxic against HeLa cervical and B16F10 melanoma cancer cell lines with a previously described method, using cis-Platin as a positive control.[26,27] Among those tested, aphanamol (**4**), exhibited the strongest cytotoxicity with an IC$_{50}$ value of 380.35 and 440.77 µM, but still considered inactive cytotoxic compound. This result indicated the value of cytotoxic activity is influenced by the presence of a cyclohexane ring and ketone group in isodaucane skeleton.[28]

References

(1) Yadav, R.; Pednekar, A.; Avalaskar, A.; Rathi, M.; Rewachandani, Y. *World J. Pharm. Sci.* **2015**, *3*, 1572-1577.

(2) Muellner, A. N.; Samuel, R.; Johnson, S. A.; Cheek, M.; Pennington, T. D.; Chase, M. W. *Am. J. Bot.* **2003**, *90*, 471-480.

(3) Grudinski, M.; Pannell, C. M.; Chase, M. W.; Ahmad, J. A.; Muellner-Riehl, A. N. *Mol. Phylogenet. Evol.* **2014**, *73*, 65-76.

(4) Harneti, D.; Supratman, U. *Phytochemistry* **2021**, *181*, 112540.

(5) Harneti, D.; Tjokronegoro, R.; Safari, A.; Supratman, U.; Loong, X. M.; Mukhtar, M. R.; Mohamad, K.; Awang, K.; Hayashi, H. *Phytochem. Lett.* **2012**, *5*, 496-499.

(6) Harneti, D.; Supriadin, A.; Ulfah, M.; Safari, A.; Supratman, U.; Awang, K.; Hayashi, H. *Phytochem. Lett.* **2014**, *8*, 28-31.

(7) Sianturi, J.; Harneti, D.; Darwati.; Mayanti, T.; Supratman, U.; Awang, K. *Nat. Prod. Res.* **2016**, *30*, 2204-2208.

(8) Sianturi, J.; Purnamasari, M.; Darwati.; Harneti, D.; Mayanti, T.; Supratman, U.; Awang, K.; Hayashi, H. *Phytochem. Lett.* **2015**, *13*, 297-301.

(9) Farabi, K.; Harneti, D.; Nurlelasari.; Maharani, R.; Hidayat, A. T.; Awang, K.; Supratman, U.; Shiono, Y. *Phytochem. Lett.* **2017**, *21*, 211-215.

(10) Farabi, K.; Harneti, D.; Nurlelasari.; Maharani, R.; Hidayat, A. T.; Supratman, U.; Awang, K.; Shiono, Y. *CMU J. Nat. Sci.* **2017**, *16*, 293-306.

(11) Farabi, K.; Harneti, D.; Nurlelasari.; Maharani, R.; Hidayat, A. T.; Awang, K.; Supratman, U.; Shiono, Y. *Rec. Nat. Prod.* **2018**, *12*, 121-127.

(12) Hidayat, A. T.; Farabi, K.; Harneti, D.; Nurlelasari.; Maharani, R.; Nurfarida, I.; Supratman, U.; Shiono, Y. *Indones. J. Chem.* **2018**, *18*, 35-42.

(13) Hutagaol, R. P.; Harneti, D.; Hidayat, A. T.; Nurlelasari. N.; Maharani, R.; Katja, D. G.; Supratman, U.; Awang, K.; Shiono, Y. *Molbank* **2020**, *2020*, M1112.

(14) Hutagaol, R. P.; Harneti, D.; Safari, A.; Hidayat, A. T.; Supratman, U.; Awang, K.; Shiono, Y. *J. Asian Nat. Prod. Res.* **2021**, *23*, 781-788.

(15) Milawati, H.; Harneti, D.; Maharani, R.; Nurlelasari.; Hidayat, A. T.; Azmi, M. N.; Shiono, Y.; Supratman, U. *Molekul* **2019**, *14*, 126-132.

(16) Milawati, H.; Sukmawati, W.; Harneti, D.; Maharani, R.; Nurlelasari.; Hidayat, A. T.; Darwati.; Supratman, U.; Shiono, Y. *Indones. J. Chem.* **2020**, *20*, 1448-1454.

(17) Kurniasih, N.; Supriadin, A.; Fajar, M.; Abdulah, R.; Harneti, D.; Supratman, U.; Taib, M. N. A. B. M. *J. physics: Conference Series* **2019**, *1402*, 055037.

(18) Kurniasih, N.; Milawati, H.; Fajar, M.; Hidayat, A. T.; Abdullah, R.; Harneti, D.; Supratman, U.; Azmi, M. N. *Molekul* **2018**, *13*, 56-62.

(19) Inada, A.; Murata, H.; Inatomi, Y.; Nakanishi, T.; Darnaedi, D. *Phytochemistry* **1997**, *45*, 1225-1228.

(20) Inada, A.; Shono, K.; Murata, H.; Inatomi, Y.; Darnaedi, D.; Nakanishi, T. *Phytochemistry* **2000**, *53*, 1091-1095.

(21) Inada, A.; Akiba, T.; Murata, H.; Inatomi, Y.; Nakanishi, T.;

Darnaedi, D. *Nat. Med.* **2002**, *56*, 147-149.

(22) Kamel, A. *J. Nat. Prod.* **1995**, *58*, 428-431.

(23) Xue, Y.; Zhang, C.; Wu, Z.; Zhang, P.; Zhao, M.; Jiang, Y.; Tu, P. *J. Chin. Pharm. Sci.* **2018**, *27*, 576-581.

(24) Hansson, T.; Wickberg, B. *J. Org. Chem.* **1992**, *57*, 5370-5376.

(25) Jares, E. A.; Pomilio, A. B. *J. High Res. Chrom.* **1989**, *12*, 565-568.

(26) Hadisaputri, Y. E.; Miyazaki, T.; Suzuki, S.; Yokobori, T.; Kobayashi, T.; Tanaka, N.; Inose, T.; Sohda, M.; Kuwano, H. *Ann. Surg. Oncol.* **2012**, *19*, S589-S596.

(27) Chavoshi, H.; Vahedian, V.; Saghaei, S.; Pirouzpanah, M. B.; Raeisi, M.; Samadi, N. *Asian Pac. J. Cancer Prev.* **2017**, *18*, 2243-2247.

(28) Pan, L.; Acuna, U. M.; Li, J.; Jena, N.; Ninh, T. N.; Pannell, C. M.; Chai, H.; Fuchs, J. R.; Carcache de Blanco, E. J.; Soejarto, D. D.; Kinghorn, A. D. *J. Nat. Prod.* **2013**, *76*, 394-404.