MOLEKUL
eISSN: 2503-0310

Articles

https://doi.org/10.20884/1.jm.2024.19.2.9823

Sesquiterpenoids from The Stem Bark of *Aglaia cucullata* (Meliaceae) and Their Cytotoxic Activity Against A549 Lung Cancer Cell Lines

Iqbal Wahyu Mustaqim[1], Desi Harneti[1*], Al Arofatus Naini[3], Erina Hilmayanti[1], Darwati[1], Nurlelasari[1], Tri Mayanti[1], Rani Maharani[1], Kindi Farabi[1,2], Ace Tatang Hidayat[1,2], Unang Supratman[1,2], Sofa Fajriah[3], Mohamad Nurul Azmi[4], Yoshihito Shiono[5]

[1]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor 45363, Indonesia
[2]Central Laboratory, Universitas Padjadjaran, Jatinangor 45363, Indonesia
[3]Research Center for Raw Materials for Medicine and Traditional Medicine, National Research and Innovation Agency (BRIN), Tangerang Selatan, Banten 15314, Indonesia
[4]School of Chemical Sciences, Universiti Sains Malaysia, 11800 Minden, Penang, Malaysia
[5]Department of Food, Life, and Environmental Science, Faculty of Agriculture, Yamagata University, Tsuruoka, Yamagata 997-8555, Japan

*Corresponding author email: desi.harneti@unpad.ac.id

Received September 25, 2023; **Accepted** February 01, 2024; **Available online** July 20, 2024

ABSTRACT. Sesquiterpenoids are a class of terpenoid compounds with a remarkable diversity of structures and biological activities. Sesquiterpenoids are primarily found in higher plants, such as the Meliaceae family's *Aglaia* genus. *Aglaia cucullata* is a species of *Aglaia* that is still rarely explored and can potentially contain sesquiterpenoid compounds with cytotoxic activity. Hence, the research intended to isolate sesquiterpenoids from the n-hexane extract of *A. cucullata* stem bark and evaluate their cytotoxic effect against A549 lung cancer cells. Compounds **1** and **2** were isolated and purified from *n*-hexane extracts utilizing various chromatographic techniques. The structure of compounds **1** and **2** was determined by analyzing various spectroscopic methods (IR, MS, and NMR) and comparing them to previously reported spectral data. Compound **1** was identified as an isodaucane-type sesquiterpenoid, 10-hydroxy-6,10-epoxy-7(14)-isodaucane, and was first reported in Meliaceae family. Compound **2** was confirmed as an eudesmane-type sesquiterpenoid, eudesm-4(15)-ene-1β,6α-dihydroxy, and was first reported in *Aglaia cucullata*. Cytotoxic activity of **1** and **2** were investigated in vitro against A549 lung cancer cells using the PrestoBlue assay and resulted in inactive and low cytotoxicity with IC_{50} values of 292.77 and 90.55 µM, respectively.

Key words: *Aglaia cucullata*, , A549 cell lines**,** cytotoxic activity, Meliaceae, sesquiterpenoids.

INTRODUCTION

Sesquiterpenoids are the most abundant group of plant secondary metabolites, consisting of 15 carbon atoms ($C_{15}H_{24}$) with over 300 different skeletons (Abu-Izneid et al. , 2020; Hussain et al., 2019; Liu et al., 2021). More than 7000 sesquiterpenoid compounds have been successfully isolated, primarily as volatile plant oils (Mai et al., 2021). Sesquiterpenoids are produced by the mevalonate pathway, which comprises three isoprene units and takes place in the endoplasmic reticulum and through farnesyl pyrophosphate (FPP) (Abbas et al. 2017; Chadwick et al., 2013). Sesquiterpenoids can be categorized as acyclic, monocyclic, bicyclic, tricyclic, or tetracyclic, depending on how many carbon rings they contain in their chemical structure (Mai et al., 2021). They can also be divided into groups based on the number of carbons are present in the rings; most rings contain five, six, seven, or even up to eleven carbon atoms (Li et al., 2019).

The largest genus in the Meliaceae family, *Aglaia*, has been reported to contain a large variety of sesquiterpenoid (Harneti et al., 2022; Izdihar et al., 2021; Saeidnia et al., 2013). More than 150 different species of *Aglaia* exist, with 65 of them only being grown in Indonesia (Harneti & Supratman 2021; Huang et al., 2022). The *Aglaia* spread in tropical and subtropical regions such as Sri Lanka, India, Southern China, Southeast Asia, Northern Australia, and the Pacific Islands (Hutagaol et al., 2021; Pannell et al. 2020; Yodsaoue et al., 2012; Farabi et al., 2022; Kavitha et al., 2022; Xia et al., 2022). In Indonesia, the Sumatera, Kalimantan, Jawa, Sulawesi, Bali, Flores, and Papua are home to the *Aglaia* genus (Pannell et al., 2020). The bark of *Aglaia* has historically been used as a traditional Indonesian

herbal remedy to treat fever, influenza, cough, and other skin issues (Milawati et al., 2019; Sianturi et al., 2016).

Since 1965, hundreds of compounds have been successively isolated and elucidated from many species of *Aglaia* genus, including determining its biological activity (Harneti & Supratman, 2021). Besides the content of sesquiterpenoids, *Aglaia* genus also contains various biologically active secondary metabolites, such as diterpenoids, triterpenoids, limonoids, steroids, flavaglines, bisamides, and lignans (Harneti & Supratman, 2021; Yan et al., 2022). Those compounds showed biological activity including, cytotoxic, insecticidal, anti-inflammataory, antifungal, and molluscicide, and cytotoxic as the most potent. The previous research on *Aglaia* species makes this plant an interesting object to explore for the discovery of new biologically active compounds including cytotoxic ones (Harneti & Supratman, 2021).

Aglaia cucullata is a unique species in *Aglaia* genus grown in mangrove environment. Previously, this plant was known as *Amoora cucullata*. This species is observed in lowland and tidal riverbanks native to Southeast Asia. In Indonesia this plant can be found in Kalimantan Island. *A. cucullata* is a tree species associated with mangrove plant (Meepol et al., 2020). Since *A. cucullata* remains unexplored yet, this species together with its unique association and environments is expected to produce remarkable secondary metabolites especially for cytotoxic compounds.

Previous phytochemical investigation revealed that *A. cucullata*, contains many potential secondary metabolites including flavaglines, bisamides, diterpenoids, and triterpenoid with cytotoxic activities against several cancer cell lines such as oral human KB, human breast cancer BC, and small cell lung NCI-H187 from the fruits, leaves, and roots (Harneti et al., 2023). On the other hand, the human A549 lung cancer cell showed high recistancy against cisplatin as a cancer drug (Harneti & Supratman 2021). Therefore, those findings inspired us to explore the sesquiterpenoid compounds from *A. cucullata* and their cytotoxic acitvity against human lung cancer A549 cell. In this study we report here the isolation, structure elucidation of sesquiterpenoids **1** and **2** from the stembark *A. cucullata* and cytotoxic activity against A549 lung cancer cell lines. Various chromatography techniques were applied including vacuum liquid chromatography (VLC) and open column chromatography. Structure elucidation of isolated compounds were using spectroscopic techniques including FTIR, mass spectroscopy, ^{1}H, and ^{13}C NMR (including one and two dimensional). Prestoblue assay were used to determine cytotoxic activity of compounds **1** and **2** against A549 lung cancer cell lines. The isolation and structure elucidation of isolated compounds **1** and **2** were conducted for the first time in this species.

EXPERIMENTAL SECTION

General Experiment Procedure

IR spectra were collected in a KBr plate using a PerkinElmer Spectrum 100 FT-IR spectrometer (PerkinElmer, Shelton, USA). Mass spectra were measured using Waters Xevo QTOF mass spectrometer (Waters, Mildford, USA). NMR spectra were retrieved using JEOL JNM-ECX500R/S1 spectrometer (JEOL, Tokyo, Japan) and Bruker Av-500 spectrometer (Bruker, Karlsruhe, Germany), both at 500 MHz for ^{1}H and 125 MHz for ^{13}C, with tetramethyl silane (TMS) as an internal standard. The column chromatography was carried out on ODS RP-18 and silica G_{60} (Merck, 70-230 mesh and 230-400 mesh) and directed by thin layer chromatography (TLC) analyses with a silica G_{60} GF_{254} (Merck, 0.25 mm) using a variety of solvent systems, and spot detection utilizing 10% H_2SO_4 in ethanol followed by heating of 100 °C and checked under UV at wavelengths 254 and 365 nm.

Material

Acetone, chloroform (pro-analyst), ethanol, ethyl acetate, *n*-butanol, *n*-hexane, methanol, methylene chloride, and water are solvents used for extraction, fractionation, isolation, and purification with pro-analyst and technical quality that has been distilled. American Type Culture Collection (ATCC® CCL-185TM, Manassas, Virginia, USA) provided the A549 cell. Roswell Memorial Park Medium (RPMI) 1640 (Cat. No.11530586, Gibco, New York, USA) was used, along with 10% Fetal Bovine Serum (FBS) (Cat. No.10082147, Gibco) and 1% Penicillin-streptomycin (Cat. No. 15140112, Gibco). The cells were incubated at 37 °C in a 5% CO_2 incubator (Cat. No. 8000DH, Thermo Fisher Scientific, Waltham, Massachusetts, USA).

Plant Material

The stem bark of *A. cucullata* was collected in December 2020 from the Manggar River in Balikpapan, East Kalimantan. This sample was assessed at the Herbarium Wanariset, Balikapapan (collection No. FF7.20), and archived at the Faculty of Forestry, Universitas Mulawarman, Samarinda, East Kalimantan, Indonesia.

Extraction, Isolation, and Characterization of Secondary Metabolite

The air-dried *A. cucullata* stem bark powder (3.1 kg) was macerated using ethanol (40 L, 7 × 24 h). The ethanol solvent was evaporated with a vacuum rotary evaporator at 40 °C to yield a concentrated ethanol extract residue (523 g). The crude ethanol extract was suspended in a mixture of ethanol:water (4:1) before partitioning with *n*-hexane, ethyl acetate, and *n*-butanol. The crude fraction of *n*-hexane (128 g), ethyl acetate (35.7 g), and *n*-butanol (13.1 g) were obtained through evaporation. The *n*-hexane crude extract was separated using vacuum-liquid chromatography (VLC) with silica G_{60} (230-400 mesh)

as a stationary phase and eluted gradient using *n*-hexane:ethyl acetate (100:0 – -0:100, 10% v/v) followed by ethyl acetate:methanol (100:0 – 0:100, 10% v/v) to yield seven fractions (A-G).

Fraction C (16.2 g) was further separated using VLC on silica G_{60} (230-400 mesh) eluted in gradient systems with *n*-hexane:ethyl acetate (100:0-50:50, 10% v/v) to result four subfractions (C1-C4). Subfraction C2 (1.28 g) was subjected to silica G_{60} (230-400 mesh) column chromatography (CC) eluted gradient using *n*-hexane:ethyl acetate (100:0 – 80:20, 1% v/v) to obtain four subfractions (C2A-C2D). Subfraction C2C (562.8 mg) was separated by CC over silica G_{60} (230-400 mesh) and eluted with *n*-hexane:ethyl acetate (90:10, v/v) to afford five subfractions (C2C1-C2C5). Subfraction C2C4 (86.5 mg) was purified further with CC over silica G_{60} (230-400 mesh) using an isocratic mixture of *n*-hexane:ethyl acetate (85:15, v/v) to give compound **1**.

Subfraction C4 (12.69 g) was separated by gradient elution of *n*-hexane:ethyl acetate (100:0 – 30:70, 2% v/v) on CC silica G_{60} (70-230 mesh) to yield ten subfractions (C4A-C4J). Subfraction C4H (200.5 mg) was subjected to reverse phase CC on ODS eluted with methanol:water (80:20, v/v) to obtain four subfractions (C4H1-C4H4). Subfraction C4H3 (111.6 mg) was subjected to CC silica G_{60} (230-400 mesh) eluted with an isocratic mixture of *n*-hexane:chloroform:ethyl acetate (30:40:30, v/v) to obtain three subfractions (C4H3A-C4H3C). Subfraction C4H3B (38.5 mg) was further purified with silica gel CC (230-400 mesh) eluted with an isocratic mixture of *n*-hexane:chloroform:ethyl acetate (30:40:30, v/v) to give compound **2**.

10-Hydroxy-6,10-epoxy-7(14)-isodaucane (**1**) – colorless oil; IR υ_{max} cm^{-1}: 3406, 2955, 1384, 1367, 1076; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 1.78 (1H, m, H-2a), 1.33 (1H, m, H-2b), 1.83 (1H, m, H-3a), 1.25 (1H, m, H-3b), 1.54 (1H, s, H-4), 1.54 (1H, s, H-5), 3.96 (1H, s, H-6), 2.50 (1H, m, H-8a), 2.33 (1H, m, H-8b), 2.01 (1H, dd, *J* = 4.0,7.0 Hz, H-9a) 1.73 (1H, m, H-9b), 1.43 (1H, m, H-11), 0.87 (3H, d, *J* = 6 Hz, CH_3-12), 0.89 (3H, d, *J* = 6.5 Hz, CH_3-13), 4.74 (1H, s, H-14a), 4.69 (1H, s, H-14b), 1.13 (3H, s, CH_3-15); ^{13}C-NMR ($CDCl_3$, 125 MHz), Table 1; HR-TOF-MS, *m/z* 237.1856 [M+H$^+$] (calcd. $C_{15}H_{25}O_2$ *m/z* = 237.1855).

eudesm-4(15)-ene-1β-6α-dihydroxy (2) – colorless oil; IR υ_{max} cm^{-1}: 3406, 2965, 1634, 1376, 1027; ^{1}H-NMR ($CDCl_3$, 500 MHz): δ_H 3.42 (1H, dd, *J* = 5.0,11.0 Hz, H-1), 1.92 (1H, m, H-2a), 1.55 (1H, m, H-2b), 2.34 (1H, dd, *J* = 4.5,13.0 Hz, H-3a), 2.07 (1H, dd, *J* = 4.5,13.5 Hz, H-3b), 1.76 (1H, d, *J* = 9.5 Hz, H-5), 3.72 (1H, t, *J* = 9.5 Hz, H-6), 1.20 (1H, t, *J* = 11.5 Hz, H-7), 1.54 (1H, m, H-8a), 1.30 (1H, m, H-8b), 1.93 (1H, dt, *J* = 3.0,14.0 Hz, H-9a), 1,24 (1H, m, H-9b), 2,25 (1H, m, H-11), 0.95 (3H, d, *J* = 7.0 Hz, CH_3-12), 0.87 (3H, d, *J* = 6.5 Hz, CH_3-13), 0.67 (3H, s, CH_3-14), 5.02 (1H, s, H-15a), 4.75 (1H, s, H-15b), 2.56 (1H, s, -OH); ^{13}C-NMR ($CDCl_3$, 125 MHz), Table 1; HR-TOF-MS, *m/z* 239.2006 [M+H$^+$] (calcd. $C_{15}H_{27}O_2$ *m/z* = 239.2011).

Cytotoxic Assay by PrestoBlue Method

The PrestoBlue cell viability method was used to test the cytotoxic activity of compounds in accordance with those previous reports (Naini et al., 2023[a,b]). The A549 lung cancer cells were cultured at a density of 2 × 10^4 cells per well into 96-well microliter plates for 24 hours at 37 °C in a humidified atmosphere of 5% CO_2 in Roswell Memorial Park Institute (RPMI) 1640 medium supplemented with 10% (v/v) Fetal Bovine Serum (FBS) and 1 µL/mL antibiotics. The compounds were put into the wells after 24 hours. Viability was assessed after 96 hours by determining the metabolic conversion reduction of resazurin substrate into pink fluorescent resofurin product produced in viable cells. The PrestoBlue assay results were read at 570 nm using a multimode reader. All compounds were evaluated at eight concentrations 3.90, 7.81, 15.63, 31.25, 62.50, 125.00, 250.00, and 500.00 µg/mL in 100 % DMSO and with a final concentration of 2 % in each well. Each compound concentration was evaluated in two parallel experiments, and IC_{50} values were determined using the linear regression method in Microsoft Excel software.

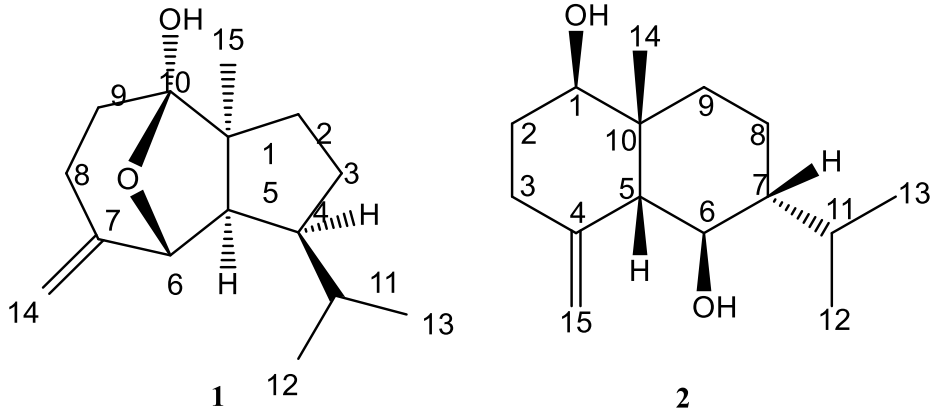

Figure 1. Chemical structure of compound **1** and **2**

RESULTS AND DISCUSSION

Compound **1** was isolated as a colorless oil that dissolves in chloroform and turns a purple-pinkish in a thin layer chromatography (TLC) plate when sprayed with 10% sulfuric acid in ethanol, representing the presence of a terpenoid structure. Compound **1** is not fluorescent under UV light at 254 nm and 365 nm wavelengths. Thus there is no conjugated double-bond system. The molecular formula determined by HR-TOFMS ([M+H$^+$]) (**Figure S1**) was $C_{15}H_{25}O_2$ with *m/z* 237.1856, calcd. *m/z* 237.1855, which needed four degrees of unsaturation. Absorption bands were observed in the IR spectra (**Figure S2**) at 3406 cm^{-1} (OH), 2955 cm^{-1} (C-H *sp*^{3}), 1384 cm^{-1} and 1367 cm^{-1} (*gem*-dimethyl), and 1076 cm^{-1} (C-O). The ^{1}H-NMR ($CDCl_3$ 500 MHz) spectrum (**Figure S3**) revealed three methyl groups consisting of two secondary methyls at δ_H/ppm 0.89 (3H, d, *J* = 6.5 Hz, CH_3-13) and 0.87 (3H, d, *J* = 6.0 Hz, CH_3-12), and one tertiary methyl at δ_H/ppm 1.13 (3H, s, CH_3-15). One olefinic methylene group resonating at δ_H/ppm 4.74 (1H, s, H-14a) and 4.69 (1H, s, H-14b), as well as one oxymethine group resonating at δ_H/ppm 3.96 (1H, s, H-6). The ^{13}C-NMR ($CDCl_3$ 125 MHz) and DEPT 135 spectra (**Figure S4**) revealed the existence of 15 carbons (**Table 1**). Assigning of carbon signals by HSQC spectrum (**Figure S5**) represented three methyls at δ_C/ppm 21.6 (CH_3-12), 21.7 (CH_3-13), and 20.5 (CH_3-15), four *sp*^{3} methylenes at δ_C/ppm 35.9 (C-2), 30.7 (C-3), 26.5 (C-8), and 33.2 (C-9), one *sp*^{2} methylenes at δ_C/ppm 108.5 (C-14), three methines at δ_C/ppm 57.7 (C-4), 58.9 (C-5), and 34.3 (C-11), one oxygenated methine at δ_C/ppm 86.1 (C-6), one quarternary *sp*^{3} carbon at δ_C/ppm 54.6 (C-1), and one quarternary oxygenated carbon at δ_C/ppm 105.2 (C-10), and one quarternary *sp*^{2} carbon at δ_C/ppm 144.9 (C-7). Thence, the ^{1}H- and ^{13}C-NMR data implicity show the presence of a sesquiterpenoid bicyclic skeleton with an epoxide ring and olefinic. The correlations between H_2-H_3-H_4-H_5, H_4-H_{11}, H_8-H_9, H_{11}-H_{12}, and H_{11}-H_{13} were observed in the ^{1}H-^{1}H COSY spectrum (**Figure S6**) indicating the isopropyl group located at C-4. Furthermore, the HMBC correlations of the methyl protons to their nearby carbons supported the interpretation of proton signals from one tertiary methyl and two secondary methyls. The correlations of CH_3-12 (δ_H/ppm 0.87) and CH_3-13 (δ_H/ppm 0.89) to δ_H/ppm 34.3 (C-11) and 57.7 (C-4) showed isopropyl group attached to C-4. Clear correlations between CH_3-15 (δ_H/ppm 1.13) to δ_C/ppm 35.9 (C-2), 54.6 (C-1), 58.9 (C-5), and 105.2 (C-10) confirmed that C-15 attached to C-1 of quarternary carbon. The correlations of methylene protons at δ_H/ppm 2.33 (H-8) to δ_C/ppm 86.1 (C-6) and 144.9 (C-7), and also at δ_H/ppm 2.01 (H-9a) and 1.73 (H-9b) to δ_C/ppm 105.2 (C-10) and 144.9 (C-7) indicated the presence of ether bridge at C-6/C-10 and double bond at C-7/C-14. The oxygenated proton correlations at δ_H/ppm 3.96 (H-6) to δ_C/ppm 105.2 (C-10) and 108.5 (C-14) verified an ether bridge at C-6/C-10 and double bond at C-7/C-14 placed adjacent to ether bridge. The correlations from 10-OH at δ_H/ppm 2.56 to δ_C/ppm 33.2 (C-9), 54.6 (C-1), and 105.2 (C-10) confirmed quarternary oxygenated carbon at C-1 with hydroxyl attached. The key ^{1}H-^{1}H COSY and HMBC correlations further confirmed the planar structure of **1** (**Figure 2**). The NOESY correlations (**Figure 3**) between H-4 (α-oriented) with CH_3-15 proposed that CH_3-15 was α-oriented. Moreover, the correlations between H-5 (α-oriented) with H-6 and 10-OH advised that both H-6 and 10-OH were α-oriented. When the NMR data from compound **1** were compared to the literature isolated from *Bursera graveolens* (Yukawa et al. 2005) (**Table 1**), it was discovered that it is 10-hydroxy-6,10-epoxy-7(14)-isodaucane, which was isolated for the first time in the Meliaceae family and *Aglaia* genus.

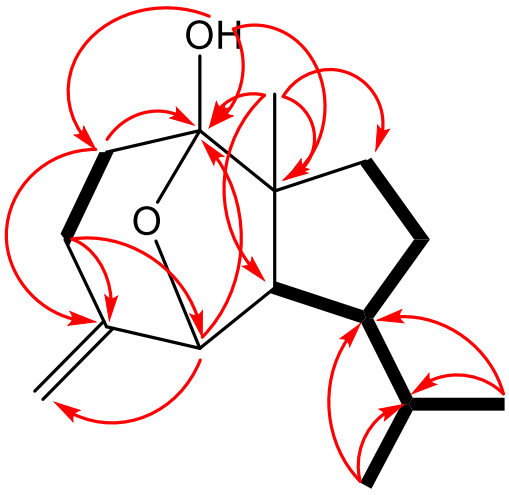

Figure 2. Selected ^{1}H-^{1}H COSY and HMBC correlations of compound **1**

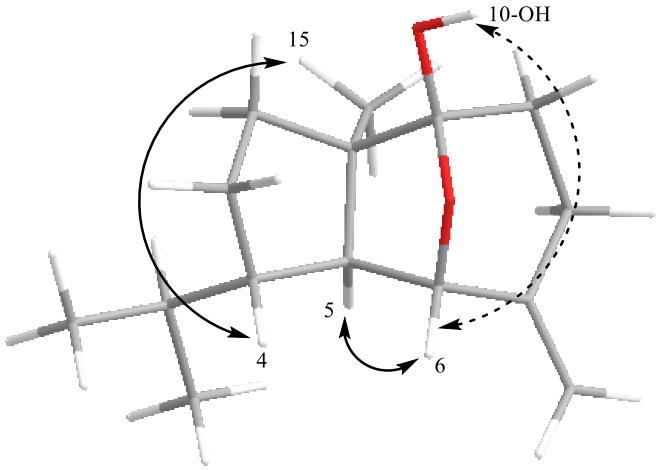

Figure 3. Selected NOESY correlations of compound **1**

Table 1. NMR data for compound **1**

Posisi	Compounds **1***		10-hidroxy-6,10-epoxy-7(14)-isodaucane*	
	δ_C (ppm)	δ_H (∑H; mult., *J*)	δ_C (ppm)	δ_H (∑H; mult., *J*)
1	54.6	-	54.5	-
2	35.9	1.78 (1H, m)	35.9	1.82 (1H)
		1.33 (1H, m)		1.37 (1H)
3	30.7	1.83 (1H, m)	30.6	1.87 (1H)
		1.25 (1H, m)		1.27 (1H)
4	57.7	1.54 (1H, s)	57.6	1.56 (1H)
5	58.9	1.54 (1H, s)	58.9	1.58 (1H)
6	86.1	3.96 (1H, s)	86.0	3.98 (1H)
7	144.9	-	144.8	-
8	26.5	2.50 (1H, m)	26.4	2.49 (1H)
		2.33 (1H, m)		2.35 (1H)
9	33.2	1.73 (1H, m)	33.2	1.76 (1H)
		2.01 (1H, dd, 7.0, 4.0)		2.02 (1H)
10	105.2	-	105.1	-
11	34.3	1.43 (1H, m)	34.1	1.45 (1H)
12	21.6	0.87 (3H, d, 6)	21.5	0.89 (3H, d, 6.6)
13	21.7	0.89 (3H, d, 6.5)	21.6	0.92 (3H, d, 6.6)
14	108.5	4.74 (1H, s)	108.4	4.76 (1H, br.s)
		4.68 (1H, s)		4.70 (1H, br.s)
15	20.5	1.13 (3H, s)	20.4	1.15 (3H, s)

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

Compound **2** is a colorless oil that is dissoluble in chloroform and change to purple in the TLC plate when sprayed with 10% sulfuric acid in ethanol, indicating the presence of a terpenoid structure. Compound **2** is not fluorescent under UV light at 254 nm and 365 nm wavelengths, indicating that it lacks a conjugated double bond system. The HR-TOF-MS spectrum (**Figure S9**) showed $[M+H^+]$ *m/z* 239.2006 (calcd. *m/z* 239.2011), which conforms to the molecular formula $C_{15}H_{27}O_2$ and thus necessary three degrees of unsaturation. The absorption peaks in the IR spectrum (**Figure S10**) of 3406 cm^{-1} (OH), 2965 cm^{-1} (C-H sp^3), 1634 cm^{-1} (C=C), 1376 cm^{-1} (*gem*-dimethyl), and 1027 cm^{-1} (C-O). The ^1H-NMR ($CDCl_3$ 500 MHz) spectrum (**Figure S11**) displayed three methyl groups composed of two secondary methyls at δ_H/ppm 0.95 (3H, d, *J* = 7.0 Hz, CH_3-12) and 0.87 (3H, d, *J* = 6.5 Hz, CH_3-13), and one tertiary methyl at δ_H/ppm 0.67 (3H, s, CH_3-14), signifying the presence of eudesmane-type sesquiterpenoid skeleton. The representation of a characteristic signal for olefinic protons at δ_H/ppm 5.02 (1H, s, H-15a) and 4.75 (1H, s, H-15b) was then demonstrated. Additionally, the signal for the oxygenated protons was spotted at δ_H/ppm 3.72 (1H, t, *J* = 9.5 Hz, H-6) and 3.42 (1H, dd, *J* = 5.0,11.0 Hz, H-1).

Table 2. NMR data for compound **2**

Position	Compound **2***		eudesman-4(15)-ena-1β,6α-diol*	
	δ_C (ppm)	δ_H (∑H; mult., *J*)	δ_C (ppm)	δ_H (∑H; mult., *J*)
1	79.0	3.42 (1H, dd, 11.0, 5.0)	78.9	3.38 (1H, dd, 11.6, 4.7)
2	31.9	1.92 (1H, m) 1.55 (1H, m)	31.8	1.82 (1H, dtd, 12.5, 5.1, 2.3) 1.51 (1H, qd, 12.7, 5.0)
3	35.1	2.34 (1H, dd, 13.0, 4.5) 2.07 (1H, dd, 13.5, 4.5)	35.0	2.29 (1H, ddd, 13.1, 5.0, 2.2) 2.02 (1H, brtd, 13.4, 5.2)
4	146.2	-	146.2	-
5	55.9	1.76 (1H, d, 9.5)	55.8	1.70 (1H, brd, 9.0)
6	66.9	3.72 (1H, t, 9.5)	67.0	3.68 (1H, t, 10.0)
7	49.3	1.20 (1H, t, 11.5)	49.2	1.26 (1H, tt, 12.5, 3.0)
8	18.1	1.54 (1H, m) 1.30 (1H, m)	18.0	1.50 (1H, m) 1.19 (1H, qd, 12.0, 2.7)
9	36.3	1.93 (1H, dt, 14.0, 3.0) 1.24 (1H, m)	36.2	1.88 (1H, dt, 12.4, 2.9) 1.12 (1H, td, 13.1, 3.5)
10	41.7	-	41.6	-
11	25.9	2.25 (1H, m)	25.9	2.20 (1H, sed, 7.0, 2.5)
12	21.1	0.95 (3H, d, 7.0)	21.1	0.91 (3H, d, 7.0)
13	21.7	0.87 (3H, d, 6.5)	16.2	0.83 (3H, d, 7.0)
14	11.6	0.67 (3H, s)	11.5	0.67 (3H, s)
15	20.5	5.02 (1H, s) 4.75 (1H, s)	107.8	4.98 (1H, brs) 4.70 (1H, brs)

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

^{13}C-NMR spectrum with DEPT 135 (**Figure S12**) detailed analysis showed the presence of fifteen carbons consisting three methyls at δ_C/ppm 21.1 (CH_3-12), 16.3 (CH_3-13), and 11.6 (CH_3-14), four sp^3 methylenes at δ_C/ppm 31.9 (C-2), 35.1 (C-3), 18.1 (C-8), and 36.3 (C-9), one sp^2 methylene at δ_C/ppm 107.8 (C-15), three methines at δ_C/ppm 55.9 (C-5), 49.3 (C-7), and 25.9 (C-11), two oxygenated methines at δ_C/ppm 79.0 (C-1) and 66.9 (C-6), one quarternary sp^3 carbon at δ_C/ppm 41.7 (C-10), and one quarternary sp^2 carbon at δ_C/ppm 146.2 (C-4). These functionalities are attributed to one of the three degrees of unsaturation. Two leftover hydrogen deficiency indexes have assigned the bicyclic sesquiterpenoid structure (Milawati et al., 2019). Compound **2** is classified as a eudesmane-type sesquiterpenoid structure due to the quantity of methyl and methylene in the ^1H-NMR, ^{13}C-NMR, and DEPT 135 spectra (Zhang et al., 2003). A comparative of the NMR data of **2** and eudesm-4(15)-ene-1β-6α-diol isolated from *Litsea verticillata* (Zhang et al., 2003) confirmed their structures were very similar (**Table 2**).. As an outcome, **2** was described as a eudesm-4(15)-ene-1β-6α-dihydroxy, isolated for the first time in this plant.

The cytotoxicity activity compounds **1** and **2** were tested against the A549 lung cancer cell lines using a method stated previously and doxorubicin (2 μM) as positive control (Xu et al., 2015). Half maximal inhibitory concentration (IC_{50}) was used to show the concentration of a molecule demanded to inhibit a particular biological process by half (50%) (Qureshi et al., 2015). Compounds **1** and **2** had IC_{50} values of 292.77 μM and 90.55 μM, respectively, indicating they were inactive and low (Naini et al., 2023[a,b]). Compound **1** has never been biologically tested (Da Silva et al., 2017; Yukawa et al., 2005), while compound **2** has been tested its cytotoxicity which showed no acitivitiy against HeLa cervical cancer cells and skin melanoma B16-F10 (Harneti et al., 2022; Zhang et al., 2003), so in this study, the first time to conduct a cytotoxic assay on lung cancer cells A549.

CONCLUSIONS

Two types of sesquiterpenoid compounds, 10-hydroxy-6,10-epoxy-7(14)-isodaucane (**1**) and eudesm-4(15)-ene-1-6-diol (**2**), were isolated from the stem bark of *A. cucullata*. Compound **1** was discovered for the first time in the Meliaceae family and *Aglaia* genus, while compound **2** was reported for *A. cucullata* for the first time. The cytotoxic activity of compounds **1** and **2** against the A549 lung cancer cell line was assessed, and the IC_{50} values revealed inactive and low cytotoxicity. The partial structural modification of the isolated compound allows for further use of it as a lead compound for potential anticancer drug candidates.

ACKNOWLEDGEMENTS

This study was funded by the Ministry of Education and Culture, Innovative and Research Council, Indonesia, Master Thesis Research (PTM) Grant, (No. 1316/UN6.3.1/PT.00/2022) by Desi Harneti and Academic Leadership Grant (ALG) from Universitas Padjadjaran, Indonesia (No. 2203/UN6.3.1/PT.00/ 2022) by Unang Supratman.

REFERENCES

Abbas, F., Ke, Y., Yu, R., Yue, Y., Amanullah, S., Jahangir, M. M., & Fan, Y. (2017). Volatile terpenoids: multiple functions, biosynthesis, modulation and manipulation by genetic engineering. *Planta*, *246*(5), 803–816.

Abu-Izneid, T., Rauf, A., Shariati, M. A., Khalil, A. A., Imran, M., Rebezov, M., … Rengasamy, K. R. R. (2020). Sesquiterpenes and their derivatives-natural anticancer compounds: An update. *Pharmacological Research*, *161*(June), 105165.

Chadwick, M., Trewin, H., Gawthrop, F., & Wagstaff, C. (2013). Sesquiterpenoids lactones: Benefits to plants and people. *International Journal of Molecular Sciences*, *14*(6), 12780–12805.

Da Silva, B. P., Nepomuceno, M. P., Varela, R. M., Torres, A., Molinillo, J. M. G., Alves, P. L. C. A., & Macías, F. A. (2017). Phytotoxicity study on *Bidens sulphurea Sch*. Bip. as a preliminary approach for weed control. *Journal of Agricultural and Food Chemistry*, *65*(25), 5161–5172.

Harneti, D., Mustaqim, I. W., Darwati, D., Naini, A. A., Purnama, P., Hilmayanti, E., Shiono, Y. (2023). Triterpenoids from the stem bark of *Aglaia cucullata* (Meliaceae) and Their cytotoxic activity against A549 Lung cancer cell line. *Indonesian Journal of Chemistry*, *23*(4), 961–973.

Harneti, D., Permatasari, A. A., Anisshabira, A., Naini, A. A., & Mayanti, T. (2022). Sesquiterpenoids from the stem bark of *Aglaia grandis*. *Natural Products Sciences*, *28*(1), 6–12.

Harneti, D., & Supratman, U. (2021). Phytochemistry and biological activities of *Aglaia* species. *Phytochemistry*, *181*(January 2021), 112540.

Heyne, K. (1987). Tumbuhan Berguna Indonesia, Jilid. 3 (Useful Plants of Indonesia, Vol. 3), terjemahan Badan Litbang Kehutanan Jakarta. *Yayasan Sarana Warna Jaya, Jakarta*.

Hussain, A., Rizwan-ul-haq, M., Aljabr, A. M., & Al-ayedh, H. (2019). Lethality of sesquiterpenes reprogramming red palm weevil detoxification mechanism for natural novel biopesticide development. *Molecules*, *24*(9), 1–13.

Izdihar, G., Naini, A. A., Harneti, D., Maharani, R., Nurlelasari, N., Mayanti, T., … Shiono, Y. (2021). Sesquiterpenoids from the stem bark of *Aglaia simplicifolia* and their cytotoxic activity against b16-f10 melanoma skin cancer cell. *Indonesian Journal of Chemistry*, *21*(6), 1560–1567.

Li, Z., Howell, K., Fang, Z., & Zhang, P. (2019). Sesquiterpenes in grapes and wines: Occurrence, biosynthesis, functionality, and influence of winemaking processes. *Comprehensive Reviews in Food Science and Food Safety*, *19*(1), 247–281.

Liu, C. L., Xue, K., Yang, Y., Liu, X., Li, Y., Lee, T. S., … Tan, T. (2021). Metabolic engineering strategies for sesquiterpene production in microorganism. *Critical Reviews in Biotechnology*, *42*(1), 73–92.

Mai, J., Li, W., Ledesma-Amaro, R., & Ji, X. J. (2021). Engineering plant sesquiterpene synthesis into yeasts: A Review. *Journal of Agricultural and Food Chemistry*, *69*(33), 9498–9510.

Milawati, H., Harneti, D., Maharani, R., Hidayat, A. T., Azmi, M. N., Shiono, Y., & Supratman, U. (2019). Caryophyllene-type sesquiterpenoids from the stembark of *Aglalia harmsiana* and their cytotoxic activity against MCF-7 breast cancer cells. *Molekul*, *14*(2), 126–132.

Pannell, C. M., Schnitzler, J., & Muellner-Riehl, A. N. (2020). Two new species and a new species record of *Aglaia* (Meliaceae) from Indonesia. *PhytoKeys*, *155*, 33–51.

Qureshi, A., Tandon, H., & Kumar, M. (2015). AVP-IC50 Pred: multiple machine learning techniques-based prediction of peptide antiviral activity in terms of half maximal inhibitory concentration (IC_{50}). *Peptide Science*, *104*(6).

Saeidnia, S., Gohari, A. R., & Haddadi, A. (2013). Biogenic trypanocidal sesquiterpenes: Lead compounds to design future trypanocidal drugs - A mini review. *DARU, Journal of Pharmaceutical Sciences*, *21*(1), 1–9.

Sianturi, J., Harneti, D., Darwati, Mayanti, T., Supratman, U., & Awang, K. (2016). A New (–)-5′,6-dimethoxyisolariciresinol-(3″,4″-dimethoxy)-3α-O-β-D-glucopyranoside from the bark of *Aglaia eximia* (Meliaceae). *Natural Product Research*, *30*(19), 2204–2208.

Xu, M., Mccanna, D. J., & Sivak, J. G. (2015). Use of the viability reagent prestoblue in comparison with alamar blue and MTT to assess the viability of human corneal epithelial cells. *Journal of Pharmacological and Toxicological Methods*, *71*, 1–7.

Yodsaoue, O., Sonprasit, J., Karalai, C., Ponglimanont, C., Tewtrakul, S., & Chantrapromma, S. (2012). Diterpenoids and triterpenoids with potential anti-inflammatory activity from the leaves of *Aglaia odorata*. *Phytochemistry*, *76*, 83–91.

Yukawa, C., Iwabuchi, H., Komemushi, S., & Sawabe, A. (2005). Mono- and sesquiterpenoids of the volatile oil of *Bursera graveolens*. *Flavour and*

Fragrance Journal, *20*(6), 653–658.

Zhang, F., Wang, J. S., Gu, Y. C., & Kong, L. Y. (2010). Triterpenoids from *Aglaia abbreviata* and their cytotoxic activities. *Journal of Natural Products*, *73*(12), 2042–2046.

Zhang, H. J., Tan, G. T., Santarsiero, B. D., Mesecar, A. D., Van Hung, N., Cuong, N. M., … Fong, H. H. S. (2003). New sesquiterpenes from *Litsea verticillata*. *Journal of Natural Products*, *66*(5), 609–615.