Cytotoxic sesquiterpene compound from the stembark of 
Aglaia simplicifolia (Meliaceae)

N Kurniasih¹, A Supriadin², M Fajar³, R Abdulah³, D Harneti³, U Supratman²,4* and M N A B M Taib⁵

¹ Department of Chemistry, Faculty of Sciences and Technology, UIN Sunan Gunung Djati Bandung, Bandung, Indonesia
² Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Padjadjaran, Jatinangor, Indonesia
³ Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Jatinangor, Indonesia
⁴ Central Laboratory, Universitas Padjadjaran, Jatinangor, Indonesia
⁵ School of Chemical Sciences, Universiti Sains Malaysia, Minden, Penang, Malaysia

* nunungkurniasih@uinsgd.ac.id

Abstract. Previous phytochemical studies from the Aglaia genus reported the presence of terpenoid compound. This research describes the isolation and structure elucidation of sesquiterpenoid compound from the stembark of Aglaia simplicifolia. Dried stembark of A. simplicifolia extracted with methanol and then partitioned with n-hexane, ethyl acetate, and n-butanol, respectively. The n-hexane extract then was separated and purified with chromatography techniques to obtain isolated compound. The chemical structure of isolated compound was elucidated by IR, NMR 1D, NMR 2D as well as mass spectra and by comparison with those previously reported spectra data. The compound identified as senecrassidiol. This compound showed cytotoxicity activity against HeLa cervical cancer cells with IC₅₀ values of 2.18 µM.

1. Introduction
The genus Aglaia (Meliaceae) consists of approximately 120 species, which are mainly distributed in the tropical rainforest of Southeast Asia and on the Pacific islands [1]. Previous phytochemical studies of Aglaia species were focused on various types of compounds, such as flavaglines, bisamides, triterpenoids and diterpenoids, and their interesting biological properties including insecticidal, anticancer and anti-inflammatory [2].

Liu et al. isolated and discribed four guaian sesquiterpenes and one eudesmene sesquiterpene from the twigs of Aglaia odorata var. microphyllina [3]. Kurniasih et al. were isolated eudesmene and aromadendrane sesquiterpene from the stem bark of Aglaia minahassae obtained in Sulawesi and Papua, Indonesia [4]. Although sesquiterpenoids of other Aglaia species have been investigated previously, the sesquiterpenoids derivatives of A. simplicifolia is yet to be reported.
2. Experimental section

2.1. General experiment procedure
The IR spectra were recorded on a Perkin-Elmer spectrum-100 FT-IR (Waltwam, MA, USA) in KBr. Mass spectra were obtained with a Synapt G2 mass spectrometer instrument (Waters, Milford, MA, USA). NMR spectral data were performed on a Bruker Topspin spectrometer at 500 MHz (Bruker BioSpin GmbH, Silberstreifen 4, D-76287 Rheinstetten, Germany), with CDCl3 as a solvent, chemical shifts were given on a δ (ppm) scale and tetramethylsilane (TMS) as an internal standard. Column chromatography was conducted on silica gel 60 (Merck, Darmstadt, Germany) and Octa Desyl Sylane (ODS, Fuji Sylisia, Japan). TLC plates were precoated with silica gel GF254 (Merck, 0.25 mm) and detection was achieved by spraying with 10% H2SO4 in EtOH, followed by heating.

2.2. Plant material
The stem bark of A. simplicifolia were collected in Bogor Botanical Garden, Bogor, West Java Province, Indonesia in January 2016. The plant was identified by the staff of the Bogoriense Herbarium, Research Center for Biology, Indonesian Institute of Science, Bogor, Indonesia and a voucher specimen (No. BO-1295311) has been deposited at the herbarium.

2.3. Extraction and isolation
The dried Stem bark of A. simplicifolia (1.1 kg) were grounded with methanol. And its methanol extract (194.97 g) was concentrated and extracted successively with n-hexane, ethyl acetate, and n-buthanol. Each extract was obtained 14.54, 28.03, and 14.56 g, respectively.

The n-hexane extract (14.54 g) was chromatographed over a vacuum-liquid chromatographed (VLC) column packed with silica gel 60 by gradient elution. The VLC fractions were repeatedly subjected to silica gel and ODS column chromatography as well as preparative thin layer chromatography (PTLC) on silica gel GF254 to afford a compounds 1 (Figure 1).

Compound 1 was obtained as a white amorphous solid. The molecular formula was determined to be C15H26O2 on the basis of HR-TOFMS spectrum showed [M-H] m/z 239.1922 (calcd. m/z 238.1932) and NMR spectral data (Table 1), thus requiring three degrees of unsaturations. The IR spectra of compound 1 showed absorption peaks due to of hydroxyl group (3421 cm⁻¹), aliphatics (2970 and 2870 cm⁻¹),

3. Result and discussion
The n-hexane extract of the stem bark of A. simplicifolia was chromatographed over a vacuum-liquid chromatographed (VLC) column packed with silica gel 60 by gradient elution. The VLC fractions were repeatedly subjected to silica gel and ODS column chromatography as well as preparative thin layer chromatography (PTLC) on silica gel GF254 to afford a compounds 1 (Figure 1).

Compound 1 was obtained as a white amorphous solid. The molecular formula was determined to be C15H26O2 on the basis of HR-TOFMS spectrum showed [M-H] m/z 239.1922 (calcd. m/z 238.1932) and NMR spectral data (Table 1), thus requiring three degrees of unsaturations. The IR spectra of compound 1 showed absorption peaks due to of hydroxyl group (3421 cm⁻¹), aliphatics (2970 and 2870 cm⁻¹),
isolated double bond (1478 cm\(^{-1}\)) and ether group (1078 cm\(^{-1}\)). \(^1\)H-NMR spectrum showed of three methyl tertiary signals, six methylene, two metines and one metin oxygenated. The \(^{13}\)C-NMR spectrum showed fifteen carbon signals, which were classified by their chemical shifts and DEPT spectra as 20 carbon signals consisting of three methyl, six methylene, three metin \(sp^3\), one metin \(sp^3\) oxygenated, two quaternary \(sp^3\) carbons and one oxygenated quaternary carbon.

![Figure 1. Chemical Structures of Compounds 1.](image)

**Table 1.** NMR data for Compounds 1 and Senecrassidiol [5].

| No | Compound 1 | Senecrassidiol* [5] |
|----|------------|-------------------|
|    | \(^{13}\)C-NMR | \(^{13}\)C-NMR | \(^{13}\)C-NMR | \(^{13}\)C-NMR |
|    | \(\delta_C/\text{ppm}\) | \(\delta_C/\text{ppm}\) | \(\delta_C/\text{ppm}\) | \(\delta_C/\text{ppm}\) |
| 1  | 69.7 s     | 72.4 s            |            |            |
| 2  | 37.1 d     | 41.3 t            | 2.84 dt (11.5/6.0) |
| 3  | 33.0 t     | 1.08 d (3.1)     | 34.2 t     |            |
|    |            | 1.06 d (3.8)     |            |            |
| 4  | 34.0 s     | -                | 33.8 s     |            |
| 5  | 42.9 d     | 1.80 m            | 48.8 d     |            |
| 6  | 19.4 t     | 1.45 m            | 21.9 t     |            |
|    |            | 1.32 m            |            |            |
| 7  | 41.1 t     | 1.39 m            | 35.4 t     |            |
|    |            | 1.34 m            |            |            |
| 8  | 38.3 s     | -                | 37.4 s     |            |
| 9  | 71.2 d     | 3.36 t (2.65)    | 73.9 d     | 3.32 br s  |
| 10 | 27.1 t     | 1.96 m            | 26.8 t     |            |
|    |            | 1.68 m            |            |            |
| 11 | 32.4 t     | 1.57 d (4.6)     | 35.9 t     |            |
|    |            | 1.43 d (2.7)     |            |            |
| 12 | 34.4 t     | 1.32 s            | 38.2 t     | 1.34 d (13.0) |
|    |            | 1.07 d (13.0)    |            | 1.85 d (13.0) |
| 13 | 19.8 q     | 0.94 s            | 24.3 q     | 0.93 s     |
| 14 | 29.5 q     | 0.92 s            | 28.5 q     | 1.18 s     |
| 15 | 25.6 q     | 0.85 s            | 29.9 t     | 1.00 s     |

*CDCl\(_3\), 500 MHz for \(^1\)H and 125 MHz for \(^{13}\)C.

In order to clarify the position of functional group in compound 1, \(^1\)H-\(^1\)H COSY and HMBC experiments were carried out and the results were shown in **Figure 2**. The \(^1\)H-\(^1\)H COSY spectrum of 1 showed correlations in C2-C3-C5-C6-C7 and C9-C10-C11, supporting the presence of three cyclic-type sesquiterpenoid skeleton in compound 1. A tertiary methyl signal at \(\delta_H 1.00\) (H-15) was correlated to \(\delta_C 41.1\) (C-7); 38.3 (C-8); 71.2 (C-9) and 34.4 (C-12), indicating that position of tertiary methyl were located at C-8. An oxygenated proton at \(\delta_H 3.36\) (H-9) was correlated to \(\delta_C 27.1\) (C-10), 32.4 (C-11), 34.4 (C-12) and 25.6 (C-15) suggesting that secondary hydroxyl group was located at C-6, whereas methylene proton at \(\delta_H 1.80\) (H-5) was correlated also to oxygenated carbon at \(\delta_C 69.7\) (C-1), indicating that tertiary hydroxyl group was located at C-1. A gem-dimethyl protons at \(\delta_H 2.13\) (H-2) was correlated...
to δC 33.0 (C-3); 34.0 (C-4) and 42.9 (C-5), methylene proton at δH 1.08 (1H, d, J=3.1; 1H, d, J=3.8; H-3) was correlated to δC 19.8 (C-13) and 42.9 (C-5), methin proton at δH 1.80 (H-5) was correlated to δC 69.7 (C-1), 37.1 (C-2), 19.8 (C-13), 29.5 (C-14), and two methyl proton at δH 0.94 (H-14) was correlated to δC 29.5 (C-14) and δH 0.92 (H-14) to δC 19.8 (C-13) indicating that a gem-dimethyl attached at cyclobutane ring at C-4. Table 1 showed a detailed comparison of NMR spectra of compound 1 with senecrassidiol [5].

Figure 2. Selected 1H-1H COSY and HMBC correlations for compound 1.

The cytotoxic effects of compounds 1 against HeLa cervical cancer cells were conducted according to the resazurin assay method [6] and were used a cisplatin (IC50 0.67 μM) as a positive control. Compounds 1 showed cytotoxic activity with IC50 values of 2.18 μM.

4. Conclusions
A sesquiterpenoid compound, senecrassidiol has been isolated from the stembark of A. simplicifolia and showed cytotoxicity activity against HeLa cervical cancer cells with IC50 values of 2.18 μM.

Acknowledgment
This research was financially supported by Directorate General of Higher Education, Ministry of Research, Technology and Higher Education, Indonesia (Postgraduate Grant, 2016-2018 by Unang Supratman) and Directorate General of Higher Islamic Education, Ministry of Religion Indonesia (Mora scholarship). We thank Dr. Mohamad Nurul Azmi Mohamad Taib and Cik Alia Syazana Binti Roslan in School of Chemical Sciences University Science Malaysia for NMR measurements. We are grateful to Miss Kusmiati Sukmana at Central Laboratory, Universitas Padjadjaran, Jatinangor, Indonesia for cytotoxicity bioassay.

References
[1] Pan L, Kardono L B S, Riswan S, Chai H, Carcache de Blanco E J, Pannell C M, Soejarto D D, McCloud T G, Newman D J and Kinghorn A D 2010 Isolation and characterization of minor analogues of silvestrol and other constituents from a large-scale re-collection of Aglaia foveolata *Journal of Natural Products* 4 1873–1878
[2] Cai X, Wang Y, Zhao P, Li Y and Luo X 2010 Dolabellane diterpenoids from Aglaia odorata *Phytochemistry* 71 1020–1024
[3] Liu S, Liu S B, Zuo W, Guo Z, Mei W and Dai H 2014 New sesquiterpenoids from Aglaia odorata var. microphyllina and their cytotoxic activity *Fitoterapia* 92 93–99
[4] Kurniasih N, Milawati H, Fajar M, Hidayat A T, Abdullah R, Harneti D, Supratman U and Azmi M N 2018 Sesquiterpenoid Compounds from The Stembark of Aglaia minahassae (Meliaceae) *Molekul*
[5] Fraga B M, Diaz C E, Amador L J, Reina M, Santana O and Coloma A G 2014 Bioactive compounds from transformed root cultures and aerial parts of Bethencourtia hermosae *Phytochemistry* 108 220-228
[6] Sittampalam G S, Coussens N P, Brimacombe K, Grossman A, Arkin M, Auld D and Chung T D Y 2004 *Measurement of β-Arrestin Recruitment for GPCR Targets—Assay Guidance Manual*