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A Cytotoxic Hydroperoxy Triterpenoid from The Stembark of *Aglaia cucullata* (Meliaceae)

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Abstract

Meliaceae is one of the plant family that is natively grown in Indonesia. The largest genus member of this family is the genus *Aglaia*. *Aglaia* consists of 55 species which are distributed in tropical and subtropical region in Indonesia. *Aglaia* has been utilized by the folks as a traditional medicine for treating many diseases including for cancer treatments. The largest metabolites isolated from *Aglaia* is triterpenoid which consists of 34% of all isolated compounds. Triterpenoids from *Aglaia* showed remarkable potency for cytotoxic activity against many human cancer cells. This research aims to isolate a triterpenoid compound that has unique hyrdoperoxy groups, (*E*)-25-hydroperoxydammar-23-en-3β,20-diol (**1**), from the *n*-hexane extract of *Aglaia cucullata* stembark. The methods included maceration, extraction, and column chromatography. The pure compound was then identified based on spectroscopic analysis, including FTIR, HRMS, and NMR (1 and 2 dimensional NMR). Compound **1** tested against MCF-7 cell lines with IC_{50} 32.27 μg/mL and categorized as moderate activity compared with a positive control.

Keywords: *Aglaia*, *Aglaia cucullata*, cytotoxic activity, hydroperoxy group, triterpenoid.

INTRODUCTION

Triterpenoids in a large group of secondary metabolites found in many organisms including plants. Triterpenoids consist of 30 carbons with additional structure modifications conjugated on their structures such as, sugar groups, ester fatty acid, hydroxyl, acetyl, olefin, and hydroperoxy [1-3]. Among all terpenoid compounds, triterpenoids showed high various structure and biological activity, including cytotoxic activity.

Aglaia is the largest member of triterpenoid compounds which can be found in tropical and subtropical regions of Asia, Australia, and Pacific islands. 65 species were grown in Indonesia from a total 150 species. The main compounds found in *Aglaia* are triterpenoids and flavaglines. The study on biological activity of this plant includes cytotoxic, antimicrobial, antiviral, and insecticidal [4-10].

Aglaia cucullata is a unique member of the genus *Aglaia* which grown in the mangrove area. This unique environment produced unique chemical structure with a high potency of biological activity [11-14]. Our continuous search of cytotoxic compounds isolated from *A. cucullata* revealed the presence of seven dammarane triterpenoid compounds with cytotoxic activity against two cancer cells (MCF-7 and B16-F10) and a normal cell (CV-1) [15]. Herein, we reported the isolation, structure elucidation, and cytotoxic assay of a hydroperoxy compound, (*E*)-25-hydroperoxydammar-23-en-3a,20-diol (**1**) (Figure 1), from the *n*-hexane extract of *Aglaia cucullata* stembark. The compound **1** was isolated for the first time in this species.

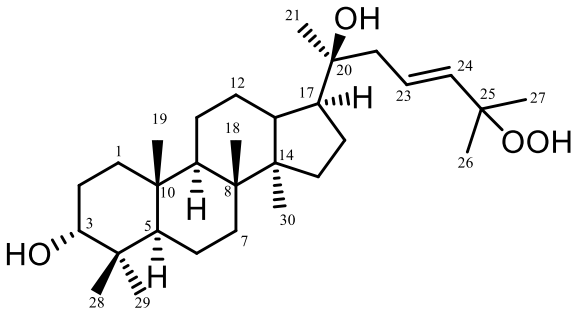

Figure 1. Chemical structure of compound **1**.

EXPERIMENTAL SECTION

Material and Instrumental

The Perkin-Elmer-type FT-IR was utilized for obtaining the FTIR spectrum in KBr. The JEOL-type NMR spectrometer was employed for the measurement of one and two-dimensional NMR (500 MHz for ^{1}H and 125 MHz for ^{13}C) in addition of tetramethylsilane (TMS, internal standard). The Waters Q-TOF type Xevo mass spectrometer was used for the determination of molecular weight together with molecular formula. Column chromatography for purifications using Silica gel (size 70-230 mesh and size 230-400 Mesh, Merck for normal phase and size 100-200 mesh for reverse phase ODS) as stationary phase. Silica gel GF_{254} and RP-18 F_{254} plates (Merck, 0.25 mm) were employed for the TLC and visualization was conducted by spraying the plates with a 10% H_2SO_4 solution in ethanol, followed by heating.

Plant Material

The stem bark of *A. cucullata* was obtained from the Manggar River in Balikpapan, East Kalimantan, Indonesia. This sample was assessed by the staff of the Herbarium Wanariset, Balikpapan in December 2020 and the specimen was deposited at the herbarium (collection No. FF7.20).

Methods

Extraction and Isolation

The fresh stembark was collected and dried in room temperature followed by crushing to give dried powder of *A. cucullata* stembark (3.5 kg). This powder then continued to maceration using ethanol for 5 days each with 5 liter of ethanol (5 x 3 L). After evaporation of solvent at reduced pressure, 523 g of thick brown ethanol extract was obtained. The partition based on different polarities was conducted to give 64 g, 35 g, and 13 g of the *n*-hexane, ethyl acetate, and 1-butanol extract, respectively.

Firstly, about 64 g of the *n*-hexane extract first was fractionated by vacuum liquid chromatography on silica gel using a gradient elution of *n*-hexane-ethyl acetate (10:0–0:10, stepwise 10%) to give eight fractions (A–H). Subsequently, 6 g of fraction C was chromatographed on silica gel using gradient elution of *n*-hexane-ethyl acetate (10:0–1:1 stepwise 2.5%) to produce five sub-fractions (C1-C5). A total of 1.5 g of fraction C3 was chromatographed on silica gel using a combination of *n*-hexane:chloroform:ethyl acetate (7:1:2) to produce seven subfractions (C3a-C3g). About 127 mg of sub-fraction C3b was chromatographed on the ODS column using a combination of methanol:water (4:1) to give pure compound **1** (10.5 mg).

Determination of Cytotoxic Activity

The methods of determination of cytotoxic activity was carried out using previous reported literature [5,6]. MCF-7 cell lines which grown in 70% confluent were harvested and counted, then diluted with complete culture RPMI medium. The cells then were transferred into 96 well-plates with a total of 170,000 cells/well. After overnight growth, the cells were treated with increasing concentrations of compound **1** (3.91, 7.81, 15.63, 31.25, 62.50, 125, 250, 500 μg/mL) with co-solvent 2% (v/v) DMSO in PBS. Cisplatin was used as the positive control. All samples were incubated at 37 °C in a 5% CO_2 incubator for 24 h. After incubation, the medium was immediately replaced by 10 μL PrestoBlue reagent in a 90 μL RPMI medium. The plates were incubated for 1–2 h until resorufin was formed (color changes from blue to purple). The absorbance was

measured at 570 nm using a microplate reader. The IC_{50} value is the concentration for 50% growth inhibition. The percentage of cytotoxicity compared to untreated cells was determined. A plot of % cytotoxicity versus sample concentrations was used to calculate the concentration, which showed 50% cytotoxicity (IC_{50}). All assay and analyses were each run-in duplicate, and all were averaged.

RESULTS AND DISCUSSION

Multiple stages of column chromatography including normal and reverse phase were applied for the purification of compound **1** (Figure 1). The chemical structure of compound **1** was carefully determined from the spectroscopic evidence (FTIR, MS, and NMR).

Compound **1** was obtained as a white amorphous powder. The chemical formula of compound **1** was identified as $C_{30}H_{52}O_4$, with an ion peak in HRMS at *m/z* 477.3951 $[M+H]^+$ (calculated as 477.3944 for $C_{30}H_{53}O_4^+$). Functional group which existed in compound **1** according to FTIR were O-H (3436 cm^{-1}), C-H sp^2 (2945 cm^{-1}), *gem*-dimethyl (1456 and 1380 cm^{-1}), C-O (1074 cm^{-1}), and O-O hydroperoxy (847 cm^{-1}). Observation of ^1H NMR of spectrum demonstrated eight tertiary methyl groups (δ_H 0.76, 0.83, 0.85, 0.94, 0.96, 1.11, 1.33, and 1.34, each 3H), one oxymethine at δ_H 3.19 (1H, dd, *J*=4.8, 11.4 Hz), and two sp^2 methine at δ_H 5.76 (1H, dd, *J*=7.8, 16.2 Hz) and 5.60 (1H, dd, *J*=4.8, 16.2 Hz). The numbers of methyl groups indicated the presence of dammarane triterpenoid skeleton [5]. The ^{13}C NMR spectrum (Table 1) showed 30 signals. The DEPT experiment identified these as eight methyls, nine methylenes, six methines (including two sp^2 methine at δ_C 127.4 and 137.4 and one oxymethine at δ_C 79.1), and six quaternary carbons (including one oxygenated quaternary carbon bearing hydroxyl group at δ_C 75.2 and one oxygenated quaternary carbon bearing hydroperoxy group at δ_C 82.2). Hydroperoxy group was attached at C-25 due to deshielded signal of C-25 (δ_C 82.2) compared with C-20 (δ_C 75.2). The alkene functional groups accounted for one out of five degrees of unsaturation, with the residual four degrees were attributed to a tetracyclic dammarane triterpenoid skeleton. The positioning of functional groups and the main skeleton of compound **1** were confirmed through the HMBC and ^1H-^1H COSY (Figure 2). The HMBC each methyl group with adjacent carbons confirmed the dammarane-type structure. The correlations of H-28,29 to C-3,4, and 5, and of H-1 to C-3, determine the position of hydroxyl group at C-3. Another hydroxyl group was attached at 20 based on HMBC cross peak of H-21,22 to C-20. The position of double bond was formed at C-24/C-24 based on HMBC of H-26 and H-27 to C-24 together with ^1H-^1H COSY crosspeak of H-22/H-23/H-24. The NMR data of compound **1** was compared with (*E*)-25-hydroperoxydammar-23-en-3β,20-diol obtained from the stem bark of *Aglaia elliptica* [8] and found to be identical. Thus, compound **1** was identified for the first time from *A. cucullata* and based on all spectroscopic evidence determined as (*E*)-25-hydroperoxydammar-23-en-3β,20-diol.

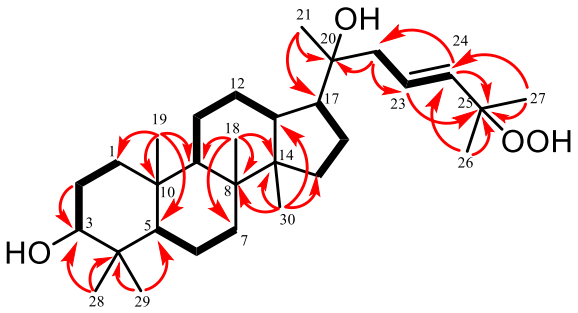

Figure 2. Selected HMBC and ^1H-^1H COSY of compound **1**.

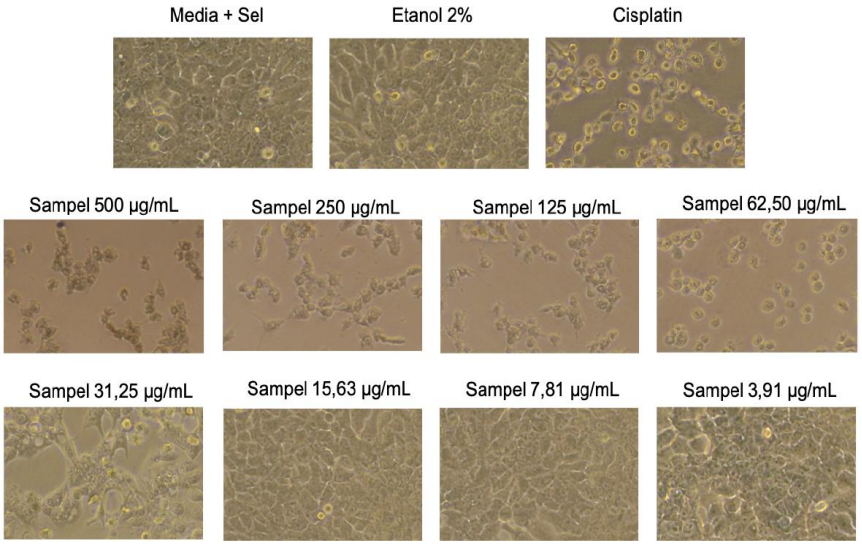

Figure 3. MCF-7 cell morphology under different concentration of compound **1**.

Table 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of compound **1** in $CDCl_3$.

No.	^{13}C NMR δc (mult.)	^{1}H NMR δ_H (Integral, mult., *J*=Hz)
1	39.1 (t)	1.68 (2H, dd, 3.6, 13.2)
2	24.9 (t)	1.44, 1.71 (each 1H, m)
3	79.1 (d)	3.19 (1H, dd, 4.8, 11.4)
4	39.0 (s)	-
5	55.9 (d)	0.71 (1H, dd, 2.4, 9.6)
6	18.3 (t)	1.40, 1.53 (each 1H, m)
7	35.3 (t)	1.25 (2H, m)
8	40.4 (s)	-
9	50.7 (d)	1.29 (1H, m)
10	37.2 (s)	-
11	21.6 (t)	1.22, 1.48 (each 1H, m)
12	27.5 (t)	1.59 (2H, m)
13	42.5 (d)	1.63 (1H, m)
14	50.4 (s)	-
15	31.2 (t)	1.07 (2H, dd, 1.8, 8.4)
16	27.6 (t)	1.21, 1.82 (each 1H, m)
17	50.3 (d)	1.72 (1H, dd, 3.6, 6.6)
18	15.6 (q)	0.94 (3H, s)
19	16.5 (q)	0.83 (3H, s)
20	75.2 (s)	-
21	25.8 (q)	1.11 (3H, s)
22	43.4 (t)	2.22 (2H, dd, 7.8, 11.4)
23	127.4 (d)	5.76 (1H, dd, 7.8, 16.2)
24	137.4 (d)	5.60 (1H, dd, 4.8, 16.2)
25	82.2 (s)	-
26	24.2 (q)	1.34 (3H, s)
27	24.5 (q)	1.33 (3H, s)
28	28.1 (q)	0.96 (3H, s)
29	15.4 (q)	0.76 (3H, s)
30	16.3 (q)	0.85 (3H, s)

Compound **1** was tested for its cytotoxicity against MCF-7 cancer cell lines using the resazurin assay method with cisplatin acting as a positive control (IC_{50} 53.0 μg/mL). The IC_{50} of compound **1** was 32.27 μg/mL. This IC_{50} value was categorized as moderate activity according to The United States National Cancer Institute [16]. Cell proliferation assay (Figure 3) showed that the growth of cancer cells decreased since the additional of 31.25 μg/mL of compound **1**, indicated by the disappearance of live cells. This result indicated that compound **1** inhibited the growth of the MCF-7 cancer cell line at 32.27 μg/mL.

CONCLUSION

A known dammarane-type triterpenoid compound with a unique hydroperoxy group was isolated from the stembark of *A. cucullata* for the first time and identified as (*E*)-25-hydroperoxydammar-23-en-3β,20-diol. The chemical structure of compound **1** was identified unambiguously with FTIR, MS, NMR (1 and 2D), as

well as comparison with previous literature. The cytotoxic assay of compound **1** against MCF-7 cell lines showed a moderate cytotoxic activity with IC_{50} 32.27 µg/mL.

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