Cytotoxic Sesquiterpenoid from the Stembark of *Aglaia argentea* (Meliaceae)

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Abstract

Aglaia argentea also known as langsat hutan in Indonesia is a higher plant traditionally used for moisturizing the lungs, reducing fever and treating contused wound, coughs and skin diseases. The stembark of A. argentea was successively extracted with methanol. The methanolic extract then partitioned by n-hexane, ethyl acetate and n-butanol. The n-hexane extract was chromatographed over a vacuum-liquid chromatographed (VLC) column packed with silica gel 60 by gradient elution.

The VLC fractions were repeatedly subjected to normal-phase column chromatography and preparative TLC on silica gel GF_{254} to afford aroma dendrane-type sesquiterpenoid compound, spathulenol. The chemical structure of the compound was elucidated on the basis of spectroscopic data and comparison with those related data previously reported. Isolated compound was tested for their cytotoxic effects against P-388 murine leukemia cells in vitro and showed cytotoxic activity with an IC_{50} value of 16.82 µg/mL.

Keywords: Meliaceae, *Aglaia argentea*, aroma dendrane-type sesquiterpenoid, spathulenol, P-388 murine leukemia cells

Introduction

The family Meliaceae, also known as mahogany family, is a high plant grown in tropical and subtropical region around the world. The largest genus from this family is *Aglaia*, comprises more than 100 species distributed mainly in India, Indonesia, Malaysia and parts of the Western Pacific[1,2]. The genus *Aglaia* is known as source of variety compounds with interesting biological activity such as antifungal and antitumor sesquiterpenoid[3,4], cytotoxic[5] and anti-inflammatory activity diterpenoid, cytotoxic and anti-retroviral triterpenoid[6-10], cytotoxic steroid[11], cytotoxic alkaloid[12], anti-inflammatory and cytotoxic rocaglamide[13-15].

In further screening for cytotoxic compounds from Indonesia *Aglaia* plants, we found that the *n*-hexane extract of *A. argentea* exhibited a cytotoxic activity against P-388 murine leukemia cells with IC_{50} values of 26.72 µg/mL. *A. argentea*, also known as *langsat hutan* in Indonesia is a higher plant traditionally used for moisturizing the lungs, reducing fever and for treating contused wound, coughs and skin diaseases[16-18]. Previous phytochemical studies of *A. argentea* have revealed the presence of compounds with cytotoxic activity including cycloartane-type triterpenoids against KB cells[19] and 3,4-secoapotirucallane-type triterpenoids against KB cells[20], but there are no reports of sesquiterpenes of this species before.

Herein we isolated, determined the chemical structure and tested at P388 murine leukemia cells of one sesquiterpenoid compound from *n*-hexane extract of *A. argentea*.

Material and Methods

General: The IR spectra were recorded on a Perkin-Elmer spectrum-100 FT-IR in KBr. Mass spectra were obtained with a Synapt G2 mass spectrometer instrument. NMR data were recorded on a Jeol ECZ-600 spectrometer at 600 MHz for ^{1}H and 150 MHz for ^{13}C and TMS as internal standard. Column chromatography was conducted on silica gel 60 (Kanto Chemical Co. Inc., Japan). TLC plates were pre-coated with silica gel GF_{254} (Merck, 0.25 mm) and detection was achieved by spraying with 80% H_2SO_4 in ethanol followed by heating.

Plant material: The barks of *A. argentea* were collected in Bogor Botanical Garden, Bogor, West Java Province, Indonesia in June 2015. The plant was identified by the staff of the Bogoriense Herbarium, Bogor, Indonesia and a voucher specimen (No. Bo-1288718) was deposited at the herbarium.

Extraction and isolation: The dried bark (2.5 kg) was extracted with methanol exhaustively (12 L) at room temperature for 5 days. After removal of the solvent under vacuum, the viscous MeOH extract (133.5 g) was first suspended in H_2O and then partitioned with *n*-hexane, EtOAc and *n*-butanol successively. Evaporation resulted in the crude extracts of *n*-hexane (26.3 g), EtOAc (12.4 g) and MeOH (12.6 g) respectively. All the extracts were tested for cytotoxic activity against P-388 murine leukemia cells and showed cytotoxic activity with IC_{50} values of 26.72, 15.49 and 85.67 µg/mL respectively.

The *n*-hexane soluble fraction (26.3 g) was fractionated by vacuum liquid chromatography on silica gel 60 using a gradient *n*-hexane and EtOAc to give fractions A–I, combined according to TLC results. Fraction A (6 g) was chromatographed on a column of silica gel, eluted successively with a gradient of *n*-hexane–CH_2Cl_2 (10:0–1:1), to give ten sub fractions (A1–A10). Sub fraction A9

was separated on preparative TLC eluted with *n*-hexane–EtOAc (9:1), to give 1 (5.3 mg).

Spathulenol (1): Colorless oil; $[\alpha]^{D}_{20}$ –3.2° (*c* 1.3, $CHCl_3$); IR (KBr) ν_{max} 3403, 2983, 1620, 1379, 1425, 1067 cm^{-1}. ^1H NMR ($CDCl_3$, 600 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 1; HR-ESI-TOFMS (positive ion mode) *m/z* 221.1810 $[M+H]^+$, (calcd. for $C_{15}H_{24}O$, *m/z* 220.1827).

Determination of Cytotoxic Activities: The P388 cells were seeded into 96-well plates at an initial cell density of approximately 3×10^4 cells cm^{-3}. After 24 h of incubation for cell attachment and growth, varying concentrations of samples were added. The compounds added were first dissolved in DMSO at the required concentration. Subsequently six desirable concentrations were prepared using PBS (phosphoric buffer solution, pH = 7.30 - 7.65). Control wells received only DMSO. The assay was terminated after 48 h incubation period by adding MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; also named as thiazol blue] and the incubation was continued for another 4 h, in which the MTT-stop solution containing SDS (sodium dodecyl sulphate) was added and another 24 h incubation was conducted.

Optical density was read by using a micro plate reader at 550 nm. IC_{50} values were taken from the plotted graph of percentage live cells compared to control (%), receiving only PBS and DMSO, versus the tested concentration of compounds (μg/mL). The IC_{50} value is the concentration required for 50% growth inhibition. Each assay and analysis was run in triplicate and averaged.

Results and Discussion

The phytochemical test with Liebermann-Burchard reagent for the *n*-hexane extract showed the presence of terpenoid compounds. By using cytotoxic assay to guide separations, the *n*-hexane fraction was separated by column chromatography over silica gel by gradient elution. The fractions were repeatedly subjected to preparative TLC on silica gel GF_{254} and yielded one cytotoxic sesquiterpenoid compound 1 (figure 1).

Compound 1 was obtained as colorless oil. The HR-TOFMS spectrum showed $[M+H]^+$ *m/z* 221.1810 (calcld *m/z* 220.1827), which corresponded to the molecular formula $C_{15}H_{24}O$ and thus required four degrees of unsaturation, originating from one pairs of C sp^2 and the remaining tricyclic aromadendrane-type sesquiterpenoid. The IR spectra showed absorption peaks at 3403 cm^{-1} (OH), 1620 cm^{-1} (C=C), 1379 and 1425 cm^{-1} (*gem*-dimethyl groups) and 1067 cm^{-1} (C-O stretch). The ^1H-NMR ($CDCl_3$ 600 MHz) spectrum showed the presence of three tertiary methyl groups resonating at δ_H 1.02 (H-12) and 1.04 (H-13), including one deshielded signal for tertiary methyl at 1.27 indicating that this methyl is attached at oxygenated carbon. All of these methyl signals indicated the presence of aroma dendrane-type sesquiterpenoid skeleton. One pair of olefinic methylene group, resonating at δ_H 4.65 and 4.67 (each 1H, s, H-14) was also observed at these spectra. The proton pairing was also confirmed with the ^1H-^1H COSY spectrum (figure 2). ^1H-^1H COSY cross peak was observed at H-3/H-2/H-1/H-5 in ring A indicating that this was a five membered ring; cross peak was also observed at H-1/H-5/H-6/H-7/H-8/H-9 in ring B indicating that this was a seven membered ring. The ^1H-^1H COSY correlation observed at H-1/H-5 showed that ring A and B fused each other to form aroma dendrane-type sesquiterpenoid skeleton.

The ^{13}C-NMR ($CDCl_3$ 150 MHz) and DEPT 135° spectra showed the presence of three methyl groups that indicate the characteristic of aroma dendrane-type sesquiterpenoid[21], one quartenary oxigenated carbon, resonating at δ_C 81.1 (C-4), one quartenary carbon at δ_C 20.4 (C-11) which was the characteristic of aroma dendrane skeleton, originating from cyclisation of guaiane skeleton at position C-6/C-11 based on biogenetic pathway of this type of compound[22], one olefinic methylene, resonating at δ_C 106.4 (C-14) and one quartenary olefinic carbon, resonating at δ_C 153.5 (C-10). These functionalities accounted for one of total four degree of unsaturation and the remaining three degrees of unsaturation were consistent with the aroma dendrane-type sesquiterpenoid.

The HMBC spectrum (figure 2) showed correlations between H-15 (δ_H 1.27) to C-3 (δ_C 41.8), C-4 (δ_C 81.1) and C-5 (δ_C 54.5) and correlation between H-3 (δ_H 1.75) to C-4 (δ_C 81.1) indicating that hydroxy group was having linkage at C-4 position. The position of double bond at C-10/C-14 was proved by HMBC correlation between H-14 (δ_H 4.65 and 4.67) to C-1 (δ_C 53.5) and C-9 (δ_C 38.9) and correlation between H-9 (δ_H 2.41) to C-10 (δ_C 153.5) and C-14 (δ_C 106.4). A comparison of the NMR data of 1 with the data for spathulenol[23] revealed that the structures of the two compounds were very similar; consequently compound 1 was identified as spathulenol.

The cytotoxicity effects of the isolated compound 1 against P388 murine leukemia cells were conducted according to the method described in previous paper[8,24,25] and used an Artonin E (IC_{50} 0.3 mg/mL) as a positive control[26]. The cytotoxic activity of isolated compound 1 in IC_{50} value as 16.82 µg/mL and categorized as weak activity, since the sesquiterpenoid compound with different functional groups from several species showed diverse biological activity[4].

Conclusion

Aroma dendrane-type sesquiterpenoid compound was success fully isolated from *n*-hexane extract of stem bark *A. argentae* identified as spathulenol. Isolated compound was tested for cytotoxic effects against P-388 murine leukemia cells *in vitro* and showed cytotoxic activity with an IC_{50} value of 16.82 µg/mL.

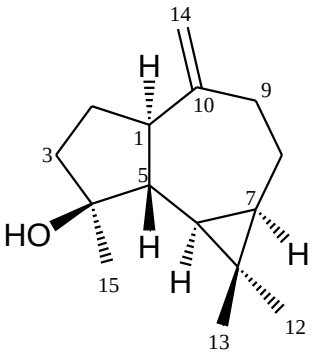

Figure 1: Structures of Compounds 1

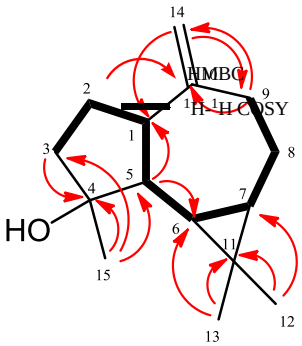

Figure 2: Selected HMBC and ^{1}H-^{1}H COSY correlations for 1

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