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Triterpenoids from *Aglaia shawiana* with their Cytotoxicity against MDA-MB-468 and MCF-7 Cancer Cell Lines

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ABSTRACT. Triterpenoids are major secondary metabolites of the genus *Aglaia* and are recognized for diverse biological activities, including anticancer potential. This study investigated the triterpenoid constituents of *Aglaia shawiana* leaves and twigs and evaluated their cytotoxic activity against breast cancer cell lines. Phytochemical investigation of the *n*-hexane extract yielded two triterpenoids, cycloartan-3β,29-diol-24-one (**1**) and 3-epi-cabraleahydroxylactone (**2**). Their structures were elucidated using infrared spectroscopy, high-resolution mass spectrometry, and one- and two-dimensional NMR techniques, supported by comparison with literature data and biosynthetic considerations. Both compounds were assessed for cytotoxicity against MCF-7 and MDA-MB-468 breast cancer cells. Compound **1** exhibited IC_{50} values of 118.27 ± 0.45 and 3.65 ± 0.10 μg/ml, while compound **2** showed IC_{50} values of 115.11 ± 0.15 and 14.53 ± 0.32 μg/ml against MCF-7 and MDA-MB-468 cells, respectively. Doxorubicin, used as a positive control, displayed IC_{50} values of 3.27 ± 0.006 and 0.03 ± 0.002 μg/ml, respectively. Compound **1** was previously reported to exhibit cytotoxicity against MCF-7 cells with IC_{50} values of > 100 μg/ml. These findings demonstrate selective cytotoxic activity of the isolated triterpenoids, particularly against MDA-MB-468 cells, highlighting their potential cytotoxic compounds warranting further investigation.

Keywords: *Aglaia shawiana*, bioactivity, cabralealactone, cycloartane, spectroscopy

INTRODUCTION

Breast cancer remains one of the most prevalent cancers worldwide and continues to be a major cause of cancer-related mortality (Barragán-Cárdenas et al., 2020). The disease is highly heterogeneous and classified into several subtypes with different biological characteristics and therapeutic responses. Among widely used in vitro models, MCF-7 cells represent luminal A, estrogen receptor–positive (ER^+) breast cancer, whereas MDA-MB-468 cells represent triple-negative breast cancer (TNBC), a more aggressive subtype lacking estrogen, progesterone, and HER2 receptors (Ramteke et al., 2025; Xu et al., 2008). Due to the limited treatment options and poor prognosis associated with TNBC, the search for new cytotoxic agents active against triple-negative breast cancer remains an important research focus.

The genus *Aglaia* (family Meliaceae) is widely distributed in tropical regions, including Indonesia, and has long been used in traditional medicine for treating fever, wounds, cough, diarrhea, and skin disorders (Heyne, 1982; Kato-Noguchi et al., 2016; Ngo et al., 2022).

Phytochemical investigations of *Aglaia* species have revealed diverse secondary metabolites, particularly rocaglate derivatives, triterpenoids, and steroids, many of which exhibit promising biological activities such as anticancer, antimicrobial, and anti-inflammatory effects (Harneti & Supratman, 2021; Hutagaol et al., 2023; 2025a; 2025b). Among these metabolites, triterpenoids represent an important group of structurally diverse natural compounds that have attracted considerable attention due to their cytotoxic and antiproliferative activities against various cancer cell lines (Li et al., 2023; Ren & Kinghorn, 2019).

Although numerous bioactive compounds have been reported from several *Aglaia* species, studies on the phytochemistry and biological activity of *Aglaia shawiana* remain relatively limited. Previous reports have identified triterpenoid compounds such as cycloartan-3β,29-diol-24-one and 3-epi-cabraleahydroxylactone from *Aglaia* species and evaluated their cytotoxic activity against MCF-7 breast cancer cells, where they exhibited weak to moderate cytotoxicity. However, to the best of our knowledge,

cytotoxicity evaluation of these compounds against triple-negative breast cancer cells, particularly MDA-MB-468, has not been reported.

In continuation of our investigation on bioactive compounds from Indonesian *Aglaia* species, this study reports the isolation and structural elucidation of two triterpenoids, cycloartan-3β,29-diol-24-one (**1**) and 3-epi-cabraleahydroxylactone (**2**), from the leaves and twigs of *A. shawiana*. Their cytotoxic activities were evaluated against MCF-7 and MDA-MB-468 breast cancer cell lines. This study expands previous findings on the cytotoxicity of these compounds in MCF-7 cells and provides the first report of their cytotoxic activity against MDA-MB-468 cells, thereby contributing new insight into their potential activity against triple-negative breast cancer.

EXPERIMENTAL SECTION

Materials

The leaves and twigs of *A. shawiana* were collected from Bogor Botanical Garden, Latitude: -6° 35' 30.59" S Longitude: 106° 47' 32.39" E, West Java Indonesia on July 2022. The following is the taxonomy of this species, kingdom: Plantae, subkingdom: Tracheobionta, superdivision: Spermatophyta, division: Mangnoliophyta, class: Mangnoliopsida, subclass: Rosidae, order: Sapindales, family: Meliaceae, genus: *Aglaia*, species: *A. shawiana* Merr. The plant was identified by Center for Plant Conservation Botanic Gardens Bogor, Indonesia, and a voucher specimen (II.K.59) deposited at the Herbarium, in Bogor Botanical Garden, Bogor, West Java Province, Indonesia.

Activity testing on the MCF-7 cancer cell line was conducted in the laboratory where the cells were stored, namely at the Center for Pharmaceutical Materials and Traditional Medicine Research, National Research and Innovation Agency (BRIN), PUSPIPTEK Area, Serpong, South Tangerang, Banten, Indonesia. Meanwhile, activity testing on the MDA-MB 468 cancer cell line was conducted at the Biomedical Laboratory of the Padjadjaran Faculty of Medicine, Bandung, where the cells were stored and cultured.

Technical solvents were distilled before maceration; isolation and spectral grade solvents (*n*-hexane, ethyl acetate (EtOAc), methanol (MeOH), and dichloride methane (DCM) from Merck, Darmstadt, Germany and Smart lab, Indonesia) were employed for spectroscopic measurements.

Instrumentation

Optical rotations were measured on a Perkin Elmer 341 Polarimeter (Waltham, MA, USA). UV was recorded on PerkinElmer UV WinLab Data Processor and Viewer Version 1.00.00. The IR spectra were recorded on a Perkin Elmer 1760 X FT-IR in KBr (Waltham, MA, USA). Mass spectra were obtained with a Water Qtof. HR-MS Xevo^{tm} mass spectrometer (Waters, Milford, MA, USA). NMR spectra was obtained with a Bruker Avance Neo (BioSpin AG, Faellanden, Switzerland) at 700 MHz for ^{1}H and 175 MHz for ^{13}C-NMR with $CDCl_3$ pure analytical grade as a solvent, chemical shift were given on a δ (ppm) scale and both using tetramethylsilane (TMS) as the internal standard. Chromatographic separations were carried out on silica gel 60 (Merck Kieselgel 60 PF 253 Art No. 7734.1000 and 9385.1000 with the particle size 0.063-0.200 mm and 0.040 – 0.063 mm), whereas the mobile phase used in the isolation stage was eluent from the redestillation of technical-grade eluent, but in the purification stage, pure analytical-grade eluent was used. Isolation steps to obtain the target compound were guided by thin-layer chromatography (TLC) with a mobile phase of pure analytical grade eluent. TLC plates were precoated with silica gel GF_{254} (Merck, Darmstadt, Germany, 0.25 mm). Spots were visualized under UV light of 254 nm and 365 nm simultaneously and by spraying with 10% Sulfuric acid (H_2SO_4) in ethanol or vanillin reagent followed by heating.

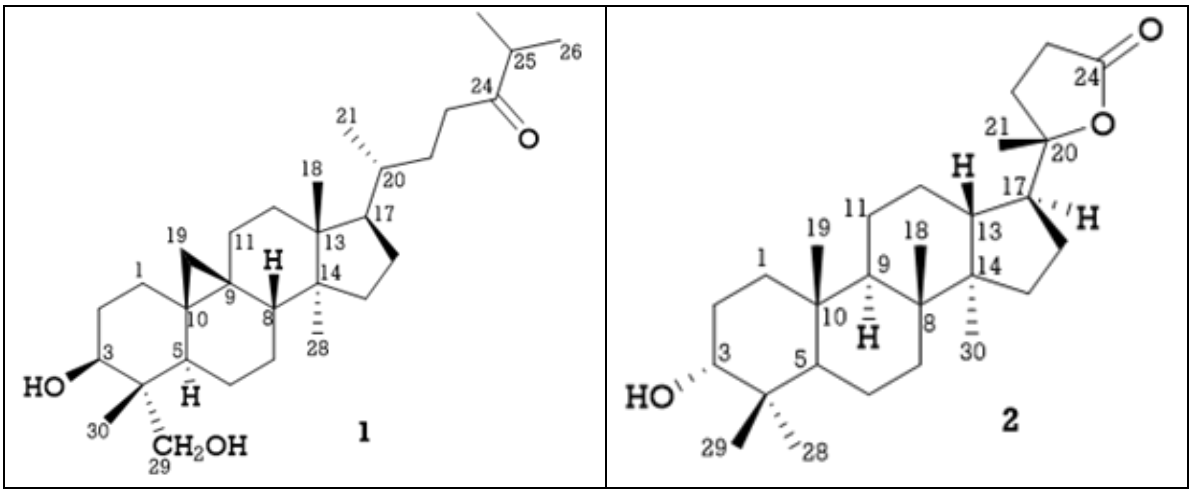

Figure 1. Structure of compounds 1 and 2

Procedure

Extraction and isolation

The leaves *n*-hexane extract (20.0 g) was subjected to vacum liquid chromatography (VLC) over silica gel using a gradient elution mixture of *n*-hexane-EA (10:0-0:10) to afford 9 fractions (A-I). Fraction H (4.49 g) was subjected to column chromatography (CC) over silica gel (200 mesh) using a gradient elution mixture of *n*-hexane-EA (10:0-8:2) and followed by gradient (8:2-0:10) as an eluent to give 16 sub-fractions (H1-H16). Sub-fraction H6 (4.16g) was subjected to CC over silica gel (200 mesh) using isocratic mixture of *n*-hexane: EA (7:3). Then the fractionation was continued with several stages of isolation with CC using silica gel and a combination of *n*-hexane/EA/DCM/MeOH eluen, at this stage, all separation processes using chromatography employ a mobile phase consisting of a redistilled technical-grade eluent. In the final stage, the isolate was purified through CC octadecyl (ODS) silica using an isocratic mixture of MeOH with analytical grade : aquadest (9.8:0.2) to obtain compound **1** (15 mg). Isolate **1** was tested for purity using the TLC method with various eluents. The spot of compound **1** on the TLC plate had an Rf value of 0.45 using the eluent composition *n*-hexane: EA (5:5).

The twigs *n*-hexane extract (21.5 g) was subjected to vacuum liquid chromatography (VLC) over silica gel using a 10% gradient elution mixture of *n*-hexane-ethyl acetate-methanol to afford 8 combined fractions (A-H). Fraction C (7.07 g), from the combination of fractions 3-5 of the VLC results was subjected to column chromatography (CC) over silica gel (200 mesh) using a gradient elution mixture of *n*-hexane-EA-MeOH (10:0:0-0:0:10). Using TLC as a guide with an eluent of *n*-hexane-EA 8:2, the isolates were combined and then fractionation was continued with several stages of isolation with CC using silica gel and a combination of *n*-hexane/EA/DCM/MeOH eluents, in the final stage, the isolate was purified through CC octadodecyl silica using an isocratic mixture of MeOH: aquadest (9.5: 0.5) to obtain compound **2** (4.5 mg). The spot of compound **2** on the TLC plate had an Rf value of 0.35 using the eluent composition *n*-hexane: EA:MeOH (6:3:1).

Cytotoxic bioassay

The cytotoxicity of the compounds against MCF-7 and MDA-MB-468 human breast cancer cells was measured using the *Methyl Thiazol Tetrazolium (MTT) Assay* but with slightly different step.

Cytotoxicity test of MCF-7 and MDA-MB-468 breast cancer cells

Complete RPMI culture media (RPMI-1640, 10% Fetal Bovine Serum (FBS) and 1% Penicillin-streptomycin antibiotics) was prepared.. Pure isolates of *A. shawiana* were dissolved in DMSO to obtain a stock solution of 10.000 μg/mL. The samples were serially diluted using complete RPMI media to obtain concentrations of 7.81, 15.63, 31.25, 62.50, 125.00, 250.00, 500.00 and 1000.00 μg/mL for MCF-7 cells test and of 100 μg/ml, 10 μg/mL, 1 μg/ml, and 0 μg/ml for MDA-MB-468 cells test. Cells to be used must reach a minimum confluence of 80%. The media in the flask is discarded, then the cells are rinsed twice with 10 mL of PBS. 3 mL of trypsin-EDTA solution is added and incubated for 5 minutes. The cells are transferred to a centrifuge tube containing complete media. The cells are centrifuged at 1500 rpm for 5 minutes. The supernatant is discarded, and the cell pellet is resuspended in fresh complete media. 5.000 cells/well were cultured (seeding) into a 96 well plate, then incubated for 24 hours (or until min 80% confluent) at 37 °C and 5% CO_2. The 96-well plates containing the cells were removed from the incubator. The plates were labeled with the sample name. The media was removed from each well, which would then be filled with sample and control cells. A 200 μL sample from each well was reintroduced into the wells at varying concentrations. Each dilution was performed in triplicate. The sample-treated cells were incubated for 24 hours for MCF-7 cells test and 48 hours for MDA-MB-468 cells test. The media containing the sample treatment was removed from each well. Each well was rinsed using PBS. 9 mL of complete medium and 1 mL of the MTT Assay kit were prepared in a 15 mL centrifuge tube, homogenized, then 100 μl of the reagent mixture was added to each well, incubated for 2-4 hours in an incubator at 37 °C and 5% CO_2 until formazan crystals formed. After formazan formation, the kit solution was discarded by immediately inverting the plate on a tissue towel. DMSO was added. Absorbance was measured on a Thermo Fisher ELISA reader at a wavelength of 550 nm.

RESULTS AND DISCUSSION

Two triterpenoids (**Figure 1**) were obtained from the *n* hexane extract leaves and twigs of *A. shawiana* using the column chromatography technique. Compound **1** was obtained as a white powder. The molecular formula of compound **1** was determined as $C_{30}H_{50}O_3$ based on the HR-TOFMS spectrum of m/z (**Figure S1**) found at 459.3875 $[M+H]^+$, (calculated for $C_{30}H_{51}O_3$ = 459.3838) with the NMR data (**Table 1**). The Index of Hydrogen Deficiency (HD) was obtained from equation, HD = ΣC − ΣH/2 + 1, so the HD index of compound **1** was six. The optical rotation of compounds **1** show values $[\alpha]_D^{29.3}$ +0.35 (c, 0.26, $CHCl_3$). The UV spectrum of compound **1** showed no conjugated double bonds based on the maximum absorption at a wavelength above 200 nm. The IR (KBr) spectrum of compound **1** (**Figure S2**) showed the presence of hydroxyl groups (3367 cm^{-1}), aliphatic C-H (2930 and 2867 cm^{-1}), C=O (1710 cm^{-1}), gem-dimethyl (1467 and 1376 cm^{-1}), and C-O (1025 cm^{-1}).

The ^1H NMR ($CDCl_3$, 700 MHz, ppm) spectrum of **1** (**Figure S3A, 3B**) displayed the presence of three tertiary methyl at [δ_H 0.88 (Me-18); 0.95 (Me-28) and

0.93 (Me-30)], three secondary methyls at [δ_H 0.81 (3H, d, *J*=6.0 Hz, Me-21), 1.08 (3H, d, *J*=7.1 Hz, Me-26) and 1.07 (3H, d, *J*=7.1 Hz, Me-27)]. Singlets spectrum seen at δ_H 0.37 (1H, s ,H-19) and 0.58 (1H, s ,H-19), these spectrum were characteristic of non equivalent protons on a cyclopropyl methylene group. The oxygenated methine and methylene signals were also observed in ^{1}H-NMR at δ_H 3.78 (1H, dd, 16.9, 11.8 Hz, H-3) and 3.76 (H, dd, 16.9, 11.8 Hz, H-29); δ_H 3.51 (1H, d; 10.4 Hz, H-29).

The ^{13}C NMR ($CDCl_3$, 175 MHz, ppm) spectrum, as can be seen in **Figure S4,** showed 30 carbon resonances, which were classified by their chemical shifts, DEPT (**Figure S5**), and HMQC (**Figure S6**), spectra as 6 methyls at δ_C 18.4, 19.4, 10.3, 18.5, 18.2, and 18.2. In the DEPT spectrum 12 methylenes were found at δ_C 30.2, 30.2, 21.1, 28.2, 25.9, 35.7, 33.0, 26.5, 30.1, 31.8, 37.7, and oxygenated methylene at 71.2. In these spectra, 6 methines were also found δ_C 42.6, 48.0, 52.4, 35.9, 41.0, and an oxygenated methine at 77.1. After that, it can be seen that there are also 5 quaternary carbons sp^3 were found at δ_C 43.8, 20.0, 25.5, 45.4, 48.9, and 1 ketone functionalities carbon group at δ_C 215.7 (six quaternary carbons in total). This functionalities accounted for 1 out of the total 6 hydrogen deficiency index were consistent with the tetracyclic structure of triterpenoid with the addition of 1 cyclopropyl group, which is a characteristic of cycloartane type triterpenoid. The ^{1}H, ^{13}C and HMQC NMR spectra implied the presence of a cyclopropyl group can be seen at [δ_H 0.37 (1H, s), 0.58 (1H, s); δ_C 30.1], one hydroxyl group at δ_H 3.78 (1H, dd, 17.4 and 12.6 Hz); δ_C 77.1 and another one at δ_H 3.76 (1H, dd, 17.4 and 12.6 Hz), and δ_H 3.51 (1H, d, 10.5 Hz); δ_C 71.2]. The chemical shifts obtained from the NMR spectrum above are very similar to the basic structure of cycloartane type triterpenoids (Dlamini et al., 2024; Wang et al., 2023), (Wang et al., 2023)

Further structural analysis was carried out on the presence and position of functional group 1, which was mostly obtained from the HMBC spectrum (**Figure S7**). There were correlations between H-18 (δ_H 0.88 (3H, s) to C-14 (δ_C 48.9), C-17 (δ_C 52.4), C-28 (δ_C 19.4), C-12 (δ_C 35.7), and also with two quaternary C sp^3 C-13 (δ_C 45.4) These correlations indicating that CH_3-18 embedded at C-13. Some HMBC cross peaks were observed from H-28 (δ_H 0.95) to C-13 (δ_C 45.4), C-8 (δ_C 48.0), and also with two quaternary C sp^3 C-14 (δ_C 48.9), these correlations suggesting that CH_3-28 embedded at C-14. The methyl protons at δ_H 0.93 (3H, s, H-30) correlate to methylene oxygenated at δ_C 71.2 (C-29), the methine at δ_C 77.1 (C-3) and also with a quaternary C sp^3 carbon at δ_C 43.8 (C-4), these correlations suggesting that CH_3-30 embedded at C-4. The methyl at δ_H 0.85 (H-21) correlate to the methine at δ_C 35.9 (C-20), δ_C 52.4 (C-17), and with methylene carbon at δ_C 31.8 (C-22). Based on the HMBC correlation analysis obtained from the spectrum of compound **1**, it was found that there were three tertiary methyl at C-4, C-13, and C-14, three secondary methyl at C-20 and C-25 (2x), as can be seen in **Figure 2A**.

In the HMBC spectrum, there are correlations between H-19 at δ_H 0.37 and 0.58 with C-9 (δ_C 20.0), C-10 (δ_C 25.5), C-1 (δ_C 30.2), C-5 (δ_C 42.6), and C-8 (δ_C 48.0) indicating that the structure had a cyclopropane. Furthermore, HMBC correlations from the methyl at δ_H 1.08 (H-26) and δ_H 1.07 (H-27) to ketone carbon group at δ_C 215.7 (C-24) indicating the presence of a ketone group at C-24. HMBC correlation from δ_H 3.76 and 3.51 (CH_2-29) to δC 43.8 (C-4). Furthermore, correlations δ_H 3.78 methine at (C-3) to δ_C 43.8 (C-4), δ_C 30.2 (C-2), δ_C 71.2 (C-29), and δ_C 10.3 (C-30) indicated the presence of a hydroxyl group embeded at C-29 and C-3.

The ^{1}H-^{1}H COSY spectrum of compound **1** (**Figure S8**), showed correlations in H_1-H_2-H_3, H_5-H_6-H_7, H_{11}-H_{12}, H_{15}-H_{16}-H_{17}-H_{20}, H_{20}-H_{21}, H_{20}-H_{22}-H_{23}, and H_{26}-H_{25}-H_{27} supporting the presence of rings A, B, C, D tetracyclic and side chain structure of **1**. Planar structure of compound **1** based on selected correlation of HMBC and COSY spectra can be seen in **Figure 2A.** To establish the position of the primary hydroxyl at C-29 or C-30, a comparison of carbon resonances of compound **1** and cycloartene-3*β*,29-diol (Nyemba et al., 1990) and cycloartane 3*β*,30-diol (Khan et al., 1994) was made. This comparison revealed that the C-29 and C-30 resonances of **1** (*δ* 71.1 and 10.1, respectively) as well as the ring-A and -B carbon resonances were in close agreement with those of cycloartene-3*β*-29-diol (*δ* 70.5 and 10.2) and were different from those of cycloartane-3*β*,30-diol (*δ* 63.1 and 22.0). Hence, the hydroxymethylene group in **1** was assigned to C-29 in an *α*-equatorial orientation. The chemical shifts of ^{13}C NMR spectrums of compound **1** from *A. shawiana* Merr. at C-29 and C-30 were 71.2 ppm and 10.3 ppm, respectively.

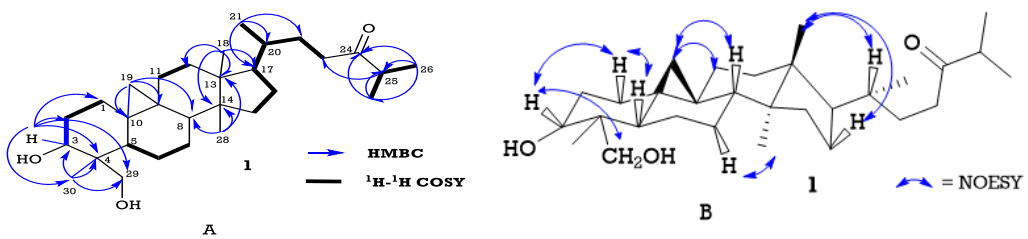

Figure 2. Selected HMBC and COSY (**A**) and NOESY (**B**) correlations of compound **1**

Table 1. ^{1}H and ^{13}C NMR spectral data (700 MHz for ^{1}H and 175 MHz, in $CDCl_3$) for **1**.

Position	Compound **1** (700 and 175 MHz, for ^{1}H and ^{13}C respectively) in $CDCl_3$)			Cycloartan-3β,29-diol-24-one. (400 and 100.5 MHz, for ^{1}H and ^{13}C respectively) in $CDCl_3$ (Inada et al., 1995)	
	^{1}H [ppm] (mult, integral; J=Hz)	^{13}C [ppm]	DEPT	^{1}H [ppm] (mult, integral; J=Hz)	^{13}C [ppm]
C-1	1.42-1.49 (1H, m) 1.31-1.38 (1H, m)	30.2	CH_2		31.7
C-2	1.53-1.59 (1H, m) 1.27-1.30 (1H, d, 5.9)	30.2	CH_2		30.2
C-3	3.78 (1H, dd, 16.9, 11.8)	77.1	CH-OH	3.75 (1H, dd, 10,5, 4,5)	77.0
C-4	-	43.8	C		43.7
C-5	2.46-2.50 (1H, d, 6.9)	42.6	CH		42.5
C-6	0.79-0.83 (1H, m) 1.20-1.24 (1H, m)	21.1	CH_2		21.0
C-7	1.88-1.94 (1H, m) 1.31-1.38 (1H, m)	28.2	CH_2		28.1
C-8	1.53-1.59 (1H, m)	48.0	CH		47.9
C-9	-	20.0	C		19.9
C-10	-	25.5	C		25.7
C-11	1.00-1.06 (1H, d, 7.2) 1.20-1.24 (1H, m)	25.9	CH_2		25.4
C-12	1.72-1.78 (1H, m) 1.27-1.30 (1H, d, 5.9)	35,7	CH_2		35.6
C-13	-	45.4	C		45.3
C-14	-	48.9	C		48.8
C-15	1,56-1,64 (1H, m, 7.9, 3.9, 2.8) 1,24-1,26 (1H, m, 4.0, 3.6, 2.8)	33.0	CH_2		32.9
C-16	1.96-2.01 (1H, dt, 17.2, 8.5, 10.5) 1.20-1.24 (1H, m)	26.5	CH_2		26.4
C-17	1.74-1.78 (1H, m)	52.4	CH		52.3
C-18	0.88 (3H, s)	18.4	CH_3	0.94 (3H, s)	18.0
C-19	0.37 (1H, s); 0.58 (1H, s)	30.1	CH_2	0.38 (1H, d, 4,2); 0.58 (1H, d, 4,2)	30.0
C-20	1.42-1.49 (1H, m)	35,9	CH		35.9
C-21	0.85 (3H, d, 6.9)	18.5	CH_3	0.86 (3H, d, 6,6)	18.4
C-22	1.60-1.64 (2H, d, 16.8)	31.8	CH_2		32.9
C-23	2.46-2.50 (1H, d, 6.9) 2.35-2.40 (1H, m)	37.7	CH_2		37.6
C-24	-	215.7	C=O		215.5
C-25	2.66 (1H, m)	41.0	CH	2.61 (1H, d, 6,8)	40.9
C-26	1.08 (3H, d, 7.1)	18.2	CH_3	1.09 (3H, d, 6,8)	18.3
C-27	1.07 (3H, d, 7.1)	18.2	CH_3	1.09 (3H, d, 6,8)	18.1
C-28	0.95 (3H, s)	19.4	CH_3	0.89 (3H, s)	19.3
C-29	3.76 (H, dd, 16.9, 11.8) 3.51 (1H, d, 10.4)	71.2	CH_2OH	3.73 (1H, dd, 10,5, 4,5); 3.52 (1H, d, 10,7)	71.1
C-30	0.93 (3H, s)	10.3	CH_3	0.96 (3H, s)	10.1

Table 2. ^{1}H and ^{13}C NMR spectral data (700 MHz for ^{1}H and 175 MHz, in $CDCl_3$) for 2.

Compound 2 (700 and 175 MHz, for ^{1}H and ^{13}C, respectively in $CDCl_3$)				3-*epi*-cabraleahydroxylacton (600 MHz for ^{1}H and 150 MHz, in $CDCl_3$) (Hutagaol et al., 2022).	
Position	^{1}H [ppm] (mult, integral; J=Hz)	^{13}C [ppm]	DEPT	^{1}H [ppm] (mult, integral; J=Hz))	^{13}C [ppm]
C-1.	1.27 (1H, m) 1.40 (1H, s)	35.1	CH_2	1.27 (1H, m) 1.35 (1H, m)	34.0
C-2	1.53 (1H, m) 1.93 (1H, m)	29.7	CH_2	1.53 (1H, m) 1.90 (1H, m)	25.8
C-3	3.40 (1H, s)	76.2	CH-OH	3.37 (1H, s)	76.3
C-4	-	37.2	C	-	37.2
C-5	1.25 (1H, s)	49.5	CH	1.25 (5H, m)	49.3
C-6	1.42 (2H, m)	18.2	CH_2	1.37 (2H, m)	18.1
C-7	1.73 (2H, m)	26.8	CH_2	1.71 (2H, m)	26.9
C-8	-	40.6	-	-	40.6
C-9	1.45 (1H, m)	50.4	CH	1.41 (1H, dd, 2.4, 13.2)	50.4
C-10	-	37.6	-	-	37.6
C-11	1.21 (1H, m) 1.74 (1H, m)	25.4	CH_2	1.20 (1H, m) 1.70 (1H, m)	25.3
C-12	1.27 (1H, m) 1.73 (1H, m)	21.3	CH_2	1.26 (1H, m) 1.71 (1H, m)	21.2
C-13	1.53 (1H, m)	43.1	CH	1.53 (1H, m)	43.1
C-14	-	50.4	-	-	50.3
C-15	1.10 (1H, dd, 9.6) 1.49 (1H, m)	31.2	CH_2	1.11 (1H, ddd, 12.8, 1.5) 1.49 (1H, m)	31.1
C-16	1.28 (1H, m) 1.81 (1H, m)	25.0	CH_2	1.25 (1H, m) 1.78 (1H, m)	25.0
C-17	1.97 (1H, dt, 8.9, 8.1)	49.5	CH	1.23 (1H, m)	49.4
C-18	0.96 (3H, s)	15.4	CH_2	0.92 (3H, s)	15.4
C-19	0.85 (3H, s)	16.0	CH_2	0.82 (3H, s)	16.0
C-20	-	90.2	CH_2	-	90.2
C-21	1.36 (3H, s)	25.4	CH_2	1.33 (3H, s)	25.4
C-22	1.95 (1H, m) 2.00 (1H, d, 3.1)	31.2	CH_2 CH_2	1.94 (1H, m) 2.12 (1H, q, 10.9)	31.1
C-23	2.53 (1H, dd, 15.1) 2.6 (1H, ddd, 9.3)	29.2	CH_2	2.52 (1H, d, 10) 2.62 (1H, d, 9.9)	29.3
C-24	-	176.8	CH_2	-	176.9
C-25	-	-	-	-	-
C-26	-	-	-	-	-
C-27	-	-	-	-	-
C-28	0.94 (3H, s)	28.3	CH_3	0.91 (3H, s)	28.4
C-29	0.84 (3H, s)	22.1	CH_3	0.81 (3H, s)	22.1
C-30	0.90 (3H, s)	16.3	CH_3	0.87 (3H, s)	16.3

NOESY spectrum (**Figure S9**) analysis was carried out to determine the stereochemistry of compound **1**, especially at the chiral C-3 atom and to confirm the stereochemistry of the hydroxyl group bound to C-3 and C-29. Based on the spectrum, it was found that the position of hydroxyl at C-3 is in *β* position (**Figure 2B**). Based on a literature search carried out, compound **1** was only found in two *Aglaia* species, it has never been found in other plants.

This compound discovery is the second discovery after the first was successfully isolated by Inada in 1995. On the basis of the interpretation of the ^{1}H and ^{13}C-nmr data, that was found by Inada, as well as on biogenetic grounds, the secondary and primary

hydroxyl groups of **1** from *Aglaia harmsiana* Perkins (Meliaceae) leaves were located at C-3*β* and C-29, respectively (Inada et al., 1995). All these features together with the data obtained from 1D and 2D NMR spectra indicate that compound 1 is a cycloartan-type triterpenoid compound named cycloartan-3β,29-diol-24-one (**Figure 1**).

The ^{1}H-NMR ($CDCl_3$, 700 MHz, ppm) spectrum of compound **2** (**Figure. S10A** and **10B**) and **Table 2**, showed six peaks corresponding to six tertiary methyls at δ_H 0.96 (Me-18), 0.85 (Me-19), 1.36 (Me-21), 0.94 (Me-28), 0.84 (Me-29) and 0.90 (Me-30). Other spectra showed one signal oxygenated methane at δ_H 3.40 (H-3). The ^{13}C NMR and DEPT 135° (175MHz, $CDCl_3$) spectra (**Figure S11** and **S12**) and Table 2, showed peaks corresponding to twenty seven carbons, consisting of six methyls, ten methylenes and five methines. The presence of six methyls at δ_C [15.4 (C-18), 16.0 (C-19), 25.4 (C-21), 28.3 (C-28), 22.1 (C-29), 16.3 (C-30)], one oxygenated methine at δ_C 76.2 (C-3), one oxygenated quaternary carbon at δ_C 90.2 (C-20) and one ester signal at 176.8 (C-24). These signals indicated release of the C-25, C-26 and C-27 atoms from molecule structure and the formation of the lactone ester at C-24/C-20. The ^{13}C and DEPT signals of compound **2** showed possibility found five double bonds equivalence on its compound which shows the characteristics of pentacyclic dammarane of trisnor-triterpenoid skeleton. These signals strengthen existence of nor-triterpenoid skeleton (Phongmaykin et al., 2008)

Based on the literature search that has been carried out, the 1D NMR spectrum of compound **2** is very similar features to the nortriterpenoid compound that was previously successfully isolated by Hutagaol from *Aglaia angustifolia* (Hutagaol et al., 2022). Stereochemistry compound **2** obtained from the ^{1}H-NMR experiment showed that *J* value of proton signal coupling on H-3 = 0 Hz at C-3 indicated the –OH at C-3 as a *α*-oriented. Then the chemical shift value δ_C 90.3 at C-20 shows that the stereochemical configuration at this carbon is 20S (Hawas et al., 2013). Therefore, compound **2** based on the data has been carefully examined and identified as a compound named as 3-*epi*-cabraleahydroxylactone, see **Figure 1**

Evaluation of Cytotoxicity

Cytotoxicity activity exploring the cytotoxic effect of different doses of the compounds **1** and **2** on the solid tumour breast cancer cell lines MCF 7 and MDA-MB-468, using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H tetrazolium bromide (MTT) assay. Based on the IC_{50} value that has been obtained indicated that both tested compounds possessed low cytotoxicity against MCF-7 cells (IC_{50} > 20 *μ*g/mL) in comparison to doxorubicin cytotoxicity, as shown in **Table 3**. It can be concluded that compound **1** and compound **2** have almost the same cytotoxic value against MCF 7 cells. The differences in structure or functional groups possessed by the two triterpenoid compounds do not have a significant effect on the activity of the two compounds on MCF-7 cells.

The enhanced cytotoxic activity of cycloartan-3β,29-diol-24-one against MDA-MB-468 cells may be related to the presence of a cyclopropane ring in its cycloartane framework. Cyclopropane moieties are known to introduce significant ring strain and conformational rigidity, which can influence molecular interactions with biological targets and membrane systems.

Table 3. Cytotoxicity (IC_{50} , *μ*g/mL) of test compounds against breast cancer cell lines

Sample	Cell Line	
	MCF-7	MDA-MBA-468
Compound **1**	118.27 ± 0.45	3.65 ± 0.10
Compound **2**	115.11 ± 0.18	14.53 + 0.32
doxorubicin	3.27 ± 0.006	0.03 ± 0.002

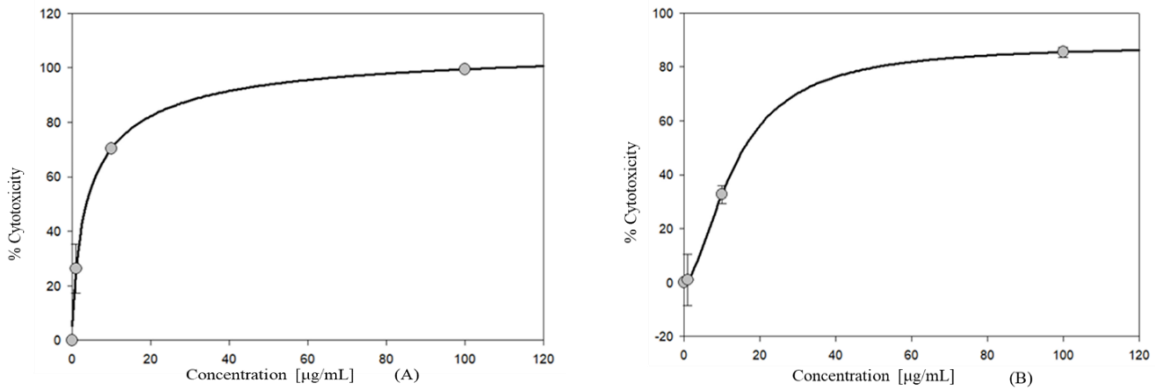

Figure 3. Dose-response curves of compounds **1** (A) and **2** (B) against MDA-MB-468 cells, data are means ± SD of triplicates (Significance level = <0.0001).

In contrast, 3-epi-cabraleahydroxylactone lacks this structural feature, which may partly explain its comparatively weaker cytotoxic activity. These observations suggest that the cyclopropane functionality may contribute to cytotoxic potency, although further structure–activity relationship studies are required to confirm this hypothesis. The dose-response curves of compounds **1** and **2** against MDA-MB-468 cells are shown in **Figure 3.**

CONCLUSIONS

Isolation of *n*-hexane extract of *Aglaia shawiana* leaves and twigs yielded two triterpenoids, which were identified as cycloartan-3*β*,29-diol-24-one (**1**), and 3-epi-cabraleahydroxylactone (**2**). The cytotoxic activity of the two compounds was tested against two breast cancer cells, namely MCF-7 and MDA-MB-468 Cell lines. Based on the IC_{50} value that has been obtained indicated that both tested compounds possessed low cytotoxicity against MCF-7 cells. Both compounds have moderate activity against MDA-MB-468 cancer cells, but Compound **1** (IC_{50} = 3.65 ± 0.10 μg/ml) has stronger activity than compound **2**. Compound **1** showed promising anticancer activity to further investigate the mechanism of its activity against the MDA-MB-468 breast cancer cell line.

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CONFLICT OF INTEREST

No potential conflict of interest was reported by the authors.

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