Cytotoxic Dammarane-Type Triterpenoids from *Aglaia cucullata* Peel Fruit

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Abstract: *Four triterpenoids, known as dammarane-type, dammaradienone (**1**), 20(S),25-epoxy-5α-dammar-20-en-3-one (**2**), 20(S)-5α-dammar-24-en-3α,20-diol-3-acetate (**3**) and 3α-acetyl-20S,24S-epoxy-25-hydroxydammarane (**4**), were isolated from* Aglaia cucullata *peel fruit. The structures of isolated compounds were identified based on their HR-TOFMS data and extensive NMR spectroscopic analysis, as well as compared with literature data. Compounds **1**-**4** were assessed for cytotoxic effects against HeLa cervical and B16-F10 melanoma skin cancer cells. All compounds showed moderate to weak activity against B16-F10 cancer cells, while compound **2** exhibited the strongest activity against HeLa cancer cells with IC_{50} of 7.10 μg/mL indicating that the existence of an epoxy moiety at the side chain increases the cytotoxicity to HeLa cells.*

Keywords: Aglaia cucullate*; B16-F10; dammarane-type; cytotoxic activity; HeLa*

■ INTRODUCTION

Triterpenoids are terpenoid compounds that are formed by six isoprene units (C_5) and have diverse chemical structures and pharmacological effects. Triterpenoids exist in several skeletal forms based on the total of their rings [1]. Dammarane-type triterpenoid is a tetracyclic triterpenoid that commonly found in genera of *Aglaia* (Meliaceae), *Sapindus* (Sapindus), *Panax* (Araliaceae), *Aralia*, *Polyscias* (Araliaceae), *Gynostemma* (Cucurbitaceae), *Bacopa* (Scrophulariaceae), *Forsynthia* (Oleaceae), *Copernicia* (Arecaceae), *Myrica* (Myrica), *Celastrus* (Celastraceae), and *Rhus* (Anacardiaceae) [2-8]. The pharmacological effects of dammarane-type triterpenoids have been investigated, such as anti-cancer, anti-HIV, antineoplastic, antihypertensive, anti-fibrotic, and anti-aging [9-11].

Aglaia genus (Meliaceae) is a large source of natural compounds which has a total of 150 species and 65 species are found in Indonesia. *Aglaia* plants are widely distributed in tropical and subtropical rainforests in Southern Asia and the Pacific regions [12]. In Asia, *Aglaia* plants are distributed in India, Malaysia, Thailand, Vietnam, and Indonesia. These plants are commonly used as folk medicines for the treatment of skin diseases, fever, diarrhea, cough, heart diseases, and bruises [13]. In addition, the pharmacological effects of *Aglaia* plants have been reported, such as anti-bacterial, insecticidal, antioxidant, anti-tumor, anti-viral, anti-fungal, and cytotoxic activities [14-15]. Based on the phytochemical study of *Aglaia* plants, ninety-six triterpenoids have been reported; there were tirucallane, apotirucallane, cycloartane, glabretal, protostane, ursane, norbaccharane, lupane, and dammarane types [10,16-18].

A. cucullata (mangrove plant that has pneumatophores roots) grows in tobacco swamps [19]. *A. cucullata* is spread in the rainforests of Southeast Asia, Vietnam, Myanmar, Pakistan, Nepal, Thailand, Bangladesh, Malaysia, Papua New Guinea, and Indonesia in the Borneo and Sumatera Islands [14]. Twenty-two secondary metabolites were found in *A. cucullata,* such as alkaloids, triterpenoids, diterpenoids, bisamides, rocaglamides, and flavonoids [20-23]. In order to explore the active compounds of the Indonesia *Aglaia* plants and observe their cytotoxicity towards HeLa cervical cancer cells and B16-F10 melanoma cells, four known triterpenoids (**1**-**4**) from *A. cucullata* peel fruit will be described.

■ EXPERIMENTAL SECTION

Materials

A. cucullata (Robx.) Pellegr. peel fruits were collected in December 2020 from Balikpapan, East Kalimantan, Indonesia and were examined with collection No. FF7.20 at the Herbarium Wanariset (WAN), Balikpapan, as well as stored at the Faculty of Forestry, Mulawarman University. The solvents used for extraction, fractionation, isolation, and purification with pro-analyst and technical quality that have been distilled are acetone, chloroform, ethanol, ethyl acetate, *n*-butanol, *n*-hexane, methanol, and water. 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).

Instrumentation

Infrared spectra were recorded on a Perkin-Elmer-100 as KBr disks (Waltham, Massachusetts, USA). Mass spectra were measured by Waters HR-ESI-QTOFMS-XEV^{otm} mass spectrometer (Milford, MA, USA), using ESI+ and ESI- mode. Meanwhile, the NMR spectra were measured by JEOL JNM-ECZ500R/S1 spectrometer at 500 MHz for ^{1}H and 125 MHz for ^{13}C, using $CDCl_3$ as a solvent. Column chromatography (CC) was carried out on silica gel 60 (Merck, Darmstadt, Germany, 70–230 and 230–400 mesh) and octadecyl silane (ODS, Fuji Sylisia Chemical LTD., 200–400 mesh). Silica gel plates GF_{254} and RP-18 F_{254S} plates (Merck) were used for thin-layer chromatography (TLC). Detection of the TLC plate was monitored under UV light at 254 and 365 nm and sprayed with 10% H_2SO_4 in EtOH.

Procedure

Extraction and isolation

An amount of 1 kg dried *A. cucullata* peel fruit was macerated, re-distillated using EtOH 70%, filtered, and evaporated to give a concentrated extract of EtOH. The concentrated EtOH extracts (352.2 g) were mixed in 8:2 of H_2O:MeOH and partitioned consecutively with *n*-hexane, EtOAc, and *n*-BuOH. Then, the organic solvent was evaporated at a temperature of 37 °C using a rotary evaporator to obtain EtOAc (40.9 g), *n*-BuOH (5.9 g), and *n*-hexane (250.7 g) extracts.

The nonpolar extract (*n*-hexane) was separated using VLC (10% gradient system of *n*-hexane-EtOAc) on silica gel to gain fractions A–E. B fraction (9.4 g) was CC on silica gel with *n*-hexane-EtOAc (100:0–90:10, 1% gradient) eluent solvent to gain fractions B1-B7. Then, the separation of B2 fraction (773.0 mg) was carried out by CC on silica gel with *n*-hexane:EtOAc (100:1) as an eluent to give ten fractions (B2A-B2J). Purification of the B2F subfraction (128.9 mg) on ODS (C_{18}, 100–200 mesh) CC eluted using acetonitrile:H_2O (25:1) yield compound **1** (26.7 mg).

As much as 2.71 g of B5 fraction was purified by CC silica gel (*n*-hexane:DCM:EtOAc, 25:25:1) to yield B5A-B5I subfractions. Furthermore, 269.0 mg of B5B fraction was purified by CC silica gel (*n*-hexane:EtOAc, 15:1) to give B5B1-B5B5 subfractions. Furthermore, 21.6 mg of B5B1 and 154.0 mg of B5B2 were purified with CC on ODS and eluted with MeOH:H_2O (9:1) and (7:3) respectively, to obtain compound **2** (5.0 mg) from B5B1 subfraction and compound **3** (22.3 mg) from B5B2 subfraction. Fraction B5C (452.0 mg) was performed by CC on silica gel using *n*-hexane:chloroform:EtOAc (30:2:1) to obtain compound **4** (52.5 mg).

Dammaradienone (1). White crystal, IR ν_{max}: 2944, 1701, 1641, 1374, 1440 cm^{-1}; ^{1}H-NMR, δ_H (ppm): 1.42

(1H, dd, *J* = 4.5 & 7.7 Hz, H-1a), 1.94 (1H, d, *J* = 7.7 Hz, H-1b), 2.42 (1H, d, *J* = 4.5 Hz, H-2a), 2.48 (1H, dd, *J* = 2.8 & 6.5 Hz, H-2b), 1.37 (1H, dd, *J* = 1.4 & 4.9 Hz, H-5), 2.18 (1H, dd, *J* = 2.2 & 5.6 Hz, H-17), 1.31 & 1.63 (1H, m, H-7a & H-7b), 1.35 & 1.50 (1H, m, H-6a & H-6b), 1.41 (2H, m, H-11), 1.40 (1H, m, H-9), 1.55 (1H, dd, *J* = 2.5 & 6.8 Hz, H-12a), 1.84 (1H, d, *J* = 2.5 Hz, H-12b), 1.67 (1H, dd, *J* = 1.6 & 7.6, H-13), 1.10 (1H, d, *J* = 4.1 Hz, H-15a), 1.57 (1H, dd, *J* = 4.1 & 8.1 Hz, H-15b), 1.89 (2H, m, H-16), 0.86 (3H, s, Me-18), 0.92 (3H, s, Me-19), 4.69 (1H, br.s, H-21a), 4.72 (1H, br.s, H-21b), 1.95 (2H, m, H-22), 2.10 (2H, q, *J* = 7.5 Hz, H-23), 5.11 (1H, tt, *J* = 7.0 & 1.5 Hz, H-24), 1.60 (3H, s, Me-26), 1.67 (3H, s, Me-27), 1.02 (3H, s, Me-28), 1.06 (3H, s, Me-29), 0.99 (3H, s, Me-30); ^{13}C-NMR, see Table 1; HRTOF-MS, found *m/z* 425.4151 $[M+H]^+$, (calcd. $C_{30}H_{49}O$, *m/z* 425.3783).

Table 1. ^{13}C-NMR data for compounds **1**-**4** (125 MHz in $CDCl_3$)

Carbon position	**1**	Lit. **1***	**2**	Lit. **2**	**3**	Lit. **3**	**4**	Lit. **4****
	δ_C							
1	40.0	40.0	40.0	39.5	35.1	34.6	34.3	34.3
2	34.1	34.1	34.2	34.1	23.0	23.2	23.0	22.9
3	218.3	218.2	218.3	218.1	78.5	78.7	78.5	78.3
4	47.5	47.4	47.5	47.4	37.2	37.5	36.8	36.7
5	55.4	55.4	55.4	55.4	50.8	50.7	50.6	50.7
6	19.7	19.7	19.7	19.7	18.1	18.4	18.2	18.1
7	34.8	34.8	34.8	34.7	34.3	35.4	35.2	35.1
8	40.4	40.4	40.4	40.4	40.6	40.8	40.6	40.6
9	50.3	50.3	50.3	50.3	50.5	51.1	50.8	50.6
10	36.9	36.9	36.9	36.9	36.8	37.1	37.2	37.1
11	21.9	21.9	21.9	21.9	21.4	21.7	21.7	21.6
12	25.0	25.0	25.0	25.0	24.9	25.1	27.1	27.0
13	45.4	45.4	45.5	45.5	42.3	42.5	42.8	42.7
14	49.4	49.4	49.5	49.4	50.5	50.7	50.2	50.1
15	31.4	31.4	30.9	31.3	31.3	31.5	31.6	31.6
16	28.9	28.9	28.9	29.0	27.6	27.9	25.9	25.8
17	47.8	47.8	47.8	47.7	49.9	50.2	49.9	49.8
18	15.8	15.8	15.8	15.4	15.6	15.8	15.6	15.5
19	16.1	16.1	16.1	16.0	16.0	16.3	16.1	16.0
20	152.6	152.6	151.8	151.8	75.5	75.8	86.7	86.5
21	107.6	107.6	107.9	107.9	25.4	25.7	27.3	27.1
22	34.2	34.2	31.4	30.9	40.5	40.9	34.8	34.7
23	27.1	27.1	27.7	27.7	22.6	22.9	26.4	26.3
24	124.4	124.4	64.3	64.2	124.8	125.1	86.4	86.2
25	131.5	131.5	58.5	58.4	131.7	131.9	70.3	70.2
26	25.8	25.7	25.0	24.9	25.8	26.1	28.0	27.8
27	17.8	17.7	18.8	18.8	17.8	18.1	24.1	24.0
28	26.8	26.8	26.8	26.7	27.9	28.2	27.9	27.9
29	21.1	21.0	21.1	21.0	21.5	22.1	21.8	21.7
30	15.4	15.4	15.4	15.8	16.7	16.9	16.7	16.6
C=O	-	-	-	-	171.0	171.2	171.0	170.8
MeC=O	-	-	-		21.8	21.7	21.5	21.7

*($CDCl_3$, 75 MHz); **($CDCl_3$, 100 MHz)

24(*S*),25-epoxy-5α-dammar-20-en-3-one (2). Colorless crystal, IR ν_{max}: 2952, 1705, 1640, 1459, 1383, 1080 cm^{-1}; ^{1}H-NMR, δ_H (ppm): 1.44 (1H, d, *J* = 4.5 Hz, H-1a), 1.92 (1H, dd, *J* = 1.6 & 4.5 Hz, H-1b), 2.27 (1H, dd, *J* = 3.4 & 5.6 Hz, H-2a), 2.51 (1H, d, *J* = 5.6 Hz, H-2b), 1.37 (1H, dd, *J* = 2.1 & 7.0 Hz, H-5), 1.34 & 1.61 (1H, m, H-7a & H-7b), 1.49 (1H, dd, *J* = 2.0 & 3.5 Hz, H-6a), 1.61 (1H, dd, *J* = 3.5 & 5.4 Hz, H-6b), 1.36 & 1.51 (1H, m, H-11a & H-11b), 2.19 (1H, q, *J* = 4.5 Hz, H-17), 1.41 (1H, m, H-9), 1.53 (1H, dd, *J* = 2.8 & 6.3 Hz, H-12a), 1.55 (1H, d, *J* = 6.3 Hz, H-12b), 1.71 (1H, d, *J* = 5.7 Hz, H-13), 1.13 (1H, dd, *J* = 2.0 & 6.0 Hz, H-15a), 1.65 (1H, d, *J* = 6.0 Hz, H-15b), 1.42 (1H, m, H-16a), 1.96 (1H, m, H-16b), 0.86 (3H, s, Me-18), 0.93 (3H, s, Me-19), 4.71 (1H, s, H-21a), 4.76 (1H, s, H-21b), 1.97 (1H, m, H-22a), 2.08 (1H, m, H-22b), 1.61 (2H, m, H-23), 1.27 (3H, s, Me-27), 1.30 (3H, s, Me-26), 2.74 (1H, t, *J* = 7.0 Hz, H-24), 1.02 (3H, s, Me-28), 1.06 (3H, s, Me-29), 1.00 (3H, s, Me-30); ^{13}C-NMR see Table 1; HRTOF-MS, found *m/z* 441.3695 $[M+H]^+$, (calcd. $C_{30}H_{49}O_2$, *m/z* 441.3732).

20(*S*)-5α-dammar-24-en-3α,20-diol-3-acetate (3). Colorless crystal, IR ν_{max}: 3507, 2945, 1733, 1455, 1375, 1036 cm^{-1}; ^{1}H-NMR, δ_H (ppm): 1.37 & 1.44 (1H, d, *J* = 6.4 Hz, H-1a & H-1b), 1.53 & 1.94 (1H, dd, *J* = 1.9 & 6.8 Hz, H-2a & H-2b), 1.25 (1H, dd, *J* =1.9 & 5.2 Hz, H-5), 1.44 (1H, dd, *J* = 2.0 & 5.6 Hz, H-6b), 1.25 (1H, d, *J* = 2.0 Hz, H-6a), 1.44 (2H, t, *J* = 7.8 Hz, H-7), 1.45 (1H, t, *J* = 7.8 Hz, H-9), 1.25 (4H, m, H-11/H-12), 1.87 (1H, dd, *J* =2.9, 7.4 Hz, H-13), 1.27 (2H, m, H-15), 1.72 (2H, m, H-16), 1.79 (1H, dd, *J* = 2.5 & 7.6 Hz, H-17), 4.60 (1H, d, *J* = 2.5 Hz, H-3), 0.84 (3H, s, Me-19), 0.81 (3H, s, Me-18), 1.44 (2H, t, *J* = 8.0 Hz, H-22), 1.12 (3H, s, Me-21), 2.03 (2H, q, *J* = 8.0 Hz, H-23), 1.60 (3H, s, Me-26), 5.10 (1H, tt, *J* = 7.0 & 1.5 Hz, H-24), 1.67 (3H, s, Me-27), 0.86 (3H, s, Me-30), 0.95 (3H, s, Me-29), 0.90 (3H, s, Me-28), 2.07 (3H, s, MeC=O); ^{13}C-NMR see Table 1; HRTOF-MS, found *m/z* $[M-H]^-$, 485.4462 (calcd. $C_{32}H_{53}O_3$, *m/z* 485.3994).

3α-acetyl-20*S*,24*S*-epoxy-25-hydroxydammarane (4). Colorless crystal, IR ν_{max}: 3490, 2960, 2873, 1735, 1459, 1375 cm^{-1}; ^{1}H-NMR, δ_H (ppm): 1.41 (1H, d, *J* = 6.7 Hz, H-1a), 1.64 (1H, d, *J* = 6.7 Hz, 1-Hb), 1.60 (1H, d, *J* = 2.1 Hz, H-2a), 1.67 (1H, ddd, *J* = 2.1 & 7.2 & 8.3 Hz, H-2b), 4.60 (1H, dd, *J* = 2.0 & 7.2 Hz, H-3), 1.43 (1H, dd, *J* = 2.0 & 12.1 Hz, H-5), 1.39 (2H, m, H-6), 1.64 (1H, dd, *J* = 2.5 & 7.9 Hz, H-7a), 1.85 (1H, d, *J* = 7.9 Hz, H-7b), 1.21 (1H, m, H-9), 1.50 (2H, m, H-11), 1.78 (2H, m, H-12), 1.64 (1H, dd, *J* = 4.0 Hz, H-13), 1.04 (1H, t, *J* = 8.0 Hz, H-15a), 1.47 (1H, m, H-15b), 1.86 (2H, m, H-16), 1.85 (1H, dd, *J* = 1.9 & 7.0 Hz, H-17), 3.63 (1H, dd, *J* = 10.2 & 4.8 Hz, H-24), 0.96 (3H, s, Me-18), 0.85 (3H, s, Me-19), 1.13 (3H, s, Me-21), 1.23 (1H, t, *J* = 5.5 Hz, H-22a), 1.30 (1H, dd, *J* = 5.5 & 8.2 Hz, H-22b),1.84 (2H, m, H-23), 1.09 (3H, s, Me-27), 1.17 (3H, s, Me-26), 0.86 (3H, s, Me-29), 0.81 (3H, s, Me-28), 2.07 (3H, s, MeC=O), 0.90 (3H, s, Me-30); ^{13}C-NMR see Table 1; HRTOF-MS found *m/z* 501.4574 $[M-H]^-$, (calcd. $C_{32}H_{53}O_4$, *m/z* 501.3943).

Cytotoxic activity test by PrestoBlue assay

Compounds **1**-**4** were tested for cytotoxic activities towards HeLa cervical and B16-F10 melanoma skin cancer cells using the PrestoBlue® method with resazurin reagent [24]. The first stage of this method was seeding cells. HeLa and B16-F10 cells were cultured/seeded into 96 well plates with the medium of RPMI and incubated for 24 h for B16-F10 cell and 48 h for HeLa cell (temperature 37 °C and 5% CO_2 gas) until the cells reached a density of 1.7×10^4 cell/well. The next stage was cell treatment using the samples, positive and negative control. The RPMI media were discarded and then the sample in medium (DMSO as solvent) with different concentrations (250.00, 125.00, 62.50, 31.25, 15.63, 7.81, 3.91, and 1.95 µg/mL), RPMI medium as negative control and cisplatin as positive control. The cells that were treated with samples and positive control were incubated for 48 h. Then, all samples were added to the PrestoBlue® reagent and incubated for 2 h until the color changed. Subsequently, the absorbance of all samples was measured using a multimode (wavelength 570 nm) and converted into cell viability to obtain the value of IC_{50} of each compound.

■ RESULTS AND DISCUSSION

Compounds **1**-**4** (Fig. 1) were obtained by several chromatography methods. Compound **1** was obtained as a white crystal with a yield of 26.7 mg. Its molecular formula was identified as $C_{30}H_{48}O$ according to the HRTOF-MS spectrum (Fig. 2) *m/z* 425.4151 $[M+H]^+$ (calcd. *m/z* 425.3783), representing seven unsaturated

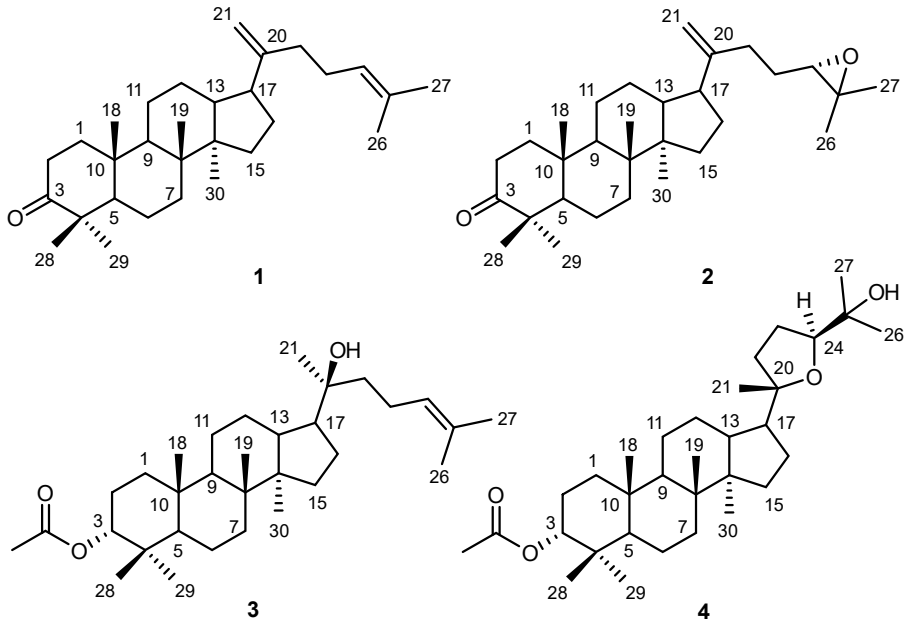

Fig 1. Structures of dammarane-type triterpenoids **1**-**4**

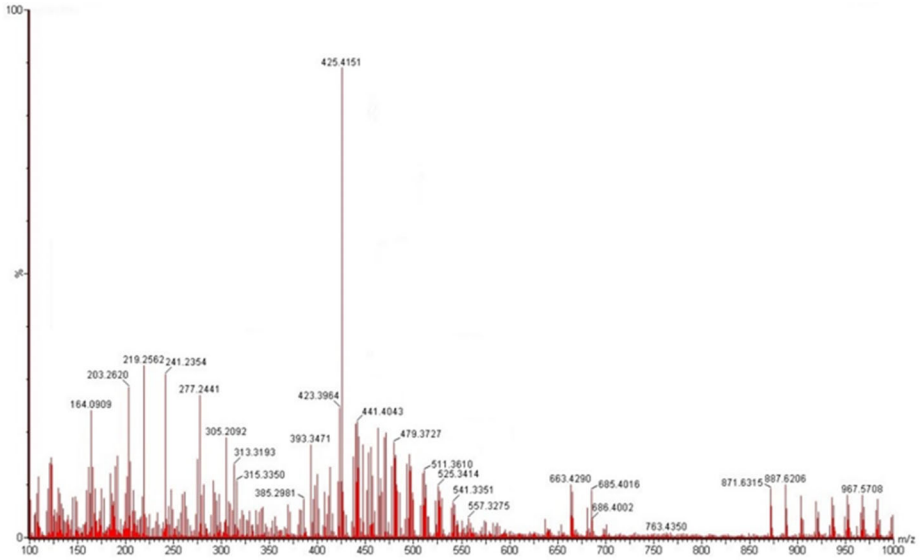

Fig 2. MS spectrum of compound **1**

degrees. The FTIR spectrum of compound **1** (Fig. S1) indicates the absorption bands of the *gem*-dimethyl group (1374 & 1440 cm^{-1}), CH sp^3 (2944 cm^{-1}), C=O ketone (1701 cm^{-1}), and C=C double bond (1641 cm^{-1}). ^{1}H-NMR spectrum of compound **1** (Fig. 3) displayed seven proton singlet resonance at δ_H 0.86 (s, Me-18), 0.92 (s, Me-19), 1.60 (s, Me-26), 1.67 (s, Me-27), 1.02 (s, Me-28), 1.06 (s, Me-29), and 0.99 (s, Me-30) for tertiary methyl groups. Then, the existence of one methylene sp^2 group, which has the chemical shift of geminal proton at δ_H 4.69 (br.s, H-21a) and 4.72 (br.s, H-21b) as well as one methine sp^2 group at δ_H 5.11 (tt, J = 7.0 & 1.5 Hz, H-24). Furthermore, the ^{13}C-NMR detailed with DEPT 135° spectra (Fig. 4) of compound **1** demonstrated 30 signals of carbons, including seven methyl carbons sp^3 at δ_C 16.1 (C-19), 25.8 (C-26), 15.8 (C-18), 17.8 (C-27), 21.1 (C-29), 26.8 (C-28), and 15.4 (C-30), eleven methylenes including ten methylene carbons sp^3 and one olefinic methylene

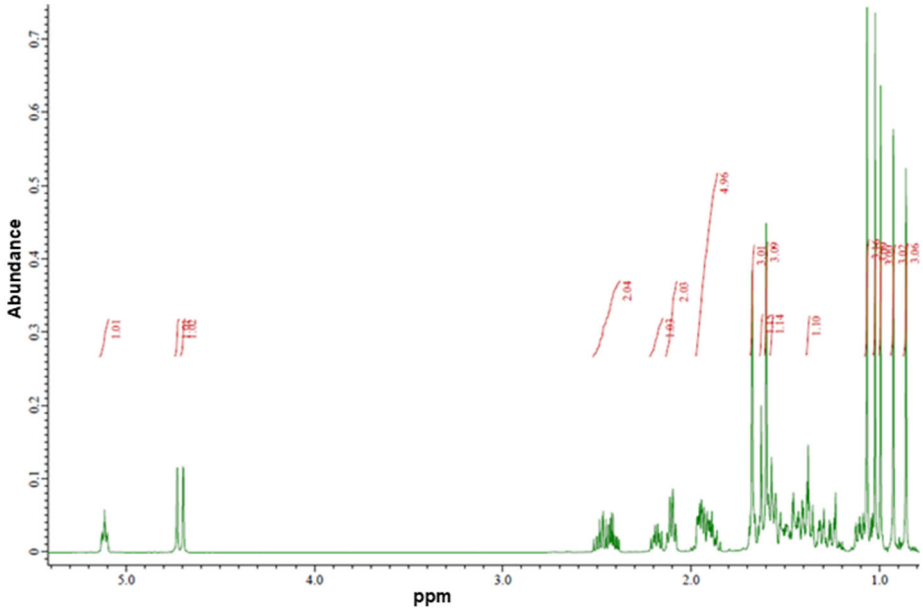

Fig 3. ^{1}H-NMR spectrum of compound **1** (500 MHz in $CDCl_3$)

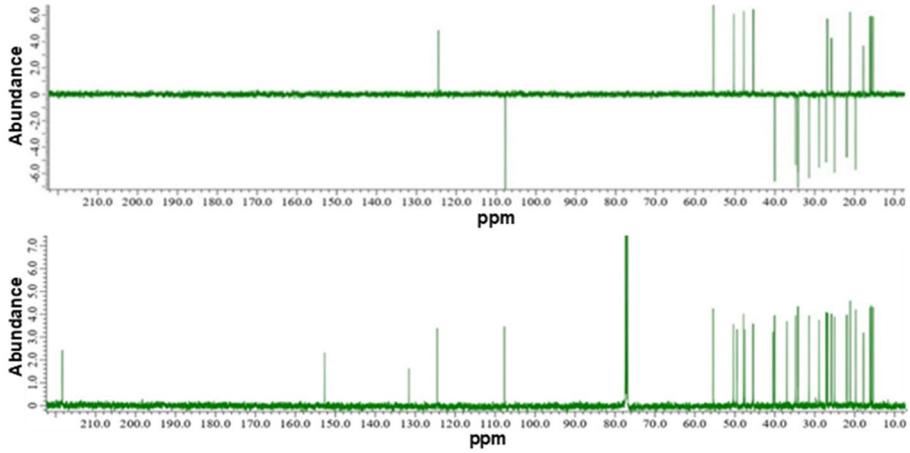

Fig 4. DEPT-135° and ^{13}C-NMR spectrum of compound **1** (125 MHz in $CDCl_3$)

carbon at δ_C 107.6 (C-21), four methine carbons sp^3, olefinic methine carbon resonating at δ_C 124.4 (C-24), and seven quaternary carbons including C=O of ketone group and two olefinic carbons resonating at δ_C 218.3 (C-3), δ_C 152.6 (C-20), and 131.5 (C-25) respectively, also four quaternary carbons sp^3. Based on NMR data, compound **1** has two pairs of double bonds and one carbonyl ketone that is calculated for three unsaturated degrees. Therefore, the four remaining unsaturated degrees corresponded to the tetracyclic triterpenoid structure. The ^{1}H chemical shift of five tertiary methyls (δ_H 0.86, 0.92, 0.99, 1.02, 1.06) showed the characteristic of dammarane-type triterpenoid [25]. Then, the position of the two double bonds is at the side chain. This is supported by the existence of two other singlet methyls at δ_H 1.60 (C-26) and 1.67 (C-27), which is the characteristic of two methyls at the side chain that bound to quaternary carbon sp^2 (C-25). In addition, the triplet of triplet multiplicity of olefinic methine (C-24) with J = 7.0 & 1.5 Hz also supports the position of one olefinic group at C-24/C-25 [25]. One remaining olefinic is positioned at C-20 and C-21, which is supported by the presence of one olefinic methylene (C-21) [25]. Subsequently, the location of the ketone group is at C-3 according to the

biosynthesis pathway of triterpenoid. Triterpenoid was derived from 2,3-epoxysqualene and it indicates that C-3 in triterpenoid is oxygenated carbon [1] and C-3 in compound **1** was oxygenated as a ketone group. A comparison of compound **1** with dammaradienone [25] showed similar NMR data. Consequently, compound **1** was obtained as dammaradienone.

Compound **2** was purified as a colorless crystal (5.0 mg) with a molecular composition $C_{30}H_{48}O_2$ which was identified by HRTOF-MS *m/z* 441.3695 $[M+H]^+$ (calcd. *m/z* 441.3732 (Fig. 5), indicating seven unsaturated degrees. The FTIR spectrum of compound **2** (Fig. S2) displayed the absorption bands of CH sp^3 (2952 cm^{-1}), C=O ketone (1705 cm^{-1}), C=C double bond (1640 cm^{-1}), and *gem*-dimethyl group (1383 & 1459 cm^{-1}). Then, Fig. 6 demonstrated the signals of 7 singlet methyl protons at δ_H 0.86 (s, Me-18), 0.93 (s, Me-19), 1.30 (s, Me-26), 1.27 (s, Me-27), 1.02 (s, Me-28), 1.06 (s, Me-29), and 1.00 (s, Me-30), one olefinic methylene which presents a geminal proton signal at δ_H 4.71 (s, H-21a) and 4.76 (s, H-21b),

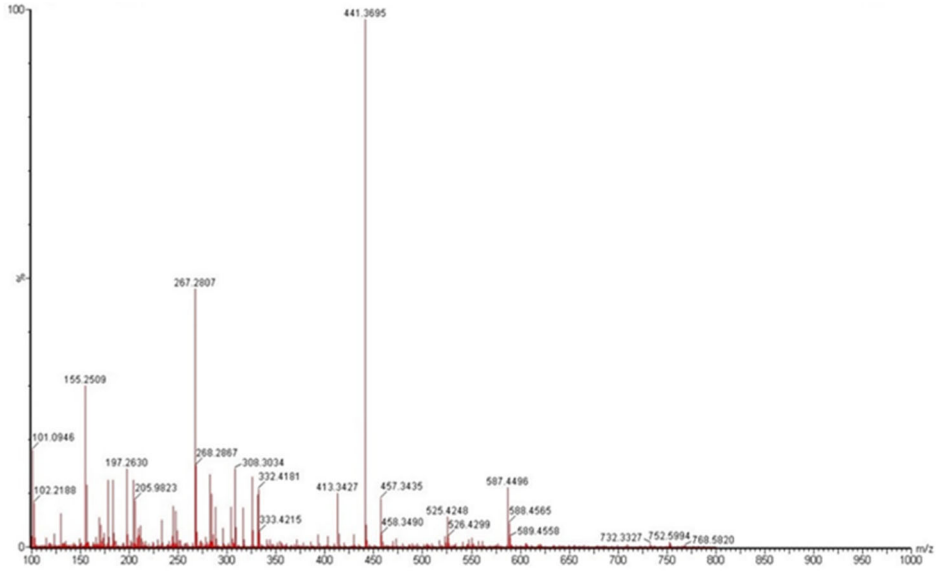

Fig 5. MS spectrum of compound **2**

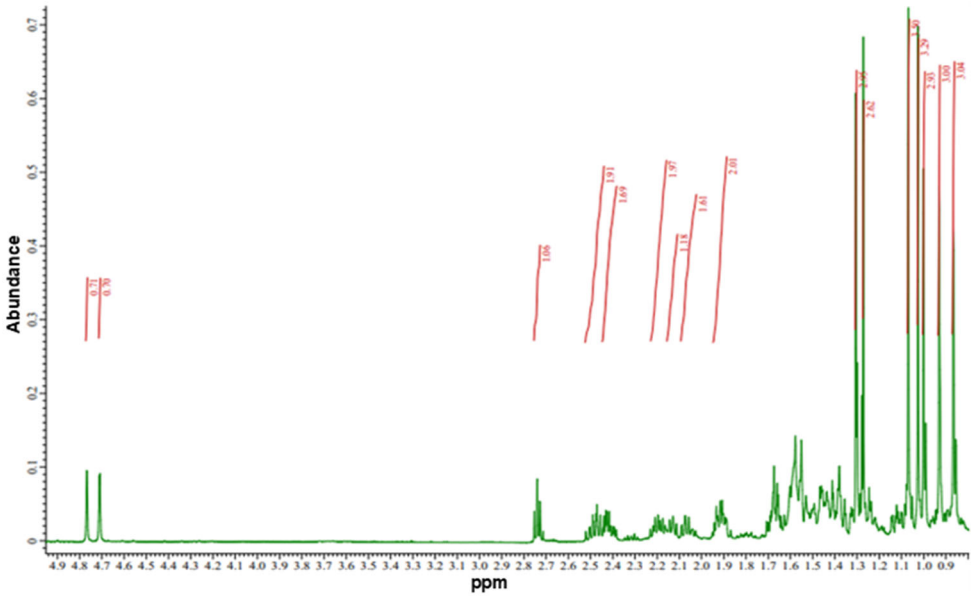

Fig 6. ^{1}H-NMR spectrum of compound **2** (500 MHz in $CDCl_3$)

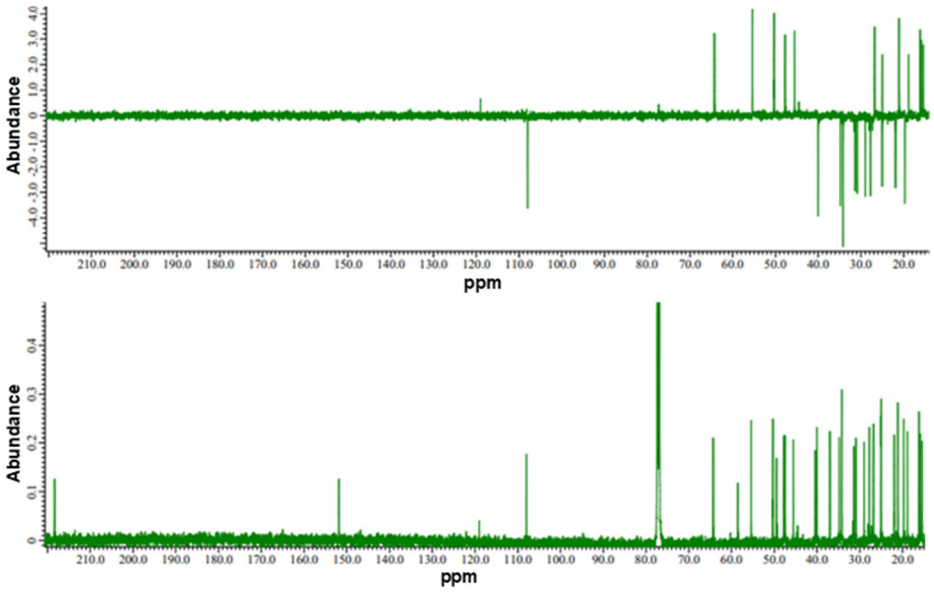

Fig 7. DEPT-135° and ^{13}C-NMR spectrum of compound **2** (125 MHz in $CDCl_3$)

and one oxymethine at δ_H 2.74 (t, *J*=7.0 Hz, H-24). In addition, ^{13}C-NMR detailed DEPT 135° spectra (Fig. 7) displayed the existence of 30 carbons, including seven methyl carbons sp^3 at δ_C 16.1 (C-19), 25.0 (C-26), 15.8 (C-18), 18.8 (C-27), 21.1 (C-29), 26.8 (C-28), and 15.4 (C-30), ten methylene carbons sp^3, one methylene carbon sp^2 at δ_C 107.9 (C-21), four methine carbons sp^3, one oxymethine carbon at δ_C 64.3 (C-24), four sp^3 carbons, one olefinic carbons at δ_C 151.8 (C-20), one oxygenated carbon at δ_C 58.5 (C-25), and one ketone at δ_C 218.3 (C-3). NMR data of compound **2** displayed the presence of one double bond and one carbonyl, which was calculated for two unsaturated degrees, so the five unassigned degrees of unsaturation corresponded to the pentacyclic ring systems. The comparison of NMR data between compounds **1** and **2** showed a similar chemical shift relatively. The main differences in their chemical shift were shown only at C-24 and C-25 whereas in compound **2**, its chemical shift indicates the existence of an epoxy group at C-24/C-25 as an additional ring at the side chain [26]. It proved by the absence of an olefinic methine (δ_C 124.4) and olefinic quaternary carbon (δ_C 131.5) replaced by oxygenated methine (δ_H 2.74) and oxygenated quaternary carbon (δ_C 58.5), respectively. In addition, the value of *J* coupling constant of oxymethine at δ_H 2.74 (t, *J* = 7.0 Hz, H-24) confirmed the stereochemistry of C-24 as *S*-configuration [26]. Therefore, compound **2** was obtained as 24(*S*),25-epoxy-5α-dammar-20-en-3-one.

Compound **3** (colorless crystal, 22.3 mg). HRTOF-MS spectra (Fig. 8) displayed the molecular composition of compound **3** *m/z* 485.4452 $[M-H]^+$ (calcd. 485.3994), with molecular formula $C_{32}H_{54}O_3$ indicating six unsaturated degrees. Fig. S3 (FTIR spectrum) exhibited the peaks of OH (3507 cm^{-1}), ester (1733 cm^{-1}), CH sp^3 (2945 cm^{-1}), and *gem*-dimethyl groups (1375; 1455 cm^{-1}). Subsequently, Fig. 9 (^{1}H-NMR spectrum of compound **3**) demonstrated nine methyl groups at δ_H 0.81 (s, Me-18), 0.84 (s, Me-19), 1.12 (s, Me-21), 1.60 (s, Me-26), 1.67 (s, Me-27), 0.90 (s, Me-28), 0.95 (s, Me-29), 0.86 (s, Me-30) and 2.07 (s, MeC=O). The most deshielded signals were those of the oxygenated methine at δ_H 4.60 (t, H-3) with *J* = 2.5 Hz, and methine sp^2 (δ_H 5.10, tt, H-24) with *J* = 7.0 & 1.5 Hz. The ^{13}C-NMR detailed with DEPT 135° (Fig. 10) exhibited the existence of nine methyl carbons sp^3 at δ_C 15.6 (C-29), 16.7 (C-30), 16.0 (C-19), 17.8 (C-18), 25.4 (C-21), 21.5 (C-27), 21.8 (MeC=O), 25.8 (C-26), 27.9 (C-28), ten methylene carbons sp^3, six methine carbons including one oxymethine and olefinic methine resonating at δ_C 78.5 (C-3); 124.8 (C-24), respectively, as well as four methine carbons sp^3. Then, four quaternary carbons sp^3, oxygenated and olefinic carbons, resonating at δ_C and 131.7 (C-25) and 75.7 (C-20), plus one carbonyl

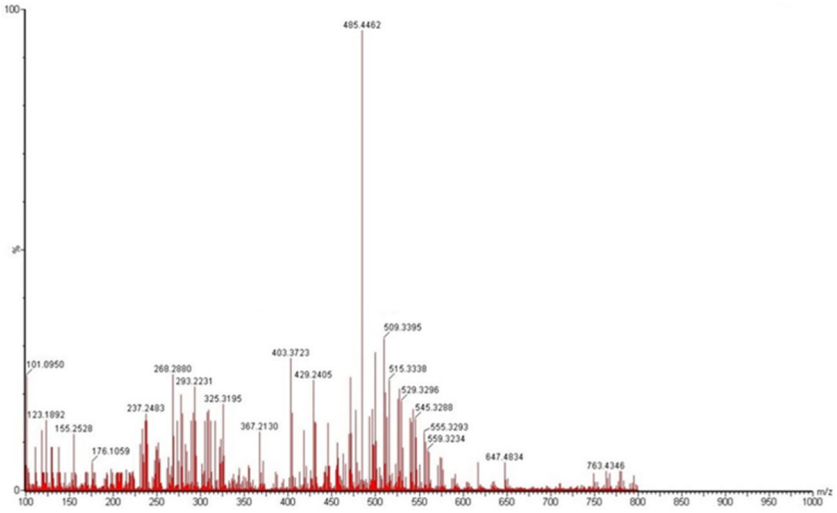

Fig 8. MS spectrum of compound **3**

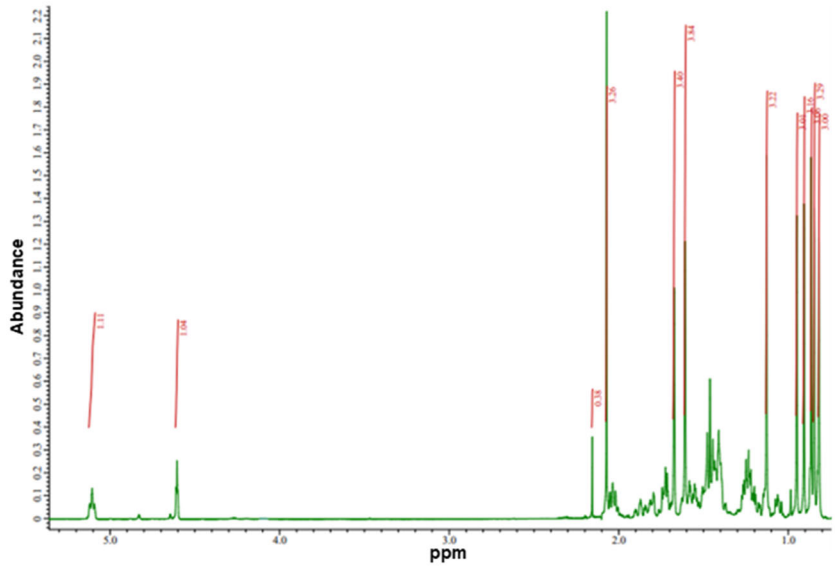

Fig 9. ^{1}H-NMR spectrum of compound **3** (500 MHz in $CDCl_3$)

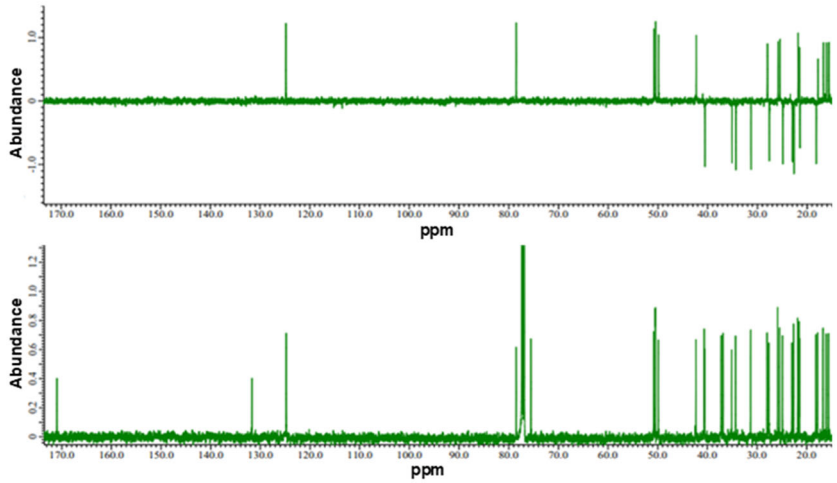

Fig 10. DEPT-135° and ^{13}C-NMR spectrum of compound **3** (125 MHz in $CDCl_3$)

of ester group at δ_C 171.0 (C=O). Based on NMR data of **3**, this compound has one olefinic group and one carbonyl, which is calculated for two unsaturated degrees. Four remaining unsaturated degrees indicated the tetracyclic ring system. In addition, the shielded proton of tertiary methyls also showed the characteristic of dammarane-type triterpenoid [16]. Furthermore, the position of the olefinic group is at the side chain (C-25/C-24) according to their chemical shift and multiplicity, which is similar to compound **1**, definitively. Then, the presence of the acetoxy group was confirmed by the presence of ester carbonyl and tertiary methyl ketone at 2.07 (s, MeC=O). The acetoxy group is positioned at C-3. This is supported by the chemical shift and multiplicity of oxygenated methine at 4.60 (t, J = 2.5 Hz, H-3) that showed characteristics of acetoxy-bound methine [27]. Subsequently, hydroxyl group is positioned at C-21 confirmed by the presence of other tertiary methyl (δ_H 1.12, s, Me-21) and oxygenated carbon (δ_C 75.7, C-20). The configuration of both the acetoxy and hydroxyl group is α-configuration according to the J coupling value of oxymetholone is 2.5 Hz which indicated α-orientation [27]. It is revealed that compound **3** was 20(*S*)-5α-dammar-24-en-3α,20-diol-3-acetate.

Compound **4** was isolated as a colorless crystal (52.5 mg), with a molecular formula $C_{32}H_{54}O_4$ which was identified by HRTOF-MS m/z 501.4574 $[M-H]^+$ (calcd. m/z 501.3943) (Fig. 11), indicating six unsaturated degrees. The FTIR spectrum of compound **4** (Fig. S4) showed the peaks of ester (1735 cm^{-1}), *gem*-dimethyl groups (1459 and 1375 cm^{-1}), CH sp^3 (2960 cm^{-1} and 2873 cm^{-1}), and OH (3490 cm^{-1}). Compound **4** has the same skeleton as compound **3** that showed the existence of an acetoxy group with α-configuration in C-3 of dammarane-type triterpenoid. The difference is the form of a side chain, whereas compound **4** has an additional cyclic tetrahydrofuran ring. The ^{1}H-NMR (Fig. 12) of compound **4** exhibited the existence of proton singlet resonance at δ_H 0.96 (s, Me-18), 0.85 (s, Me-19), 1.13 (s, Me-21), 1.17 (s, Me-26), 1.09 (s, Me-27), 0.81 (s, Me-28), 0.86 (s, Me-29), 0.90 (s, Me-30), and 2.07 (s, MeC=O). The most deshielded signals were those of the oxygenated methines at δ_H 3.63 (dd, H-24) with coupling constant J = 4.8 & 10.2 Hz, and 4.60 (t, J = 3.0 Hz, H-3). Then, DEPT 135 & ^{13}C-NMR spectra (Fig. 13 and 14) of compound **4** demonstrated 32 signals of carbon, including nine methyl carbons sp^3 at δ_C 15.6 (C-18), 16.1 (C-19), 16.7 (C-30), 21.5 (MeC=O), 21.8 (C-29), 24.1 (C-27), 27.3 (C-21), 27.9 (C-28), 28.0 (C-26), ten methylene carbons sp^3, four methines sp^3, two oxymethines resonating at δ_C 78.5 (C-3); 86.4 (C-24), four quaternary carbons sp^3, two oxygenated carbons at

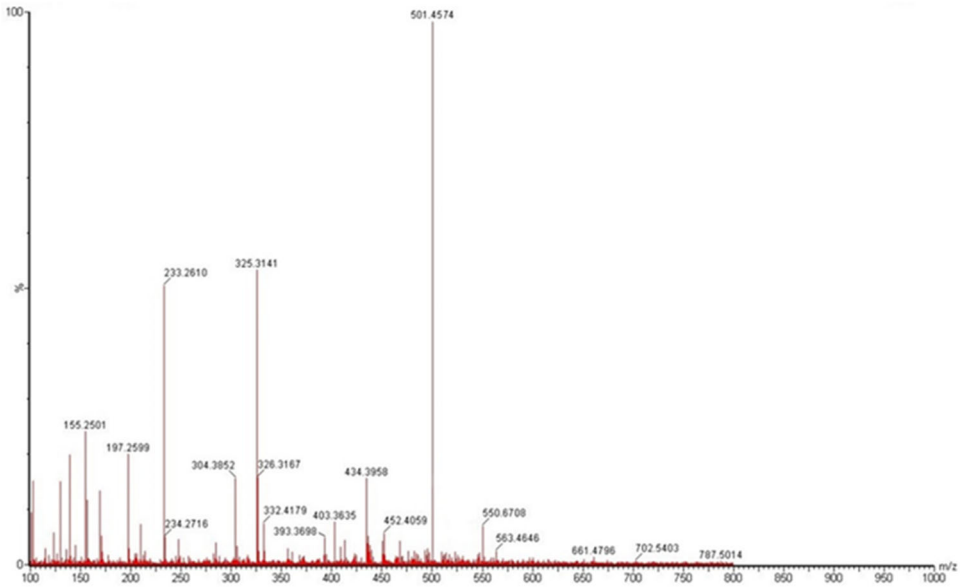

Fig 11. MS spectrum of compound **4**

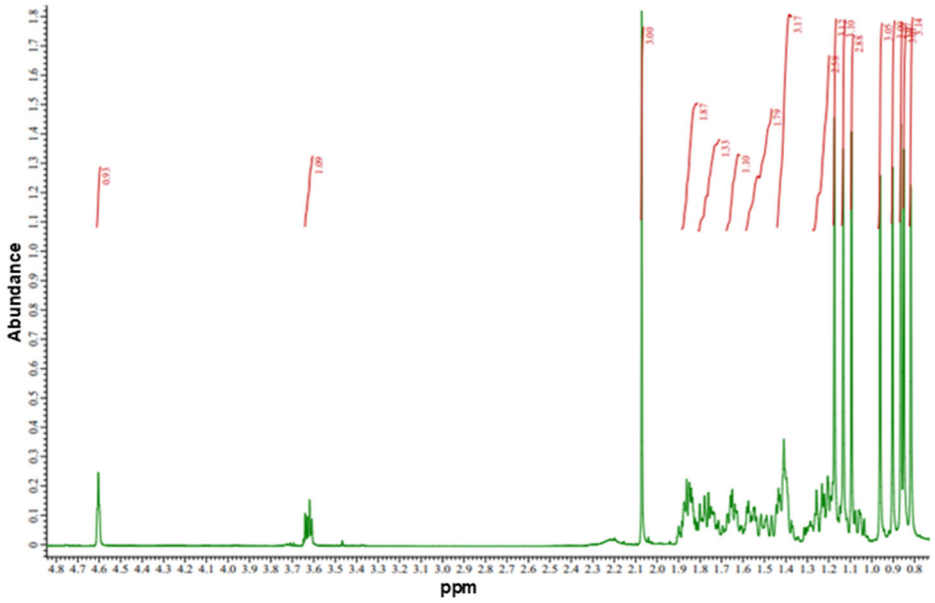

Fig 12. ^{1}H-NMR spectrum of compound **4** (500 MHz in $CDCl_3$)

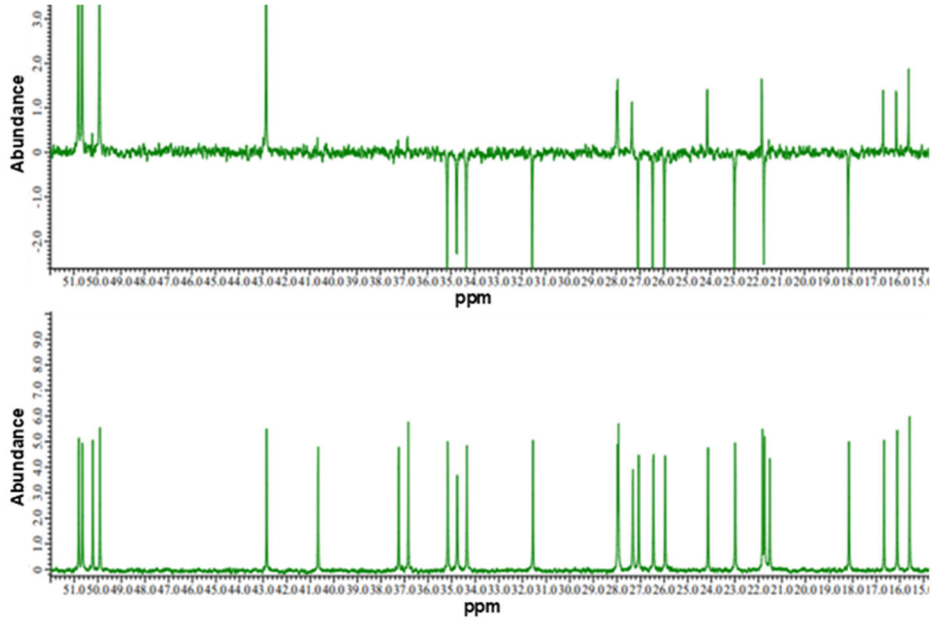

Fig 13. DEPT-135° and ^{13}C-NMR spectrum of compound **4** (125 MHz in $CDCl_3$)

δ_C 70.3 (C-25); 86.7 (C-20), plus a carbonyl of ester group at δ_C 171.0 (C=O). According to NMR data, compound **4** has one carbonyl which is calculated for one unsaturated degree. Five remaining of it indicated the pentacyclic ring system, including four cyclic dammarane-type skeletons and one additional cyclic as tetrahydrofuran at the side chain. The tetrahydrofuran ring was confirmed by the presence of oxygenated methines and oxygenated carbon at δ_C 86.4 (C-24) and 86.7 (C-20), respectively [28]. In addition, this chemical shift indicated the stereochemistry of both C-20 and C-24 as the *S*-configuration [28]. Furthermore, the position of the hydroxyl group suggested at C-25, proved by the presence of oxygenated carbon at δ_C 70.3 (C-25) and two tertiary methyls at δ_H 1.17 (s, Me-26) and 1.09 (s, Me-27) which is characteristic of methyl-bound oxygenated quaternary carbon in tetrahydrofuran ring [28]. The comparison with previous literature data [28] indicated

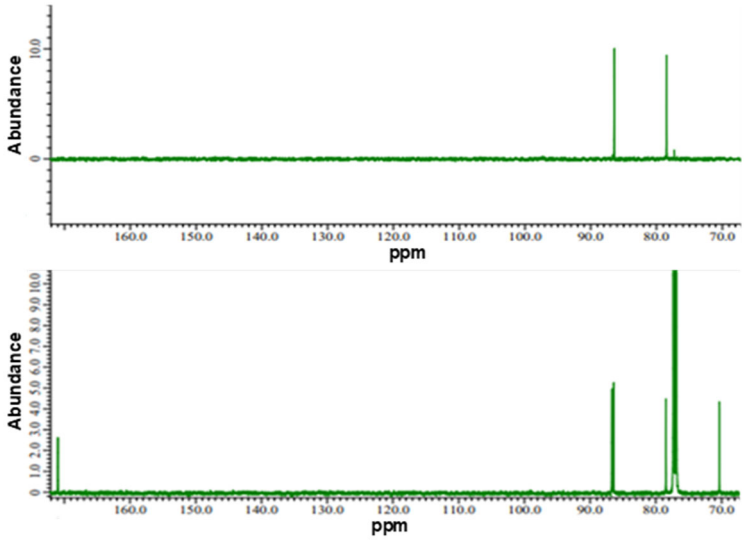

Fig 14. DEPT-135° and ^{13}C-NMR and DEPT 135° spectrum of compound **4** (125 MHz in $CDCl_3$)

Table 2. Cytotoxicity of compounds **1**-**4** against HeLa and B16-F10 cell lines

Compound	IC_{50} (µg/mL)	
	HeLa	B16-F10
Dammaradienone (**1**)	83.54	41.83
24(*S*),25-epoxy-5α-dammar-20-en-3-one (**2**)	7.10	41.50
20(*S*)-5α-dammar-24-en-3α,20-diol-3-acetate (**3**)	99.14	33.35
3α-acetyl-20*S*,24*S*-epoxy-25-hydroxydammarane (**4**)	98.29	104.70
Cisplatin	2.71	12.90

that compound **4** was 3α-acetyl-20*S*,24*S*-epoxy-25-hydroxydammarane commonly known as cabraleadiol monoacetate.

The cytotoxicity of all triterpenoids **1**-**4** was evaluated against cervical HeLa and B16-F10 cells corresponding to a method described previously with cisplatin as positive control [25,29]. The results exhibited that compound **2** exhibited the strongest activity against HeLa cancer cells with IC_{50} value of 7.10 µg/mL, followed by compounds **1**, **4**, and **3** with IC_{50} of 83.54, 98.29, and 99.14 µg/mL respectively. Meanwhile, all compounds showed moderate to weak activity levels against B16-F10 cancer cells, where compound **3** showed the highest value in moderate level with IC_{50} value of 33.35 µg/mL, followed by compounds **2**, **1**, and **4** with IC_{50} of 41.50, 41.83, and 104.70 µg/mL, respectively (Table 2). An epoxy group of **2** significantly increased the cytotoxicity against HeLa cancer cells compared to compound **1**. However, compounds **1** and **2** have the same IC_{50} value against B16-F10 cancer cells, indicating the changes of the alkene group in C-24 and C-25 to the epoxy group did not affect the cytotoxicity against B16-F10 cancer cells significantly. In addition, the changes of side chain form between compounds **3** and **4** showed that the presence of tetrahydrofuran ring can decrease the cytotoxicity against B16-F10 cancer cells yet not give a significant effect on HeLa cancer cells.

■ CONCLUSION

Four known dammarane-type triterpenoids, namely, dammaradienone (**1**), 24(*S*),25-epoxy-5α-dammar-20-en-3-one (**2**), 20(*S*)-5α-dammar-24-en-3α,20-diol-3-acetate (**3**), and 3α-acetyl-20*S*,24*S*-epoxy-25-hydroxydammarane (**4**) were obtained from the *A. cucullata* peel fruit for the first time. The cytotoxic effect of compounds **1**-**4** was assessed towards B16-F10 skin cancer cell lines and HeLa cervical cancer cells. Among all these triterpenoids, compound **2** exhibited the

strongest cytotoxic activity at a strong level against HeLa cancer cells with IC_{50} of 7.0 µg/mL, suggesting that the existence of epoxy group at C-24 and C-25 increased the cytotoxicity against HeLa cells, compared with compound **1**. In addition, **3** exhibited the highest cytotoxic activity against B16-F10 cancer cells with IC_{50} of 33.35 µg/mL, indicating that the opening of the tetrahydrofuran ring influenced the cytotoxicity against B16-F10 cancer cells, compared with compound **4**.

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■ AUTHOR CONTRIBUTIONS

Conceptualization, Unang Supratman; methodology, Intan Hawina Anjari; formal analysis, Desi Harneti and Kindi Farabi; investigation, Intan Hawina Anjari and Al Arofatus Naini; resources, Hadi Kuncoro; data curation, Ace Tatang Hidayat; writing–original draft preparation, Intan Hawina Anjari and Risyandi Anwar; writing–Mohamad Nurul Azmi and Unang Supratman.

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