Steroids from *Atactodea striata* and Their Cytotoxic Activity against MCF-7 Breast Cancer Cell Lines

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Abstract: *Marine environment is known as a source of potential steroids with multiple biological activities, one of which is an anticancer agent.* Atactodea striata *are one of the seashells distributed in Indonesia located in the Kei Islands, Southeast Maluku. During the course of our continuing search for biologically active substances from Indonesia seashells, seven steroids have been isolated from the* n*-hexane fraction of* A. striata *and they were identified as 7β-hydroxy-sitosterol (**1**), campesterol (**2**), β-sitosterol (**3**), cholesterol (**4**), 5α,8α-epidioxycholest-6-en-3-β-ol (**5**), 7-keto-cholesterol (**6**), and 7α-hydroxy-cholesterol (**7**). The structure was identified by spectroscopic methods including 2D NMR techniques, FTIR, HRTOFMS, and chemical shift comparison with previously reported spectral data. Compounds **1-7** were evaluated for their cytotoxic effects against MCF-7 breast cancer cells and showed weak or no anticancer activity.*

Keywords: Atactodea striata*; cytotoxic activity; MCF-7; steroids*

■ INTRODUCTION

Sterol belongs to steroid group and is widely distributed in plants, animals, and marines [1]. Marine is known as a source of interesting secondary metabolites, especially as a natural resource of sterols. Several studies indicate that sterols, isolated from marine products, exhibit several bioactivities such as anti-inflammation, antimicrobial, anti-HIV, and anticancer [2-5]. Several sterols with various structures have been isolated from the seashells of *Villorita cyprinoides*, which are two new cholestane type (22*E*),(24^1*E*)-24^1,24^{2-}dihomocholesta-5,22,24^1-trien-3β-ol and (22*E*)-24^1-homocholesta-5,22-dien-(3β,24^{1}β)-diol showed interesting bioactivity as antioxidant and anti-inflammation agents against DPPH and COX-1 with the IC_{50} values of 0.91 and 0.93 µg/mL (DPPH), 1.16 and 1.19 µg/mL (COX-1), respectively [6].

The *Atactodea striata*, known as Mas Ngur shells in Indonesia, are one of the seashell groups belonging to the mollusc phylum, geographically distributed in the Indo-Pacific from East Africa, including Madagascar and the Red Sea, to eastern Polynesia, to northern Japan, and also distributed in Indonesia located in the Kei Islands, Southeast Maluku [7-8]. *A. striata* are small, short-lived bivalves, have a high population, and are usually found in the intertidal zone along sandy beaches [9]. Previously reported that secondary metabolites have

been isolated from Mas Ngur Shells are vicenin-2 and apigenin. Both vicenin-2 and apigenin showed potent cytotoxic activity against breast cancer cells: MCF-7, MDA-MB-231, and Hs578T [10-12].

In our continuous search for biologically active compounds from marine sources, we have isolated two known stigmastane-type steroids (**1**-**2**), one known campestane-type steroid (**3**), and four known cholestane-type steroids (**4**-**7**) from the *n*-hexane fraction of *A. striata*. Compounds **1**-**7** showed weak activity and no cytotoxic activity against MCF-7 breast cancer cells through *in vitro* assay. Here, we report the isolation and structure elucidation of compounds **1**-**7** along with their cytotoxic activity against MCF-7 breast cancer cells.

■ EXPERIMENTAL SECTION

Materials

The meat of *Atactodea striata* was collected in Ohoi, Kei Island, Southeast Maluku, Indonesia in June 2019. The animal sample was identified and classified by Deep Sea Research Center BRIN, with a voucher B-1209/III/DI/2/2022.

Instrumentation

IR spectra were recorded by Perkin Elmer Spectrum 100 FTIR spectrometer (Shelton, Connecticut, USA) using a NaCl plate. High-resolution mass spectra (HR-TOFMS) were determined on a Waters Xevo Q-TOF direct probe/MS system, utilizing ESI mode and microchannel plates MCPs detector (Milford, MS, USA). The NMR spectra were recorded on JEOL JNM-ECX500R/S1 spectrometer (Tokyo, Japan) at 500 MHz for ^{1}H and 125 MHz for ^{13}C with TMS as an internal standard. The column chromatography was conducted on silica gel 60 (70–230 and 230–400 mesh, Merck, Darmstadt, Germany). The TLC analysis was implemented with silica GF_{254} (Merck, 0.25 mm) and spot detection was obtained by spraying with 10% H_2SO_4 in EtOH, followed by heating and irradiating under ultraviolet-visible light (λ 254 and 365 nm).

Procedure

Extraction and isolation

Dried meat of Mas Ngur shells (*A. striata*) 1.2 kg was extracted with ethanol (10 L) at room temperature for 3 d and then concentrated under vacuum to yield EtOH extract (407.7 g). The concentrated extract was suspended in H_2O and then partitioned with *n*-hexane, EtOAc and *n*-BuOH.

The *n*-hexane fraction (168.8 g) was subjected to vacuum liquid chromatography in silica gel 60 using a gradient of elution of *n*-hexane-EtOAc-MeOH (10% stepwise) to obtain eight fractions (A-H) combined according to TLC profile. Fraction B (10.2 g) was chromatographed on a column of silica gel, eluted with a gradient of elution of *n*-hexane-EtOAc-MeOH (10% stepwise) to give thirteen subfractions (B1-B13) combined based on TLC control. Fraction B3 and B4 were combined (921.9 mg) and then chromatographed on a column of silica gel, eluted with a gradient of eluent of *n*-hexane: EtOAc (9:1) to obtain **1** (62.2 mg), *n*-hexane:EtOAc (7:3) to obtain **2** (12.5 mg), *n*-hexane:EtOAc (8:2) to obtain **3** (14.7 mg), *n*-hexane:EtOAc (6:4) to obtain **4** (11.8 mg). Fraction B7 (408.5 mg) was separated using column chromatography on silica gel (230−400 mesh) and eluted using 1% gradient of elution of *n*-hexane:EtOAc to yield **5** (15.2 mg). Fractions B8 and B9 were combined (943.4 mg) and then separated using column chromatography on silica gel (230–400 mesh) eluted with *n*-hexane:DCM: EtOAc (8:1:1) to give ten subfractions (B8.9a-j). Fractions B8.9g-h were combined (23.1 mg) and then separated using column chromatography on silica gel (230–400 mesh) eluted with *n*-hexane:DCM:EtOAc (7:2:1) to yield **6** (6.8 mg). Fractions C and D were combined (8.1 g) then chromatographed on a column of silica gel (70–230 mesh), eluted with a gradient of *n*-hexane-EtOAc-MeOH (10% stepwise) to give ten subfractions (CD1-10). Fraction CD4 (263.4 mg) was separated using column chromatography on silica gel (230–400 mesh) eluted using 1% gradient of elution of *n*-hexane:EtOAc to give nine subfractions (CD4.a-i). Fraction CD4.i (35 mg) was separated using column chromatography on silica gel (230–400 mesh) eluted with *n*-hexane:acetone (9:1) to obtain **7** (4.4 mg).

7β-Hydroxy-sitosterol (1). White solid, IR ν_{max} 3409, 2853, 1458, 1084 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz), δ_H (ppm): 1.18 (1H, m, H-1a), 1.89 (1H, m, H-1b), 1.85 (1H,

m, H-2a), 1.56 (1H, m, H-2b), 3.53 (1H, m, H-3), 2.28 (2H, m, H-4), 5.35 (1H, d, *J* = 3.5 Hz, H-6), 4.01 (1H, d, *J* = 3.5 Hz, H-7), 1.93 (1H, m, H-8), 0.98 (1H, m, H-9), 1.51 (1H, 2H, H-11), 1.19 (1H, m, H-12a), 2.03 (1H, m, H-12b), 1.00 (1H, m, H-14), 1.13 (1H, m, H-15a), 1.61 (1H, m, H-15b), 1.35 (1H, m, H-16a), 1.86 (1H, m, H-16b), 1.10 (1H, m, H-17), 0.68 (3H, s, CH_3-18), 1.01 (3H, s, CH_3-19), 1.37 (1H, m, H-20), 0.99 (3H, d, *J* = 6.0 Hz, CH_3-21), 1.04 (1H, m, H-22), 1.20 (2H, m, H-23), 0.97 (1H, m, H-24a), 1.70 (1H, m, H-24b), 1.35 (1H, m, H-25), 0.82 (3H, d, *J* = 4.0 Hz, CH_3-26), 0.79 (3H, d, *J* = 6.5 Hz, CH_3-27), 1.27 (2H, m, H-28), 0.83 (3H, t, *J* = 6.5 Hz, CH_3-29); ^{13}C-NMR ($CDCl_3$, 125 MHz) see Table 1; HRTOF-MS (positive ion mode) *m/z* 431.3669 $[M+H]^+$, (calculated $C_{29}H_{51}O_2$, *m/z* 431.3660).

β-Sitosterol (2). White amorphous solid, IR ν_{max} 3409, 2853, 1459, 1084 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H (ppm): 1.18 (1H, m, H-1a), 1.89 (1H, m, H-1b), 1.85 (1H, m, H-2a), 1.56 (1H, m, H-2b), 3.53 (1H, m, H-3), 2.28 (2H, m, H-4), 5.35 (1H, d, *J* = 3.5 Hz, H-6), 1.53 (2H, m, H-7), 1.93 (1H, m, H-8), 0.98 (1H, m, H-9), 1.51 (2H, m, H-11), 1.19 (1H, m, H-12a), 2.03 (1H, m, H-12b), 1.00 (1H, m, H-14), 1.13 (1H, m, H-15a), 1.61 (1H, m, H-15b), 1.35 (1H, m, H-16a), 1.86 (1H, m, H-16b), 1.10 (1H, m, H-17), 0.68 (3H, s, CH_3-18), 1.01 (3H, s, CH_3-19), 1.37 (1H, m, H-20), 0.99 (3H, d, *J* = 6.0 Hz, CH_3-21), 1.04 (1H, m, H-22a), 1.35 (1H, m, H-22b), 1.20 (2H, m, H-23), 0.97 (1H, m, H-24a), 1.70 (1H, m, H-24b), 1.35 (1H, m, H-25), 0.82 (3H, d, *J* = 6.5 Hz, CH_3-26), 0.79 (3H, d, *J* = 6.5 Hz, CH_3-27), 1.27 (2H, m, H-28), 0.83 (3H, t, *J* = 6.5 Hz, CH_3-29); ^{13}C-NMR ($CDCl_3$, 125 MHz) see Table 1; HRTOF-MS (positive ion mode) *m/z* 415.3744 $[M+H]^+$, (calculated $C_{29}H_{51}O$, *m/z* 415.3790).

Campesterol (3). White waxy solid, IR ν_{max} 3380, 2890, 1459, 1084 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H (ppm): 1.15 (1H, m, H-1a), 1.89 (1H, m, H-1b), 1.85 (1H, m, H-2a), 1.56 (1H, m, H-2b), 3.53 (1H, m, H-3), 2.28 (2H, m, H-4), 5.34 (1H, d, *J* = 5.0 Hz, H-6), 1.53 (2H, m, H-7), 1.93 (1H, m, H-8), 0.98 (1H, m, H-9), 1.51 (2H, m, H-11), 1.19 (1H, m, H-12a), 2.03 (1H, m, H-12b), 1.00 (1H, m, H-14), 1.13 (1H, m, H-15a), 1.61 (1H, m, H-15b), 1.35 (1H, m, H-16a), 1.86 (1H, m, H-16b), 1.10 (1H, m, H-17), 0.68 (3H, s, CH_3-18), 1.00 (3H, s, CH_3-19), 1.37 (1H, m, H-20), 0.92 (3H, d, *J* = 7.0 Hz, CH_3-21), 1.04 (1H, m, H-22a), 1.35 (1H, m, H-22b), 1.20 (2H, m, H-23), 0.97 (1H, m, H-24), 1.70 (1H, m, H-25), 0.82 (3H, d, *J* = 6.5 Hz, CH_3-26), 0.85 (3H, d, *J* = 6.5 Hz, CH_3-27), 0.84 (3H, d, *J* = 6.5 Hz, CH_3-28); ^{13}C-NMR ($CDCl_3$, 125 MHz) see Table 1; HRTOF-MS (positive ion mode) *m/z* 401.3744 $[M+H]^+$, (calculated $C_{28}H_{49}O$, *m/z* 401.3790).

Cholesterol (4). White amorphous solid, IR ν_{max} 3409, 2853, 1459, 1084 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H (ppm): 1.15 (1H, m, H-1a), 1.83 (1H, m, H-1b), 1.81 (1H, m, H-2a), 1.56 (1H, m, H-2b), 3.53 (1H, m, H-3), 2.28 (2H, m, H-4), 5.34 (1H, d, *J* = 5.5 Hz, H-6), 1.58 (2H, m, H-7), 1.93 (1H, m, H-8), 1.93 (1H, m, H-9), 1.51 (2H, m, H-11), 1.16 (1H, m, H-12a), 2.00 (1H, m, H-12b), 1.04 (1H, m, H-14), 1.13 (1H, m, H-15a), 1.77 (1H, m, H-15b), 1.85 (2H, H-16), 1.10 (1H, m, H-17), 0.66 (3H, s, CH_3-18), 0.99 (3H, s, CH_3-19), 1.36 (1H, m, H-20), 0.90 (3H, d, *J* = 6.5 Hz, CH_3-21), 1.06 (1H, m, H-22a), 1.32 (1H, m, H-22b), 1.25 (2H, m, H-23), 0.94 (2H, m, H-24), 1.80 (1H, m, H-25), 0.85 (3H, s, CH_3-26), 0.83 (3H, s, CH_3-27);^{13}C-NMR ($CDCl_3$, 125 MHz) see Table 1; HRTOF-MS (positive ion mode) *m/z* 387.3669 $[M+H]^+$, (calculated $C_{27}H_{47}O$, *m/z* 387.3680).

5α,8α-Epidioxycholest-6-en-3-β-ol (5). White solid, IR ν_{max} 3383, 2866, 1459, 1070, 930 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H (ppm): 1.95 (1H, m, H-1a), 1.68 (1H, m, H-1b), 1.51 (m, H-2a), 1.82 (1H, m, H-2b), 3.93 (1H, m, H-3), 1.90 (1H, m, H-4a), 2.10 (H-4b), 6.22 (1H, d, *J* = 8.5 Hz, H-6), 6.49 (1H, d, *J* = 8.5 Hz, H-7), 1.48 (1H, m, H-9), 1.01 (1H, m, H-11a), 1.60 (1H, m, H-11b), 1.20 (2H, m, H-12), 1.57 (1H, m, H-14), 1.21 (1H, m, H-15a), 1.50 (1H, m, H-15b), 1.42 (2H, m, H-16), 1.95 (1H, m, H-17), 0.78 (3H, s, CH_3-18), 0.85 (3H, s, CH_3-19), 1.32 (1H, m, H-20), 0.88 (3H, d, *J* = 6.5 Hz, CH_3-21), 1.51 (2H, m, H-22), 1.25 (2H, m, H-23), 1.33 (2H, m, H-24), 1.49 (1H, m, H-25), 0.85 (3H, d, *J* = 6.5 Hz, CH_3-26), 0.84 (3H, d, *J* = 6.5 Hz, CH_3-27); ^{13}C-NMR ($CDCl_3$, 125 MHz) see Table 1; HRTOF-MS (positive ion mode) *m/z* 439.3188 $[M+Na]^+$, (calculated $C_{27}H_{44}O_3Na$, *m/z* 439.3188).

7-keto-cholesterol (6). White solid, IR ν_{max} 3430, 2853, 1674 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H (ppm): 1.15 (1H, m, H-1a), 1.83 (1H, m, H-1b), 1.81 (1H, m, H-2a), 1.56 (1H, m, H-2b), 3.67 (1H, m, H-3), 2.50 (1H, m, H-4a), 2.38 (1H, m, H-4b), 5.68 (1H, d, *J* = 2.0 Hz, H-6),

2.23 (1H, dd, *J* = 10.5 and 8.5 Hz, H-8), 0.97 (1H, m, H-9), 1.51 (2H, m, H-11), 1.16 (1H, m, H-12a), 2.00 (1H, m, H-12b), 1.03 (1H, m, H-14), 1.13 (1H, m, H-15a), 1.76 (1H, m, H-15b), 1.33 (1H, m, H-16a), 1.85 (1H, m, H-16b), 1.10 (1H, m, H-17), 0.67 (3H, s, CH_3-18), 1.18 (3H, s, CH_3-19), 1.35 (1H, m, H-20), 0.90 (3H, d, *J* = 6.5 Hz, CH_3-21), 1.06 (1H, m, H-22a), 1.32 (1H, m, H-22b), 1.25 (2H, m, H-23), 0.95 (1H, m, H-24a), 1.80 (1H, m, H-24b), 1.37 (1H, m, H-25), 0.84 (3H, d, *J* = 6.5 Hz, CH_3-26), 0.86 (3H, d, *J* = 6.5 Hz, CH_3-27); ^{13}C-NMR ($CDCl_3$, 125 MHz) see Table 1; HRTOF-MS (positive ion mode) *m/z* 401.3439 $[M+H]^+$, (calculated $C_{27}H_{45}O_2$, *m/z* 401.3420).

7α-Hydroxy-cholesterol (7). White solid, IR ν_{max} 3430, 2850 cm^{-1}, ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H (ppm): 1.13 (1H, m, H-1a), 2.02 (1H, m, H-1b), 1.52 (1H, m, H-2a), 1.82 (1H, m, H-2b), 3.57 (1H, m, H-3), 2.33 (2H, m, H-4), 5.58 (1H, d, *J* = 5.5 Hz, H-6), 3.83 (1H, m, H-7), 1.39 (1H, m, H-8), 1.04 (1H, m, H-9), 1.51 (2H, m, H-11), 1.02 (2H, m, H-12), 1.16 (1H, m, H-14a), 1.43 (1H, m, H-14b), 1.80 (1H, m, H-15a), 1.29 (1H, m, H-15b), 1.30 (2H, m, H-16) 1.41 (1H, m, H-17), 1.00 (3H, s, CH_3-18), 0.66 (3H, s, CH_3-19), 1.35 (1H, m, H-20), 0.92 (3H, d, *J* = 6.5 Hz, CH_3-21), 1.05 (1H, m, H-22a), 1.86 (1H, m, H-22b), 1.15 (1H, m, H-23a), 1.14 (1H, m, H-23b), 1.15 (2H, m, H-24), 1.44 (1H, m, H-25), 0.86 (3H, d, *J* = 6.5 Hz, CH_3-26), 0.84 (3H, d, *J* = 6.5 Hz, CH_3-27); ^{13}C-NMR ($CDCl_3$, 125 MHz) see Table 1; HRTOF-MS (positive ion mode) *m/z* 403.1793 $[M+H]^+$, (calculated $C_{27}H_{47}O_2$, *m/z* 403.1793).

Cytotoxic activity test by PrestoBlue assay

The cytotoxicity of all isolated compounds against MCF-7 human breast cancer cells was measured using the PrestoBlue cells viability assay [13]. The cells were maintained in a Roswell Park Memorial Institute (RPMI) medium supplemented with 10% (*v*/*v*) Fetal Bovine Serum (FBS) and 1 µL/mL antibiotic. Cultures were incubated at 37°C in a humidified atmosphere of 5% CO_2. The cells were seeded in 96-well microliter plates at 1.7×10^4 cells per well. After 24 h, compounds **1**-**7** were separately added to the wells. After 96 h, cell viability was determined by measuring the metabolic conversion of resazurin substrate into pink fluorescent resorufin product resulting from the reduction in viable cells. The PrestoBlue assay results were read using a multimode reader at 570 nm. IC_{50} values were taken from the plotted graph of the percentage of living cells compared to control (%), receiving DMSO, versus the tested concentration of compounds (µg/mL). The IC_{50} values mean concentration required for 50% growth inhibition. PrestoBlue assay and analysis were run in triplicate and averaged. The crude extract of ethanol (407.7 g), *n*-hexane (168.8 g), EtOAc (1.7 g), and *n*-butanol (42.2 g) were tested for their cytotoxic activity against MCF-7 breast cancer cells and showed cytotoxic activity with IC_{50} values of were 450.90, 176.02, 580.32 and 5,088.12 µg/mL, respectively.

■ RESULTS AND DISCUSSION

The *n*-hexane extract of *A. striata* was separated and purified using the column chromatography method repeatedly, to obtain compounds **1**-**7** (Fig. 1).

Compound **1** was obtained as a white solid with the yield of mass 62.2 mg (6.74%) of B3-4 fraction (921.9 mg).

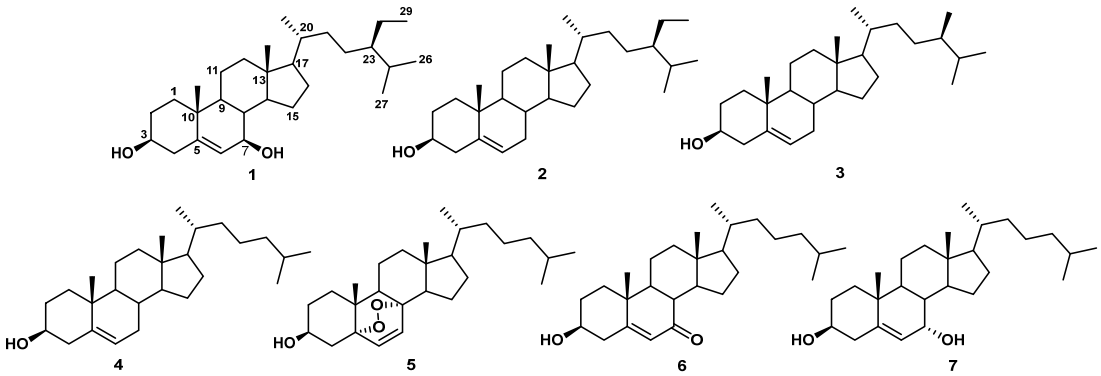

Fig 1. Structures of steroids **1**-**7**

Result of HRTOF-MS spectra (Fig. S1), obtained the molecular weight of **1** $[M+H]^+$ *m/z* 431.3669 (calculated *m/z* 431.3680) with molecular formula $C_{29}H_{50}O_2$ indicating five degrees of unsaturation, consisting of 1 double bound and tetracyclic ring system. The FTIR spectrum of **1** (Fig. S2) showed the absorption of the hydroxyl group (3409 cm^{-1}), CH sp^3 (2853 cm^{-1} and 1458 cm^{-1}) and C-O stretching (1084 cm^{-1}). The ^{1}H-NMR spectrum of **1** (Fig. S3) showed the presence of two tertiary methyl groups at δ_H 0.68 (s, CH_3-18) and 1.01 (s, CH_3-19), three secondary methyl groups at 0.79 (d, *J* = 6.5, CH_3-27), 0.82 (d, *J* = 6.5, CH_3-26) and 0.99 (d, *J* = 6.0, CH_3-21), one primary methyl group at 0.83 (t, *J* = 6.5, CH_3-29) indicating the presence of sitosterol groups [14], ten methylene protons sp^3 and also seven methine protons, two oxygenated methine protons at δ_H 3.53 (m, H-3), 4.01 (d, *J* = 3.5, H-7), and one olefinic methine at δ_H 5.35 (d, *J* = 3.5, H-6). The ^{13}C (Fig. S4) and DEPT 135° (Fig. S5) NMR spectrum of compound **1** showed 29 signals of carbons, presence of six methyl carbons sp^3 at δ_c 12.0 (C-29), 12.1 (C-18), 18.9 (C-21), 19.1 (C-27), 19.5 (C-19), and 20.0 (C-26), ten methylene carbons sp^3, seven methine carbons, two oxygenated methine carbons at δ_c 71.9 (C-3); 75.0 (C-7), one olefinic methine carbon at δ_c 121.9 (C-6), two quaternary carbons sp^3 at δ_c 36.6 (C-10), 45.8 (C-13) and one quaternary carbon olefinic at δ_c 140.9 (C-5).

The HMBC correlation of **1** (Fig. S7) showed the correlation of H-3 (δ_H 3.53) to C-4 (δ_c 42.4), C-2 (δ_c 31.8), and C-1 (δ_c 37.4), correlation of H-6 (δ_H 5.35) to C-8 (δ_c 31.8), C-10 (δ_c 36.6) and C-4 (δ_c 42.4), correlation between H-21 (δ_H 0.99) to C-17 (δ_c 56.1), C-20 (δ_c 36.3), and C-22 (δ_c 34.0), correlation of H-25 (δ_H 1.70) with C-26 (δ_c 20.0) and C-27 (δ_c 19.1), correlation between H-29 (δ_H 0.83) to C-28 (δ_c 23.2) and C-24 (42.5), correlation of H-18 (δ_H 0.68) to C-12 (δ_c 39.9) and C-13 (45.9), H-19 (δ_H 1.01) to C-10 (δ_c 36.6) and C-11 (21.2) showed the characteristic of tetracyclic of stigmastane-type steroid. The presence of hydroxyl group at C-7 showed by correlation of H-7 (δ_H 4.01) to C-8 (δ_c 31.8), C-6 (δ_c 121.9) and C-5 (δ_c 140.9). The cross peak of ^{1}H-^{1}H-COSY (Fig. S8) spectra observed that H1/H2/H3/H4 and H6/H7/H8, indicate that hydroxy group at C-3 and C-7 also confirm that double bond at C-5/C-6, observed that H25/H24/H28/H29 confirm that primary methyl group at C-29, H17/20/21 and H26/H25/H27 indicated that secondary methyl group at C-21, C-26, and C-27. A comparison with the previous NMR data of **1** with β-sitosterol [15] revealed that the structures of the two compounds were very similar, except for C-7, the β oriented of hydroxyl at C-7 confirmed with the literature [16]; thus, compound **1** was identified as 7β-hydroxy-sitosterol.

Compound **2** was obtained as a white solid, with the yield of the mass 14.7 mg (1.59%) from B3-4 fraction (921.9 mg). The result of HRTOF-MS spectra (Fig. S9) obtained molecular weight of **2** $[M+H]^+$ *m/z* 415.3744, (calculated *m/z* 415.3790), with molecular formula $C_{29}H_{50}O$, which required five degrees of unsaturation consisting of 1 double bound and tetracyclic ring system. The FTIR spectrum of **2** (Fig. S10) showed the absorption of the hydroxyl group (3409 cm^{-1}), CH sp^3 (2870 and 1459 cm^{-1}), and C-O stretching (1084 cm^{-1}). The ^{1}H, ^{13}C, and DEPT 135° NMR spectrum of **3** (Fig. S11-S13) similar with **1**, the main difference was that **2** was not substituted with hydroxyl at C-7, proved by the absence of an oxygenated methine (δ_c 75.0) replaced by methylene carbon sp^3 (δ_c 32.0). The selected HMBC correlation (Fig. 2) of **2** showed the correlation of H-3 (δ_H 3.53) to C-4 (δ_c 42.4), C-2 (δ_c 31.6 ppm) and C-1 (δ_c 37.2 ppm), correlation of H-6 (δ_H 5.35) to C-7 (δ_c 32.0), C-8 (δ_c 32.0); C-10 (δ_c 36.6) and C-4 (δ_c 42.4), correlation between H-21 (δ_H 0.99) to C-17 (δ_c 56.1), C-20 (δ_c 36.3), and C-22 (δ_c 34.0), H-25 (δ_H 1.70) to C-26 (δ_c 20.0) and C-27 (δ_c 19.1), correlation of H-29 (δ_H 0.83) with C-28 (δ_c 23.2) and C-24 (42.5), correlation CH_3-18 (δ_H 0.68) to C-12 (δ_c 39.9 ppm) and C-13 (45.8 ppm), CH_3-19 (δ_H 1.01) to C-10 (δ_c 36.6) and C-11 (21.1) showed the characteristic of tetracyclic of stigmastane-type steroid. The cross peak of ^{1}H-^{1}H COSY spectra (Fig. 2) observed that H1/H2/H3/H4 indicate that hydroxy group at C-3, observed that H6/H7/H8 confirm that double bond at C5/C6, H25/H24/H28/H29 confirm that primary methyl at C-29; H17/20/21 and H26/H25/H27 indicated that secondary methyl group at C21, C-26, and C-27. Compound **2** was confirmed by data comparison with previously isolated compound and identified as β-sitosterol [15].

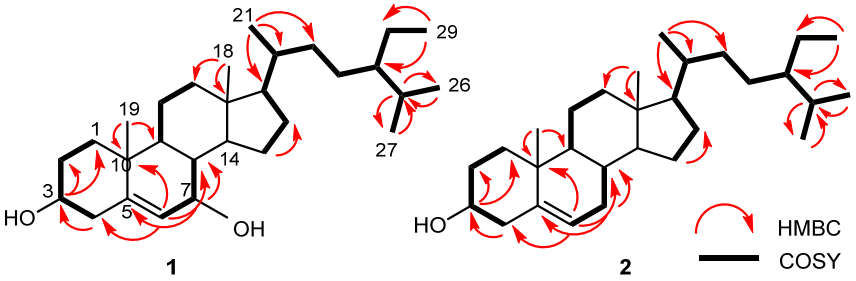

Fig 2. Selected HMBC and ^{1}H-^{1}H-COSY Correlations for **1** and **2**

Compound **3** was obtained as a white waxy solid, with the yield of mass 12.2 mg (1.36%) from B3-4 fraction (921.9 mg). Result of HRTOF-MS spectra (Fig. S17), obtained molecular weight of **3** $[M+H]^+$ *m/z* 401.3744, (calculated *m/z* 401.3790) with molecular formula $C_{28}H_{48}O$, which required five degrees of unsaturation consisting of 1 double bound and tetracyclic ring system. The FTIR spectrum of **3** (Fig S.18) showed the absorption of the hydroxyl group (3380 cm^{-1}), CH sp^3 (2890 and 1459 cm^{-1}), and C-O stretching (1084 cm^{-1}). The ^{1}H-NMR (Fig. S19) spectrum of compound **3** showed the presence of two tertiary methyl groups at δ_H 0.68 (s, CH_3-18) and 1.00 (s, CH_3-19), four secondary methyl groups at δ_H 0.82 (d, *J* = 6.5, CH_3-27), 0.84 (d, *J* = 6.5, CH_3-26), 0.85 (d, *J* = 6.5, CH_3-28) and 0.92 (d, *J* = 7.0, CH_3-21), ten methylene protons sp^3, seven methine protons sp^3, one oxygenated methine proton at δ_H 3.53 (m, H-3), and one olefinic methine at δ_H 5.34 (br.d, *J* = 5.0, H-6). The ^{13}C (Fig. S20) and DEPT 135° (Fig. S21) NMR spectra of compound **3** showed 28 signals of carbons, presence of six methyl sp^3 carbons at δ_c 11.9 (C-18), 18.8 (C-21), 19.4 (C-26, C-19), 22.6 (C-27), 22.8 (C-28), ten methylene sp^3 carbons, seven methine sp^3 carbons, one oxygenated methine carbon at δ_c 71.9 (C-3), one olefinic methine carbon at δ_c 121.8 (C-6), two quaternary sp^3 carbons at δ_c 36.6 (C-10), 42.3 (C-13) and one quaternary olefinic carbon at δ_c 140.8 (C-5). Compound **3** was confirmed with data from the literature identified as a campesterol [17].

Compound **4** was obtained as a white solid, with the yield of mass 15.2 mg (1.65%) from B3-4 fraction (921.9 mg). The result of HRTOF-MS spectra (Fig. S22), obtained molecular weight of **4** $[M+H]^+$ *m/z* 387.3669, (calculated *m/z* 387.3680), with molecular formula $C_{27}H_{46}O$, which required five degrees of unsaturation consisting of 1 double bound and tetracyclic ring system. The FTIR spectrum of **4** (Fig. S23) showed the absorption of the hydroxyl group (3409 cm^{-1}), CH sp^3 (2853 and 1459 cm^{-1}), and C-O stretching (1084 cm^{-1}). The ^{1}H, ^{13}C, and DEPT 135° NMR spectrum of **4** similar with **2**, the main difference was that **4** was not substituted with methyl at C-24. The ^{1}H-NMR spectrum (Fig. S24) of compound **4** showed the presence of two tertiary methyl groups at δ_H 0.66 (s, CH_3-18), and 0.99 (s, CH_3-19), three secondary methyl groups at δ_H 0.83 (d, *J* = 6.5, CH_3-27), 0.85 (d, *J* = 6.5, CH_3-26), and 0.90 (d, *J* = 6.5, CH_3-21), eleven sp^3 methylene protons, and also six sp^3 methine protons, one oxygenated methine proton at δ_H 3.53 (m, H-3), and one olefinic methine at δ_H 5.34 (d, *J* = 5.5, H-6). The ^{13}C-NMR spectrum (Fig. S25) of compound **4** showed 27 signals of carbons, presence of five sp^3 methyl carbons at δ_c 11.9 (C-18), 18.8 (C-21), 19.5 (C-19), 22.6 (C-26), and 22.9 (C-27), eleven sp^3 methylene carbons, six sp^3 methine carbons at δ_c 31.9, 50.2, 56.9, 56.2, 36.2 and 28.1, one oxygenated methine carbon at δ_c 71.9 (C-3), one olefinic methine carbon at δ_c 121.8 (C-6), two sp^3 quaternary carbons at δ_c 36.6 (C-10), 42.4 (C-13) and one olefinic quaternary carbon at δ_c 140.8 (C-5). Compound **4** was confirmed with data from the literature identified as a cholesterol [18].

Compound **5** was obtained as a white solid, with the yield of mass 15.2 mg (3.72%) from B7 fraction (408.5 mg). The result of HRTOF-MS spectra (Fig. S26), obtained molecular weight of **5** $[M+Na]^+$ *m/z* 439.3188, (calculated *m/z* 439.3188), with molecular formula $C_{27}H_{44}O_3$, which required six degrees of unsaturation consisting of 1 double bound and pentacyclic ring system. The FTIR of **5** (Fig. S27) spectrum showed the absorption of the hydroxyl group (3383 cm^{-1}), CH sp^3

(2866 and 1459 cm^{-1}), C-O stretching (1070 cm^{-1}), and C-O-O of peroxide stretching (930 cm^{-1}). Compound **5** have same skeleton with **4**, the main difference was **5** have double bond at C-6 and C-7, addition of one peroxide cyclic at C-5 and C-8. The ^{1}H-NMR spectrum (Fig. S28) of compound **5** showed the presence of two tertiary methyl groups at δ_H 0.78 (s, CH_3-18), and 0.85 (s, CH_3-19), three secondary methyl groups at δ_H 0.84 (d, J = 6.5, CH_3-27), 0.85 (d, J = 6.5, CH_3-26), and 0.88 (d, J = 6.5, CH_3-21), ten methylene protons sp^3, five methine protons sp^3, one oxygenated methine proton at δ_H 3.93 (m, H-3), and two olefinic methine protons at δ_H 6.22 (d, J = 8.5, H-7) and 6.49 (d, J = 8.5, H-6). The ^{13}C-NMR (Fig. S29) and DEPT 135° (Fig. S30) spectra of compound **5** showed 27 carbon signals, presence of five methyl sp^3 carbons at δ_c 12.7 (C-18), 18.2 (C-21),18.6 (C-19), 22.6 (C-26), and 22.8 (C-27), ten methylene sp^3 carbons, five methine sp^3 carbons, one oxygenated methine carbon at δ_c 66.5 (C-3), two olefinic methine carbons at δ_c 130.8 (C-7), 135.5 (C-6), two quaternary sp^3 carbons at δ_c 37.0 (C-10), 44.8 (C-13) and two oxygenated quaternary carbons at δ_c 79.5 (C-8), 82.2 (C-5). Compound **5** was confirmed by data comparison with previously isolated compound, identified as a 5α,8α-epidioxycholest-6-en-3β-ol [19].

Compound **6** was obtained as a white solid, with the yield of mass 6.8 mg (29.44%) from B8.9g-h fraction (23.1 mg). The result of HRTOF-MS spectra (Fig. S31), obtained molecular weight of **6** $[M+H]^+$ m/z 401.3439, (calculated m/z 401.3420), with molecular formula $C_{27}H_{44}O_2$, which required six degrees of unsaturation consisting of 1 double bound, 1 carbonyl, and tetracyclic ring system. The FTIR spectrum of **6** (Fig. S32) showed the absorption of the hydroxyl group (3430 cm^{-1}), CH sp^3 (2853 cm^{-1}), and C=O stretching (1674 cm^{-1}). Compound **6** have same skeleton with **4**, the main difference that **6** was substituted with carbonyl at C-7, the presence of carbonyl confirmed with ^{13}C-NMR spectrum of **6** at δ_c 202.4 ppm. The ^{1}H-NMR (Fig. S33) spectrum of compound **6** showed the presence of two tertiary methyl groups at δ_H 0.67 (s, CH_3-18), and 1.18 (s, CH_3-19), three secondary methyl groups at δ_H 0.84 (d, J = 6.5, CH_3-27), 0.86 (d, J = 6.5, CH_3-26), and 0.90 (d, J = 6.5, CH_3-21), ten methylene sp^3 protons, six methine sp^3 protons, one oxygenated methine proton at δ_H 3.67 (m, H-3), and one olefinic methine at δ_H 5.68 (d, J = 2.0, H-6). The ^{13}C-NMR (Fig. S34) and DEPT 135° (Fig. S35) spectrum of compound **6** showed 27 signals of carbons, presence of five methyl sp^3 carbons at δ_c 12.0 (C-18), 17.9 (C-19), 18.9 (C-21), 22.7 (C-26), and 22.9 (C-27), ten methylene sp^3 carbons, six methine sp^3 carbons, one oxygenated methine carbon at δ_c 70.6 (C-3), one olefinic methine carbon at δ_c 126.2 (C-6), two quaternary sp^3 carbons at δ_c 38.4 (C-10), 43.2 (C-13), one olefinic quaternary carbon at δ_c 165.3 (C-5), and one quaternary carbon of carbonyl at δ_c 202.4 (C-5). Compound **6** was confirmed with data from the literature identified as a 7-keto-cholesterol [20].

Compound **7** was obtained as a white solid, with the yield of mass 4.4 mg (12.57%) from CD4i fraction (35 mg). The result of HRTOF-MS spectra (Fig. S36), obtained molecular weight of **7** $[M+H]^+$ m/z 403.1793, (calculated m/z 403.1793), with molecular formula $C_{27}H_{46}O_2$, which required five degrees of unsaturation consisting of 1 double bound and tetracyclic ring system. The FTIR spectrum of **7** (Fig. S37), showed the absorption of the hydroxyl (3430 cm^{-1}) and CH sp^3 (2850 cm^{-1}) groups. Compound **7** have same skeleton with **4**, the main difference that **7** was substituted with hydroxyl at C-7. The ^{1}H-NMR (Fig. S38) spectrum of compound **7** showed the presence of two tertiary methyl groups at δ_H 0.66 (s, CH_3-18) and 1.00 (s, CH_3-19), three secondary methyl groups at δ_H 0.84 (d, J = 6.5, CH_3-26), 0.86 (d, J = 6.5, CH_3-27) and 0.92 (d, J = 6.5, CH_3-21), ten methylene sp^3 protons, six methine sp^3 protons, two oxygenated methine protons at δ_H 3.57 (m, H-3) and 3.83 (bs, H-7), and one olefinic methine at δ_H 5.58 (d, J = 5.5, H-6). The ^{13}C-NMR (Fig. S39) and DEPT 135° (Fig. S40) spectra (Fig. S33) of compound **7** showed 27 signals of carbons, presence of five methyl sp^3 carbon at δ_c 11.9 (C-18), 18.3 (C-19), 18.8 (C-21), 22.6 (C-27), and 22.9 (C-26), ten methylene sp^3 carbons, six methine sp^3 carbons, two oxygenated methine carbons at δ_c 65.4 (C-7) and 70.6 (C-3), one olefinic methine carbon at δ_c 123.9 (C-6), two quaternary sp^3 carbons at δ_c 37.4 (C-10) and 42.1 (C-13), one olefinic quaternary carbon at δ_c 146.3 (C-5). A comparison with the previous NMR data of **7** with 7β-hydroxy-cholesterol [21], that the structures of

Table 1. ^{13}C NMR data (125 MHz for ^{13}C, in $CDCl_3$) for **1**-**7**

Position	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Carbon	δ_c (mult.)	δ_c (mult.)	δ_c (mult.)	δ_c (mult.)	δ_c (mult.)	δ_c (mult.)	δ_c (mult.)
1	37.4 (t)	37.2 (t)	37.3 (t)	37.3 (t)	34.7 (t)	36.4 (t)	39.5 (t)
2	31.8 (t)	31.6 (t)	31.7 (t)	31.7 (t)	30.1 (t)	31.2 (t)	31.4 (t)
3	71.9 (d)	71.8 (d)	71.9 (d)	71.9 (d)	66.5 (d)	70.6 (d)	71.4 (d)
4	42.4 (t)	42.4 (t)	42.3 (t)	42.4 (t)	37.0 (t)	41.9 (t)	42.0 (t)
5	140.9 (s)	140.9 (s)	140.8 (s)	140.8 (s)	82.2 (s)	165.2 (s)	146.3 (s)
6	121.9 (d)	121.9 (d)	121.8 (d)	121.8 (d)	135.5 (d)	126.2 (d)	123.9 (d)
7	75.0 (d)	32.0 (t)	31.9 (t)	31.9 (t)	130.8 (t)	202.4 (s)	65.4 (s)
8	31.8 (d)	32.0 (d)	31.9 (d)	31.9 (d)	79.5 (s)	45.9 (d)	37.5 (d)
9	50.2 (d)	50.2 (d)	50.1 (d)	50.2 (d)	51.1 (d)	50.0 (d)	49.5 (d)
10	36.6 (s)	36.6 (s)	36.6 (s)	36.6 (s)	37.0 (s)	38.4 (s)	37.4 (s)
11	21.2 (t)	21.1 (t)	21.1 (t)	21.2 (t)	23.4 (t)	21.3 (t)	20.7 (t)
12	39.9 (t)	39.9 (t)	39.5 (t)	39.9 (t)	39.5 (t)	38.8 (t)	36.2 (t)
13	45.9 (s)	45.8 (s)	42.3 (s)	42.4 (s)	44.8 (s)	43.2 (s)	42.3 (s)
14	45.9 (d)	56.9 (d)	56.8 (d)	56.9 (d)	51.7 (d)	50.0 (d)	55.9 (d)
15	24.4 (t)	24.4 (t)	24.3 (t)	24.4 (t)	20.6 (t)	26.4 (t)	24.3 (t)
16	28.4 (t)	28.4 (t)	28.3 (t)	28.3 (t)	28.3 (t)	28.7 (t)	28.3 (t)
17	56.1 (d)	56.1 (d)	56.2 (d)	56.2 (d)	56.5 (d)	54.9 (d)	55.9 (d)
18	12.1 (q)	12.1 (q)	11.9 (q)	11.9 (q)	12.7 (q)	12.0 (q)	11.9 (q)
19	19.5 (q)	19.5 (q)	19.4 (q)	19.5 (q)	18.6 (q)	17.3 (q)	18.3 (q)
20	36.3 (d)	36.3 (d)	36.3 (d)	36.2 (d)	35.3 (d)	35.8 (d)	35.8 (d)
21	18.9 (q)	18.9 (q)	18.8 (q)	18.8 (q)	18.2 (q)	18.9 (q)	18.8 (q)
22	34.0 (t)	34.0 (t)	34.0 (t)	35.9 (t)	36.0 (t)	36.3 (t)	37.0 (t)
23	26.1 (t)	26.1 (t)	23.9 (t)	23.9 (t)	23.8 (t)	23.9 (t)	23.8 (t)
24	42.5 (t)	42.5 (t)	39.4 (t)	39.6 (t)	39.5 (t)	39.6 (t)	39.2 (t)
25	29.2 (d)	29.2 (d)	28.1 (d)	28.1 (d)	28.0 (d)	28.1 (d)	28.1 (d)
26	20.0 (q)	20.0 (q)	19.4 (q)	22.6 (q)	22.6 (q)	22.7 (q)	22.9 (q)
27	19.1 (q)	19.1 (q)	22.6 (q)	22.9 (q)	22.8 (q)	22.9 (q)	22.6 (q)
28	23.2 (t)	23.2 (q)	22.8 (q)	-	-	-	-
29	12.0 (q)	12.0 (q)	-	-	-	-	-

the two compounds were very similar, except for C-7, the α oriented of hydroxyl at C-7 confirmed with the literature, compound **7** was identified as a 7α-hydroxy-cholesterol [22].

The cytotoxic activity of the steroids **1**-**7** was tested against the MCF-7 cancer cell according to a method described [13]. Cisplatin (53 μM) was used as a positive control. Among all steroid compounds, 5α,8α-epidioxycholest-6-en-3β-ol (**5**) showed the highest cytotoxic activity with IC_{50} value of 164.08 μM, followed by campesterol (**3**), β-sitosterol (**2**), 7*β*-hydroxy-sitosterol (**1**), 7α-hydroxy-cholesterol (**7**), cholesterol (**4**) and 7-keto-cholesterol (**6**) with IC_{50} of 256.36, 264.83, 282.33, 439.17, 517.39, and 4246.01 μM, respectively (Table 2). Based on the results, the cytotoxic activity value of the steroids **1**-**7** against MCF-7 breast cancer cells is affected by the skeleton type of steroids and changes in substituents can reduce the IC_{50} value, the resonance of double bond at C-6, C-7, and addition of one peroxide cyclic at C-5 and C-8 of (**5**) significantly increased cytotoxic activity, compared to compound **1**-**4**, **6**-**7** which has double bond at C-5 and C-6 reduced cytotoxic activity, the α and β-oriented of hydroxyl group at C-7 of (**7**) and (**1**) gives significant difference of cytotoxic activity with α-oriented decreases the cytotoxic activity, the presence of carbonyl at C-7 of (**6**) showed the weakest activity.

Table 2. Cytotoxic activity of compounds **1**-**7** against MCF-7 cells

Compounds	IC_{50} (μM)
7β-hydroxy-sitosterol (**1**)	282.33
β-Sitosterol (**2**)	264.83
Campesterol (**3**)	256.36
Cholesterol (**4**)	517.39
5α,8α-epidioxycholest-6-en-3β-ol (**5**)	164.08
7-keto-cholesterol (**6**)	4246.01
7α-hydroxy-cholesterol (**7**)	439.17
Cisplatin*	53.00

*Positive control

CONCLUSION

Seven steroids have been isolated from the *n*-hexane fraction of *Atactodea striata*, two known stigmastane-type steroids, 7β-hydroxy-sitosterol (**1**) and β-sitosterol (**2**), one known campestane-type steroid, campesterol (**3**), and four known cholestane-type steroids, cholesterol (**4**), 5α,8α-epidioxycholest-6-en-3-β-ol (**5**), 7-keto-cholesterol (**6**) and 7α-hydroxy-cholesterol (**7**). All compounds were firstly reported from genus *Atactodea*. The cytotoxic activity of the steroids **1**-**7** was tested against the MCF-7 cancer cell. Compound (**5**) showed the highest cytotoxic activity, followed by campesterol (**3**), β-sitosterol (**2**), 7β-hydroxy-sitosterol (**1**), 7α-hydroxy-cholesterol (**7**), cholesterol (**4**) and 7-keto-cholesterol (**6**). The resonance of double bond at C-6, C-7, and addition of one peroxide cyclic at C-5 and C-8 of (**5**) significantly increased cytotoxic activity, compared to compounds **1**-**4**, **6**-**7** which has double bond at C-5 and C-6 reduced cytotoxic activity, the α and β-oriented of hydroxyl group at C-7 of (**7**) and (**1**) gives significant difference of cytotoxic activity with α-oriented decreases the cytotoxic activity, the presence of carbonyl at C-7 of (**6**) showed the weakest activity. One of these steroids showed weak activity (**5**) and the remaining had no activity. The recommendation for future study is the isolated steroids need further research to determine other bioactivities including other cytotoxic activities, such as antioxidant and anti-inflammation activities.

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