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Catomentosaponin, a new triterpene saponin from the roots of *Catunaregam tomentosa*

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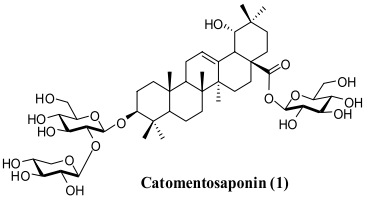
ABSTRACT

A new triterpene saponin, catomentosaponin (**1**) and 11 known analogues (**2**–**12**) were isolated from the roots of *Catunaregam tomentosa*. The structures of **1**–**12** were determined on the basis of extensive NMR and MS data analysis. The sugar residues were identified by co-TLC and HPLC analysis after hydrolysis. All isolated compounds were evaluated for their cytotoxicity against KB and HeLa cell lines. Compound **2** showed moderate cytotoxicity against KB cell with IC_{50} value of 24.84 μM.



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1. Introduction

Catunaregam tomentosa (Blume ex DC.) Tirveng (synonymous with *Randia tomentosa*), also known as 'Khet' in Thai is a tree widely distributed in the north-east region of Thailand. The fruits are used in Thai for washing the hair and clothes, assuredly because of the presence of saponins (Kanchanapoom et al. 2002). In previous study, iridoid glycosides have been reported from the leaves and branches of this plant (Kanchanapoom et al. 2002). The genus *Catunaregam* (Rubiaceae) comprises 12 species distributed over tropical and subtropical regions of Southeast Asia and Africa. This genus has interesting pharmacological activities, such as anti-inflammatory, antispasmodic, antidysenteric, antifertility, immunomodulatory

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and nephrotoxicity properties (Li et al. 2015; Adikay and Sravanthi 2016). Previous phytochemical investigation on this genus revealed the presence of triterpene saponins, iridoid glucosides, coumarin glucosides and lignans (Gao et al. 2011). In this report, we discuss the isolation and structural elucidation of a new triterpene saponin, catomentosaponin (**1**) and eleven known analogues (**2**–**12**) from the methanol extract of the roots of *C. tomentosa*. The cytotoxic activities against KB (human epidermoid carcinoma) and Hela S–3 (human cervix adenocarcinoma) cell lines were evaluated for all isolated compounds.

2. Results and discussion

The methanol extract of the dried roots (10.0 kg) of *C. tomentosa* was subjected to multiple chromatographic steps over silica gel, and Sephadex LH-20. Twelve triterpene saponins were obtained, including a new compound, catomentosaponin (**1**) and 11 known compounds, araliasaponin V (**2**), araliasaponin IV (**3**), araliasaponin VI (**4**); (Miyase et al. 1996), taibaienoside VI (**5**) (Kong and Min 1996), elatoside K (**6**), elatoside A (**7**), elatoside C (**8**); (Yoshikawa et al. 1995), quinovic acid 3-*O*-*α*-L-rhamnoside (**9**) (Asase et al. 2008), gongganoside B (**10**) (Ohashi et al. 1994), cinchonaglycoside C (**11**) (Li et al. 2007) and 3-*O*-*β*-D-glucopyranosyl quinovic acid-28-*β*-D-glucopyranosyl ester (**12**) (Aquino et al. 2001). The structures of **1**–**12** (Figure 1) were identified by physical data analyses, including 1D and 2D NMR, and HRESIMS. The sugar residues were identified by co-TLC and HPLC analyses after hydrolysis.

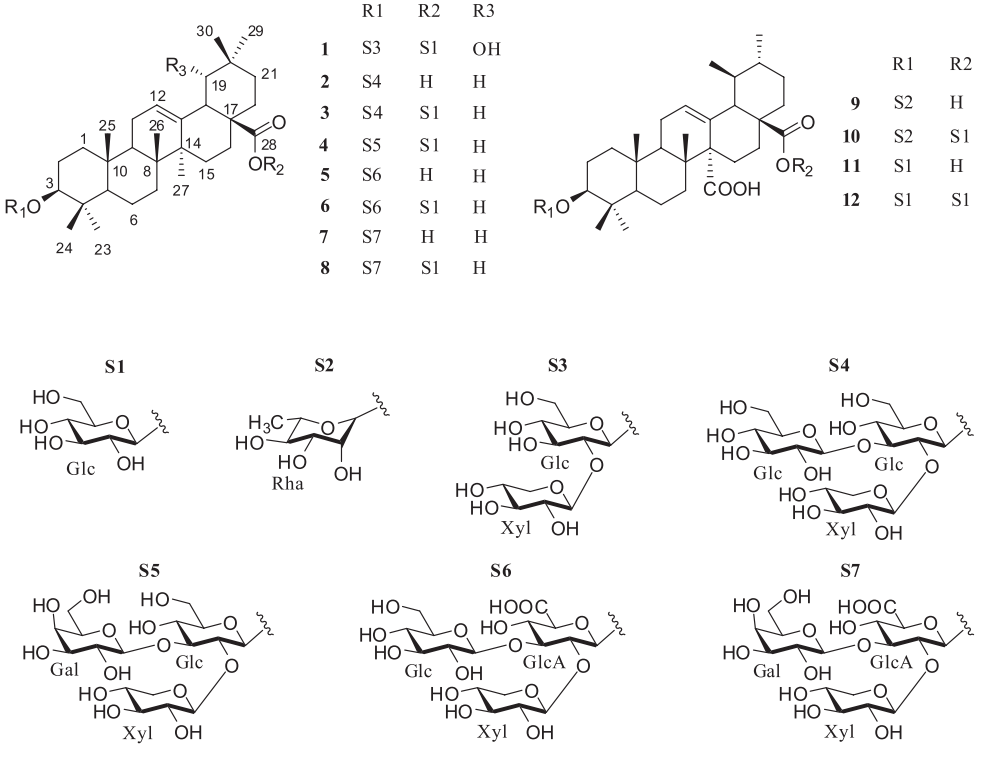

Figure 1. Chemical structures of **1**–**12**.

Catomentosaponin (**1**) was obtained as white amorphous powder with $[\alpha]_D^{20}$ + 117.8 (*c* 0.90, MeOH). Its molecular formula was determined as $C_{47}H_{76}O_{18}$ from the positive HRESIMS $[M + Na]^+$ ion peak at *m/z* 951.4915 (calcd. for $C_{47}H_{76}O_{18}Na$, 951.4929). The IR spectrum showed absorption bands at 3425, 1731 and 1643 cm^{-1}, suggesting the presence of hydroxyl, carbonyl and olefinic groups, respectively. The ^1H and ^{13}C NMR data of **1** exhibited signals for seven methyl groups at δ_H 0.75 (3H, s, H-26), 0.85 (3H, s, H-24), 0.95 (3H, s, H-29), 0.96 (3H, s, H-25), 0.96 (3H, s, H-30), 1.06 (3H, s, H-23) and 1.30 (3H, s, H-27), one olefinic proton at δ_H 5.33 (1H, br s, H-12) with two typical olefinic carbon signals at δ_C 125.0 (C-12) and 144.2 (C-13), indicative of an olean-12-ene skeleton. The spectroscopic data also showed signals for two oxymethines at δ_H 3.16 (1H, dd, *J* = 5.0, 10.7 Hz, H-3) with δ_C 91.0 (C-3) and δ_H 3.28 (1H, d, *J* = 3.3 Hz, H-19) with δ_C 82.4 (C-19), and one carbon of carbonyl group at δ_C 178.6 (C-28). The NOESY correlations (Figure S8) between H-3 and H-23, and between H-19 and H-30 indicated the *α*-orientation of H-3 and *β*-orientation of H-19. Thus, the aglycone was identified as 3*β*,19*α*-dihydroxyolean-12-ene-28-oic acid (siaresinolic acid) (Barton et al. 1952). The presence of three sugar residues was confirmed by the observation of three anomeric protons at δ_H 4.43 (1H, d, *J* = 7.7 Hz, H-1′), δ_H 4.53 (1H, d, *J* = 7.4 Hz, H-1″) and δ_H 5.39 (1H, d, *J* = 8.1 Hz, H-1‴), which were correlated in the HSQC spectrum with anomeric carbons at δ_C 105.2, 106.2 and 95.8, respectively. After acid hydrolysis, the sugar units were confirmed to be D-glucose and D-xylose, which were identified by co-TLC and HPLC analysis. The configuration of glucose and xylose glycosidic bonds were established as *β*-glucose and *β*-xylose by the coupling constants (*J* = 7.4–8.1 Hz) and the NOESY correlations (Figure S8) between the anomeric proton at H-1′ (δ_H 4.43) with the proton at H-23 (δ_H 1.06), between the anomeric proton at H-1″ (δ_H 4.53) with the proton at H-3″ (δ_H 3.36) and between the anomeric proton at H-1‴ (δ_H 5.39) with the proton H-5‴ (δ_H 3.37). The HMBC correlations (Figure S8) between the anomeric proton at H-1′ (δ_H 4.43) with the carbon at C-3 (δ_C 91.0), between the anomeric proton at H-1″ (δ_H 4.53) with the carbon at C-2′ (δ_C 83.2) and C-5″ (δ_C 67.1), between the anomeric proton at H-1‴ (δ_H 5.39) with the carbon at C-28 (δ_C 178.6), and between the oxymethine proton at H-2′ (δ_H 3.44) with the carbons at C-1′ (δ_C 105.2) and C-1″ (δ_C 106.2) confirmed the *β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranosyl chain was linked at C-3 and the *β*-D-glucopyranosyl was substituted at C-28 of aglycone. The comparison of the ^1H and ^{13}C NMR spectroscopic data of **1** showed similar resonances to the known compound, 3-*O*-*β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranosyl-28-*O*-*β*-D-glucopyranosyl-3-*β*-olean-12-en-28-oic acid (Jayasinghe et al. 2002), apart from the lack of a hydroxyl group at C-19. On the basis of the above results, the structure of compound **1** was identified as 3-*O*-[*β*-D-xylopyranosyl-(1→2)-*β*-D-glucopyranosyl]-28-*O*-(*β*-D-glucopyranosyl)-3*β*,19*α*-dihydroxyolean-12-en-28-oic acid.

The *in vitro* cytotoxic activities of all compounds are shown in Table 1, compound **2** showed moderate cytotoxicity against KB cell with IC_{50} value of 24.84 μM, compounds **1**, **5** and **7** showed weak cytotoxicity against KB cell with IC_{50} values in the range of 51.10–55.77 μM, compounds **1**, **2**, **7**, **8** and **9** showed weak cytotoxicity against HeLa S–3 cell with IC_{50} values in the range of 44.99–77.24 μM, and other compounds were inactive.

3. Experimental

3.1. General experimental procedures

For structural elucidation: The IR spectra were measured on a Nicolet 6700 FT-IR spectrometer using KBr discs. Optical rotations were detected by a Jasco P-1010 polarimeter. The NMR

Table 1. *In vitro* cytotoxicity of compounds **1**–**7** against KB and HeLa S-3 cell lines.

	IC_{50} (μM)			IC_{50} (μM)	
Compound	KB	HeLa S-3	Compound	KB	HeLa S-3
1	51.44	58.58	**7**	55.77	77.24
2	24.84	52.57	**8**	> 100	71.39
3	> 100	> 100	**9**	> 100	44.99
4	> 100	> 100	**10**	> 100	> 100
5	51.10	> 100	**11**	> 100	> 100
6	> 100	> 100	**12**	> 100	> 100
Doxorubicin	0.15	0.02			

Notes: $IC_{50} \leq 10$ = good activity, $10 < IC_{50} \leq 30$ = moderate activity, $IC_{50} > 100$ = inactive.

spectra were recorded on a Bruker 400 AVANCE spectrometer (400 MHz for ^{1}H and 100 MHz for ^{13}C). The HRESIMS was obtained using a Bruker MICROTOF model mass spectrometer. HPLC analysis were obtained using a Alltech System equipped with model 626 binary gradient pumps, select TM degasser, 580 autosampler, 2000 ES evaporative light scattering detector (Alltech) and peak simple chromatography data system software. For isolation: Column chromatography was performed by silica gel 60 (0.063–0.200 mm), and Sephadex LH-20 (25–100 μm, GE Healthcare). For bioassay: Microplate reader TECAN infinite M200PRO.

3.2. Plant material

The roots of *C. tomentosa* were collected from Sahatsakhan district, Kalasin province, Thailand, in January 2016. The plant material was identified by Ms. Suttira Khumkratok, a botanist at the Walai Rukhavej Botanical Research Institute, Mahasarakham University and a specimen retained as a reference (Khumkratok No. 6–12).

3.3. Extraction and isolation

The air-dried roots of *C. tomentosa* (10.0 kg) were extracted with MeOH over a period of 3 days at room temperature, respectively (2 × 25 L). Removal of the solvent under reduced pressure provided MeOH (85.5 g) crude extract. The MeOH crude extract was further separated by column chromatography (CC) over silica gel (40 × 10 cm, 2.5 kg) and eluted with a gradient of EtOAc-MeOH (100% EtOAc, 80, 60, 40, 20 and 100% MeOH each 5 L, respectively) to give four fractions (A–D). Fraction A (15 g) was purified by silica gel CC (45 × 5 cm, 0.5 kg) and eluted with 80 and 60% CH_2Cl_2-MeOH (each 10L, respectively) to give two subfraction (A1 and A2). Subfraction A1 (3 g) was separated by Sephadex LH-20 column (50 × 5 cm, 150 g) eluted with 50% CH_2Cl_2-MeOH (2 L) to yield **2** (20 mg) and **10** (15 mg). Subfraction A2 (5 g) was subjected to silica gel CC (40 × 4 cm, 0.4 kg) and eluted with 80% CH_2Cl_2-MeOH (5 L) to provide **9** (12 mg) and **11** (15 mg). Fraction B (10 g) was fractionated by silica gel CC (45 × 5 cm, 0.5 kg) and eluted with 80 and 60% CH_2Cl_2-MeOH (each 10L, respectively) to afford two subfraction (B1 and B2). Compound **5** (10 mg) was purified from subfraction B1(2 g) by Sephadex LH-20 column (50 × 5 cm, 150 g) eluted with 50% CH_2Cl_2-MeOH (2 L). Subfraction B2 (3 g) was separated by Sephadex LH-20 column (50 × 5 cm, 150 g) eluted with 50% CH_2Cl_2-MeOH (2 L) to obtain **1** (15 mg) and **4** (10 mg). Fraction C (5 g) was separated by Sephadex LH-20 column (50 × 3 cm, 150 g) eluted with 50% CH_2Cl_2-MeOH (2 L) to yield **5** (15 mg) and **7** (12 mg). Fraction D (7 g) was chromatographed over silica gel (40 × 4 cm,

0.4 kg) and eluted with 70 and 50% CH_2Cl_2-MeOH (each 10L, respectively) to provide two subfractions (D1 and D2). Compound **4** (13 mg) was purified from subfraction D1 (2 g) by Sephadex LH-20 column (50 × 3 cm, 150 g) eluted with 20% CH_2Cl_2-MeOH (2 L). Finally, Subfraction D2 (5 g) was separated by silica gel CC (40 × 4 cm, 0.4 kg) and eluted with 70% CH_2Cl_2-MeOH (3 L) to yield **6** (11 mg) and **8** (12 mg).

3.3.1. *Catomentosaponin* (1)

White amorphous powder; $[\alpha]_D^{20}$ + 117.8 (*c* 0.90, MeOH); IR (KBr) ν_{max} 3425, 1731, 1643 cm^{-1}; ^1H NMR (400 MHz, in MeOH-d_4) δ_H: 1.00 (1H, m, H-1), 1.61 (1H, m, H-1), 1.72 (1H, m, H-2), 1.93 (1H, m, H-2), 3.16 (1H, dd, *J* = 5.0, 10.7 Hz, H-3), 0.79 (1H, m, H-5), 1.42 (1H, m, H-6), 1.56 (1H, m, H-6), 1.31 (1H, m, H-7), 1.50 (1H, m, H-7), 1.73 (1H, m, H-9), 1.95 (2H, m, H-11), 5.33 (1H, br s, H-12), 1.02 (1H, m, H-15), 1.73 (1H, m, H-15), 2.33 (2H, m, H-16), 3.06 (1H, br s, H-18), 3.28 (1H, d, *J* = 3.3 Hz, H-19), 1.68 (2H, m, H-21), 1.67 (1H, m, H-22), 1.79 (1H, m, H-22), 1.06 (3H, s, H-23), 0.85 (3H, s, H-24), 0.96 (3H, s, H-25), 0.75 (3H, s, H-26), 1.30 (3H, s, H-27), 0.95 (3H, s, H-29), 0.96 (3H, s, H-30), 4.43 (1H, d, *J* = 7.7 Hz, H-1′), 3.44 (1H, m, H-2′), 3.37 (1H, m, H-3′), 3.47 (1H, m, H-4′), 3.56 (1H, m, H-5′), 3.69 (1H, m, H-6′), 3.84 (1H, m, H-6′), 4.53 (1H, d, *J* = 7.4 Hz, H-1″), 3.26 (1H, m, H-2″), 3.37 (1H, m, H-3″), 3.35 (1H, m, H-4″), 3.15 (1H, m, H-5″), 3.82 (1H, m, H-5″), 5.39 (1H, d, *J* = 8.1 Hz, H-1‴), 3.34 (1H, m, H-2‴), 3.44 (1H, m, H-3‴), 3.37 (1H, m, H-4‴), 3.37 (1H, m, H-5‴), 3.69 (1H, m, H-6‴), 3.84 (1H, m, H-6‴); ^{13}C NMR (100 MHz, in MeOH-d_4) δ_C: 39.7 (C-1), 27.1 (C-2), 91.0 (C-3), 40.9 (C-4), 57.2 (C-5), 19.4 (C-6), 33.9 (C-7), 40.3 (C-8), 49.4 (C-9), 38.0 (C-10), 24.8 (C-11), 125.0 (C-12), 144.2 (C-13), 42.6 (C-14), 29.5 (C-15), 28.4 (C-16), 47.1 (C-17), 45.0 (C-18), 82.4 (C-19), 35.9 (C-20), 29.4 (C-21), 33.2 (C-22), 28.2 (C-23), 16.5 (C-24), 15.9 (C-25), 17.8 (C-26), 25.0 (C-27), 178.6 (C-28), 28.6 (C-29), 25.2 (C-30), 105.2 (C-1′), 83.2 (C-2′), 78.2 (C-3′), 71.1 (C-4′), 78.6 (C-5′), 62.4 (C-6′), 106.2 (C-1″), 76.2 (C-2″), 78.2 (C-3″), 71.4 (C-4″), 67.1 (C-5″), 95.8 (C-1‴), 73.9 (C-2‴), 77.7 (C-3‴), 71.1 (C-4‴), 77.4 (C-5‴), 62.7 (C-6‴); positive HRESIMS *m/z* 951.4915 $[M + Na]^+$ (calcd. for $C_{47}H_{76}O_{18}Na$, 951.4929).

3.4. Acidic hydrolysis

A solution of catomentosaponin (**1**) (5 mg) in 2 M HCl (1 mL) was heated at reflux for 24 h. The reaction mixture was neutralised with 2 M NaOH and extracted by partition with EtOAc (5 × 1 mL). The sugar residues were identified by co-TLC (TLC Silica gel 60 F_{254}) by comparison with standard sugar. The solvent system was CH_2Cl_2-MeOH-H_2O (2:1:0.2), and spots were visualised by spraying with EtOH-H_2SO_4-anisaldehyde (9:0.5:0.5, v/v), then heated at 150 °C. The R_f values of D-glucose and D-xylose by TLC was 0.30 and 0.44, respectively. In addition, the sugar were identified by HPLC analysis (column: lichrocart-NH_2 250 × 4.0 mm, carrier: 82% ACN in H_2O 1.5 mL/min, retention time: 8.13 and 9.25 min) in comparison with the authentic D-glucose and D-xylose, respectively. Actually, the natural occurring of glucose and xylose are in the D-isomer.

3.5. Cytotoxicity assay

All isolated compounds (**1**–**12**) were subjected to cytotoxic evaluation against KB (human epidermoid carcinoma) and HeLa S–3 (human cervical carcinoma) cell lines employing the colorimetric method (Skehan et al. 1990), (Kongkathip et al. 2003). Doxorubicin was used as the reference substance which exhibits activity against five cancer cell lines. The

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (Sigma Chemical Co., USA) was dissolved in saline to make a 5 mg/mL stock solution. Cancer cells (3 × 103 cells) suspended in 100 µg/wells of MEM medium containing 10% fetal calf serum (FCS, Gibco BRL, Life Technologies, NY, USA) were seeded onto a 96-well culture plate (Costar, Corning Incorporated, NY 14831, USA). After 24 h pre-incubation at 37 °C in a humidified atmosphere of 5% CO_2/95% air to allow cellular attachment, various concentrations of test solution (10 µL/well) were added and these were then incubated for 48 h under the above conditions. At the end of the incubation, 10 µL of tetrazolium reagent was added into each well followed by further incubation at 37 °C for 4 h. The supernatant was decanted, and DMSO (100 µL/well) was added to allow formosan solubilisation. The optical density (OD) of each well was detected using a Microplate reader at 550 nm and for correction at 595 nm. Each determination represented the average mean of six replicates. The 50% inhibition concentration (IC_{50} value) was determined by curve fitting.

4. Conclusion

The MeOH crude extract from the roots of *C.tomentosa* comprise a new catomentosaponin (**1**) and eleven known triterpene saponins (**2**–**12**). Compound **2** showed moderate cytotoxicity against KB cell with IC_{50} values of 24.84 µM, compounds **1**, **5** and **7** showed weak cytotoxicity against KB cell with IC_{50} values in the range of 51.10–55.77 µM, compounds **1**, **2**, **7**, **8** and **9** showed weak cytotoxicity against HeLa S-3 cell with IC_{50} values in the range of 44.99–77.24 µM, and other compounds were inactive. Therefore, we believe that this plant is an important source for the diverse structure of triterpene saponins and should be further investigated for other biological activities.

Disclosure statement

No potential conflict of interest was reported by the authors.

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