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Secondary metabolites from *Sarcosperma kontumense*

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Abstract. *Sarcosperma, which belongs to the Sapotaceae family,* is composed of 11 species distributed in Asia, five of which are found in Viet Nam. The phytochemical and biological investigations of these plants are limited. Up to now, the species *Sarcosperma kontumense* Gagnep. ex Aubrév. has not been studied yet. The biological activity screening of Vietnamese plants showed the methanol extract of the leaves of this plant possessing strong cytotoxic activity against KB cells. Therefore, this plant was collected in Lam Dong province for its phytochemical study. From the leaves, six ursane-type triterpenes (**1** - **6**) and two flavonoids (**7**, **8**) were isolated. By spectroscopic analyses including ESI-MS, 1D, and 2D NMR spectra and comparison with those reported in the literature, their chemical structures were elucidated as 2*α*,3*α*,19*α*,23-tetrahydroxy-13,27-cyclours-11-en-28-oic acid (**1**), rotundic acid (**2**), euscaphic acid (**3**), ursolic acid (**4**), pomolic acid (**5**), jacoumaric acid (**6**), (+)-catechin (**7**), and (-)-epi-catechin (**8**). Compounds **1**, **2**, **4**, **6**, and **7** were isolated for the first time from the genus *Sarcosperma*.

Keywords: Sarcosperma kontumense, ursane-type triterpenes, flavonoid, cytotoxicity.

Classification numbers: 1.1.1, 1.1.6.

1. INTRODUCTION

The genus *Sarcosperma* (Sapotaceae) includes 11 species distributed in Asia, five of which are found in Viet Nam, namely *S. affinis, S. angustifolium, S. kachinense, S. kontumense*, and *S. laurinum* [1, 2]. The phytochemical and biological investigations of these plants are limited. Up to now, there has been only one publication on the chemical constituents of *S. affinis*, which reported the isolation and identification of some triterpenes, lignans, and polyphenols [3]. However, the species *Sarcosperma kontumense* Gagnep. ex Aubrév. has not been studied yet. In

our project for the biological activity screening of Vietnamese plants, the methanol extract of the leaves of this plant showed cytotoxic activity against KB cells with 32.31 % inhibition at 1 µg/mL. Therefore, a sample of this plant was collected in Lam Dong province for further study. As a result, in a recent paper, we reported on the isolation of two new compounds, sarcokontums A and B, from the stems and leaves of this plant [4]. The isolation and structural elucidation of six ursane-type triterpenes (**1 - 6**) and two flavonoids (**7**, **8**) (Figure 1) from its leaves were described in this paper.

2. MATERIALS AND METHODS

2.1. General experimental procedures

^{1}H-NMR, ^{13}C-NMR, and 2D-NMR spectra were recorded on a Bruker AM600 FT-NMR spectrometer. Optical rotations were recorded on a JASCO P-2000 Polarimeter. ESI-MS spectra were measured on an Agilent 1100 Series LC/MSD Trap SL. Column chromatography (CC) was performed using silica gel 60 (230 - 400 mesh, Merck) or RP-18 resin (30 - 50 µm, Fuji Silysia Chemical Ltd., Aichi, Japan). Percolated silica gel 60 F_{254} (Merck) and RP-18 F254S plates (Merck) were used for thin-layer chromatography (TLC).

2.2. Plant materials

The leaves of *Sarcosperma kontumense* were collected from the Bidoup-Nui Ba National Park, Lam Dong province, Viet Nam, during May 2021 and identified by botanist Nguyen Quoc Dat at the Bidoup-Nui Ba National Park. The voucher specimen (VN-1436) has been deposited at the Institute of Marine Biochemistry (IMBC), VAST.

2.3. Extraction and isolation

The dried and ground leaves of *S. kontumense* (5.0 kg) were ultrasonically extracted with 70 % MeOH (15 L × 4) and the solvent was removed *in vacuo* to yield MeOH crude extract (SKM, 350 g). The SKM extract was suspended in H_2O (1 L) and successively partitioned with *n*-hexane and ethyl acetate. The obtained extracts were concentrated under decreased pressure to yield the corresponding residues, SKH (140 g) and SKE (157 g) The residue SKE was chromatographed on a silica gel column eluting with the gradient solvent system of dichloromethane/methanol (20/1, 10/1, 2/1, 1/1, v/v) to give four fractions, SE1-SE4.

The SE2 fraction (35.0 g) was chromatographed on a silica gel column eluting with dichloromethane/ethyl acetate (10/1, v/v) to yield four sub-fractions (SE2.1–SE2.4). SE2.1 (3.8 g) was chromatographed on a silica gel column eluting with dichloromethane/methanol (20/1, v/v) to give three sub-fractions (SE2.1.1-SE2.1.3). SE2.1.1 (250 mg) was chromatographed on a silica gel RP-18 column eluting with MeOH/H_2O (4/1, v/v), then purified by Sephadex LH-20 CC eluting with MeOH to get compound **4** (30 mg). SE2.1.2 (110 mg) was chromatographed on a silica gel RP-18 column eluting with MeOH/H_2O (4/1, v/v), then purified by Sephadex LH-20 CC eluting with MeOH to give compound **5** (10 mg). SE2.3 (12 g) was chromatographed on a silica gel column eluting with dichloromethane/methanol (20/1, v/v) to give five sub-fractions (SE2.3.1-SE2.3.5). SE2.3.2 (2.5 g) was chromatographed on a silica gel RP-18 column eluting with MeOH/H_2O (3/1, v/v), then purified by Sephadex LH-20 CC eluting with MeOH to give compound **6** (45 mg).

SE3 (37.0 g) was chromatographed on a silica gel RP-18 column eluting with MeOH/H_2O (2/1, v/v) to give three sub-fractions (SE3.1–SE3.5). SE3.1 (11.0 g) was chromatographed on a silica gel column eluting with dichloromethane/acetone (4/1, v/v) to yield four sub-fractions

(SE3.1.1- SE3.1.4). SE3.1.1 (2.0 g) was separated by Sephadex LH-20 CC eluting with MeOH to give compound **1** (7.6 mg). SE3.3 (27 g) was chromatographed on a silica gel RP-18 column eluting with MeOH/H_2O (3/1, v/v) to give four sub-fractions (SE3.3.1–SE3.3.4). SE3.3.1 (150 mg) was purified by Sephadex LH-20 CC eluting with MeOH to give compound **2** (17 mg). SE3.3.4 (210 mg) was purified by Sephadex LH-20 CC eluting with MeOH to yield compound **3** (7.5 mg).

SE4 (15 g) was chromatographed on a silica gel RP-18 column eluting with MeOH/H_2O (1/1) to give four sub-fractions (SE4.1-SE4.4). SE4.4 (3.5 g) was chromatographed on silica gel RP-18 CC eluting with MeOH/H_2O (2/1) to yield four fractions (SE4.4.1-SE4.4.4). SE4.4.3 (170 mg) and SE4.4.2 (150 mg) were purified by Sephadex LH-20 CC eluting with MeOH to give compounds **7** (35 mg) and **8** (41 mg), respectively (Figure 1).

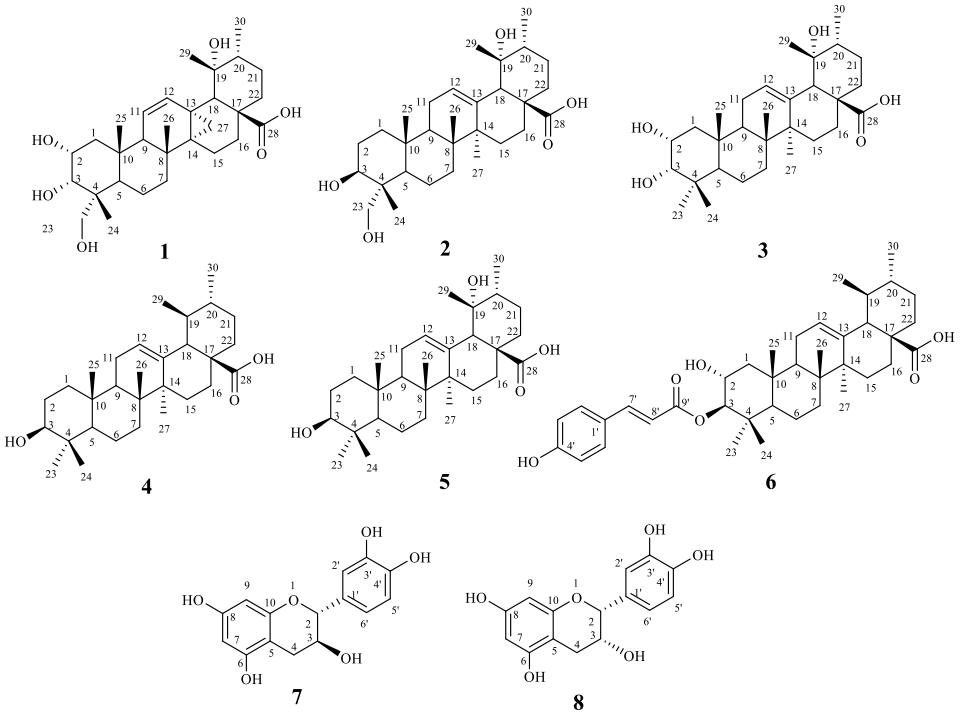

Figure 1. The chemical structures of compounds **1**–**8**.

Compound **1** (2*α*,3*α*,19*α*,23-Tetrahydroxy-13,27-cyclours-11-en-28-oic acid): white powder, ESI-MS: *m/z* 525 $[M+Na]^+$. ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), see Table 1.

Compound **2** (Rotundic acid): white powder. ESI-MS: *m/z* 511 $[M+Na]^+$. ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), see Table 1.

Compound **3** (Euscaphic acid): white powder. ESI-MS: *m/z* 511 $[M+Na]^+$. ^1H NMR (DMSO-d_6, 500 MHz) and ^{13}C NMR (DMSO-d_6, 125 MHz), see Table 1.

Compound **4** (Ursolic acid): white powder. ESI-MS: *m/z* 457 $[M+H]^+$. ^1H NMR (CD_3OD, 600 MHz): δ_H 5.13 (t, *J* = 3.0 Hz, H-12), 3.06 (dd, *J* = 11.4, 4.2 Hz, H-3), 2.11 (br d, *J* = 11.4 Hz, H-18), 1.83 (d, *J* = 9.0, 4.2 Hz, H_2-11), 0.65 (dd, *J* = 12.6, 1.2 Hz, H-5α), 0.88 (3H, s, H_3-23), 0.68 (3H, s, H_3-24), 0.86 (3H, s, H_3-25), 0.76 (3H, s, H_3-26), 1.02 (3H, s, H_3-27), 0.79 (3H,

d, J = 6.6 H, H_3-29), 0.87 (3H, d, J = 6.6 Hz, H_3-30); and ^{13}C NMR (CD_3OD, 150 MHz), see Table 2.

Compound **5** (Pomolic acid): white powder. ESI-MS: *m/z* 473 $[M+H]^+$. ^{1}H NMR (CD_3OD + $CDCl_3$, 600 MHz): δ_H 5.35 (t, J = 3.6 Hz, H-12), 3.20 (dd, J = 10.8, 4.8 Hz, H-3), 2.56 (s, H-18β), 1.28 (3H, s, H_3-29), 1.22 (3H, s, H_3-27), 0.98 (3H, s, H_3-25), 0.95 (3H, d, J = 6.6 Hz, H_3-30), 0.92 (3H, s, H_3-26), 0.78 (3H, s, H_3-23), 0.77 (3H, s, H_3-24), 0.75 (1H, dd, J = 11.5, 1.8 Hz, H-5); and ^{13}C NMR (CD_3OD + $CDCl_3$, 150 MHz), see Table 2.

Compound **6** (Jacoumaric acid), ESI-MS: *m/z* 641 $[M+Na]^+$. ^{1}H NMR ($CDCl_3$, 600 MHz): δ_H 7.65 (1H, d, J = 16.0 Hz, H-7'), 7.41 (2H, dd, J = 8.4, 1.8 Hz, H-2' and H-6'), 6.83 (2H, dd, J = 8.4, 1.8 Hz, H-3'and H-5'), 6.33 (1H, d, J = 16.0 Hz, H-8'), 5.25 (1H, br s, H-12), 4.61 (1H, dd, J = 10.2, 1.8 Hz, H-3), 3.86 (1H, ddd, J = 13.2, 10.2, 3.0 Hz, H-2), 2.20 (1H, d, J = 10.8 Hz, H-18), 0.92 (3H, s, H_3-23), 0.94 (3H, s, H_3-24), 1.03 (3H, s, H_3-25), 0.82 (3H, s, H_3-26), 1.11 (3H, s, H_3-27), 0.87 (3H, d, J = 6.0 Hz, H_3-29), 0.96 (3H, d, 6.0 Hz, H_3-30). ^{13}C NMR ($CDCl_3$, 150 MHz), see Table 2.

Compound **7** [(+)-Catechin]: yellow powder. $[\alpha]_D^{25}$ +17 (*c* 0.1, MeOH). ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), see Table 3.

Compound **8** [(−)-*epi*-Catechin]: yellow powder. $[\alpha]_D^{25}$-39 (*c* 0.1, MeOH). ^{1}H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), see Table 3.

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white powder. The NMR spectra of **1** (Table 1) showed signals of an ursane-type triterpene [5]. In the ^{1}H NMR spectrum of **1**, two vinylic methines (CH=CH) at δ_H 5.15 (dd, J = 10.2, 2.4 Hz, H-11) and 5.88 (dd, J = 10.2, 3.0 Hz, H-12), two oxymethines at δ_H 3.93 (ddd, J = 12.0, 4.2, 2.4 Hz, H-2β) and 3.63 (d, J = 2.4 Hz, H-3), one methine at δ_H 2.34 (s, H-18), and one oxymethylene at δ_H 3.54 and 3.41 (2H, each d, J = 10.8 Hz, H_2-23) were observed. In addition, five methyl groups, including four singlet methyls at δ_H 0.79 (s, H_3-24), 0.98 (s, H_3-25), 0.82 (s, H_3-26), 1.38 (s, H_3-29), and one doublet methyl at δ_H 0.92 (d, J = 6.6 Hz, H_3-30) were shown. The ^{13}C NMR, HSQC, HMBC, and DEPT spectra of **1** indicated the signals of 30 carbons: one carbonyl at δ_C182.5 (C-28), which was identified by HMBC correlation (Figure 2) from H-18 (δ_H 2.34) to C-28; one oxygenated tertiary carbon at δ_C 76.6 (C-19) and six quaternary carbons; eight methines, including two vinylic methines at δ_C 119.5 (C-11) and 141.9 (C-12); and two oxymethines at δ_C 67.1 (C-2) and 78.8 (C-3); nine methylenes, including one oxymethylene at δ_C 71.3 (C-23); and five methyls. The spin-spin correlations in the COSY spectrum of **1** (Figure 2*)* between H-1/H-2, H-2/H-3, and H-11/H-12, together with the HMBC correlations between H-3 and C-2/C-4/C-24, H_2-23 and C-3/C-5/C-24, H-11 and C-13/C-10/C-8, and H-12 and C-9/C-18/C-14/C-13 (Figure 2) confirmed the positions of 2-OH, 3-OH, 23-OH, and the double bond C_{11}=C_{12}. Further, the singlet signals of CH_3-29 and H-18, and the downfield chemical shifts of C-19 (δ_C 76.6), together with the HMBC correlations (Figure 2) between H-18 and C-19/C-28/C-13/C-29, between CH_3-29 (δ_H 1.38) and C-19/C-18/C-20, as well as the NOESY correlation of H-18/H-29 (Figure 3) revealed the last hydroxy group as 19α-OH. The small coupling constant of H-3 with H-2 (d, J = 2.4 Hz) indicated its equatorial orientation, whereas the large coupling constants of H-2 with H-1 (J = 12.0 Hz) exhibited that H-2 was in an axial orientation. Additionally, the NOESY correlations of H-3 with H-2/H-24, H-2 with H-25/H-24/H-3 revealed that H-2, H-3, H-24, and H-25 were placed on the β-face, and 2-OH, 3-OH, and H_2-23 groups were placed on the α-face (Figure 3). Comparing to

the ursan-12-ene compounds, the 27-methyl group was replaced by a cyclopropane methylene group (δ_C 16.5) and the double bond was moved to C-11/C-12 [5, 6]. Two vinylic methines at δ_H 5.15 (H-11)/δ_C 119.5 (C-11) and δ_H 5.88 (H-12)/δ_C 141.9 (C-12) and two quaternary carbons resonanced at δ_C 28.4 (C-13) and δ_C 33.8 (C-14), together with the HMBC correlations between H-18 (δ_H 2.34)/H-9 (δ_H 1.83)/H-15 (δ_H 1.49) and C-27 (δ_C 16.5) indicated a 13,27-cyclopropane ring and the C-11/C-12 double bond [5, 6]. Thus, the structure of **1** differs from the structure of sarcokontum A [4] only by the replacement of the 3β-OH group in sarcokontum A with 3*α*-OH in **1**. The ESI-MS spectrum of **1** exhibited an ion peak at *m/z* 525 $[M+ Na]^+$, corresponding to the molecular formula of $C_{30}H_{46}O_6$. From the above evidence, **1** was determined as 2*α*,3*α*,19*α*,23-tetrahydroxy-13,27-cyclours-11-en-28-oic acid [6]. Triterpenes of 13,27-cycloursane-type are very rare in nature [6, 7]. Thus, the structure of **1** differs from the structure of sarcokontum A [4] only by the replacement of the 3β-OH group in sarcokontum A with 3*α*-OH in **1**. The ESI-MS spectrum of **1** exhibited an ion peak at *m/z* 525 $[M+ Na]^+$, corresponding to the molecular formula of $C_{30}H_{46}O_6$. From the above evidence, **1** was determined as 2*α*,3*α*,19*α*,23-tetrahydroxy-13,27-cyclours-11-en-28-oic acid [6]. Triterpenes of 13,27-cycloursane-type are very rare in nature [6, 7].

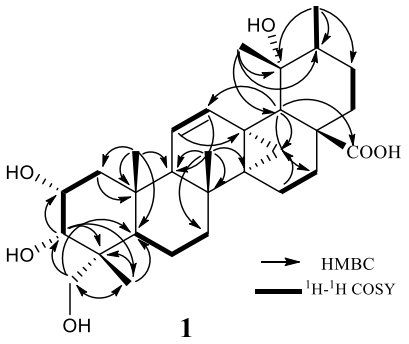

Figure 2. Key ^{1}H-^{1}H-COSY and HMBC correlations of **1**.

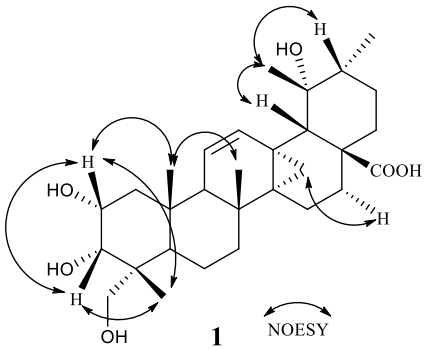

Figure 3. Key NOESY correlations of **1**.

Compound **2** was obtained as a white powder. In the ^{1}H and ^{13}C NMR spectra of **2**, one carbonyl at δ_C 183.3 (C-28), one oxygenated tertiary carbon at δ_C 73.7 (C-19), one olefinic methine at δ_H 5.30 (t, J = 3.6 Hz, H-12)/δ_C 129.3 (C-12), one oxymethine at δ_H 3.64 (dd, J = 12.0, 4.8 Hz, Hax-3)/δ_C 74.2 (C-3), one oxymethylene at δ_H 3.56 and 3.33 (each d, J = 10.8, H_2-23)/δ_C 67.7 (C-23) were observed. In addition, six methyls including five singlet methyls at δ_H 0.74 (s, H_3-24), 1.00 (s, H_3-25), 0.84 (s, H_3-26), 1.37 (s, H_3-27), 1.29 (s, H_3-29) and one doublet methyl at δ_H 0.97 (d, J = 6.6 Hz, H_3-30) were recognized. These suggested that **2** was a trihydroxyurs-12-en-28-oic acid [5]. The large coupling constant of H-3 (J = 12.0 Hz) indicated that H-3 was *α*/axial oriented. The downfield chemical shifts of C-3, C-23, C-12, C-13, C-19 and the HMBC correlations between H-12 (δ_H 5.30) and C-13/C-18/C-9/C-14/C-11, H-3 (δ_H 3.64) and C-23/C-4/C-2/C-24, H_2-23 (δ_H 3.56 and 3.33) and C-3/C-5/C-4/C-24, H-18 (δ_H 2.55) and C-28/C-13/C-12/C-19/C-17/C-20/C-16 indicated the locations of the three hydroxyl groups at C-3, C-23, and C-19, and the C-12/C-13 double bond [8]. The ESI-MS spectrum of **2** gave the ion peak at *m/z* 511 $[M+Na]^+$, corresponding to the molecular formula of $C_{30}H_{48}O_5$. Thus, **2** was determined as rotundic acid (3*β*,19*α*,23-trihydroxyurs-12-en-28-oic acid) [8].

Compound **3** was obtained as a white powder. The NMR spectra of **3** showed similar signals to those of **2**, except for the addition of a hydroxyl group at C-2 (Table 1) and the 23-oxymethylene being replaced by a methyl group. The small coupling constants of H-3 (d, J = 1.2 Hz) indicated that H-3 was in *β*/equatorial orientation. The carbon chemical shifts of **3** were

compared to those of euscaphic acid and found to match (Table 1). In addition, the ESI-MS spectrum of **3** gave the ion peak at *m/z* 511 $[M + Na]^+$, corresponding to the molecular formula of $C_{30}H_{48}O_5$. Therefore, **3** was identified as euscaphic acid (2*α*,3*α*,19*α*-trihydroxyurs-12-en-28-oic acid) [5].

Table 1. ^{13}C NMR spectral data of compounds **1**-**3** and the references.

C	1			2			3		
	δ_C [*d] [6]	δ_C [a,]	δ_H [a,c] (*J* in Hz)	δ_C [*e] [8]	δ_C [a,]	δ_H [a,c] (*J* in Hz)	δ_C [**d] [5]	δ_C [a,b]	δ_H [a,c] (*J* in Hz)
1	43.4	42.4	1.23 (dd, 12.0, 2.0) 1.75 (dd, 12.0, 4.2)	38.9	39.1	1.01 (m) 1.64 (m)	42.6	41.4	1.15 (m) 1.39 (m)
2	66.0	67.1	3.93 (ddd, 12.0, 4.2, 2.4)	21.1	27.4	1.60 (m) 1.70 (m)	66.2	64.7	3.77 (m)
3	79.9	78.8	3.63 (d, 2.4)	73.7	74.2	3.64 (dd, 12.0, 4.8)	79.3	77.9	3.15 (d, 1.2)
4	42.4	42.1	-	42.9	43.2	-	38.9	38.0	-
5	44.2	44.4	1.58 (m)	48.8	48.7	1.18 (m)	48.8	47.6	1.15 (m)
6	18.9	19.0	1.48 (m) 1.50 (m)	18.9	19.2	1.42 (m) 1.45 (m)	18.8	17.7	1.28 (m) 1.35 (m)
7	37.7	38.2	1.61 (m) 1.74 (m)	33.4	33.8	1.30 (m) 1.65 (m)	33.7	32.6	1.21 (m) 1.43 (m)
8	34.9	34.9	-	40.4	41.0	-	40.8	39.5	-
9	53.8	53.9	1.83 (d, 2.4)	47.9	48.0	1.75 (m)	47.8	46.5	1.66 (m)
10	38.2	38.0	-	37.2	37.9	-	38.8	37.8	-
11	119.0	119.9	5.15 (dd, 10.2, 2.4)	24.2	24.7	1.98 (m) 2.02 (m)	24.3	23.1	1.90 (m)
12	142.2	141.9	5.88 (dd, 10.2, 3.0)	128.2	129.2	5.30 (t, 3.6)	128.7	126.7	5.17 (br s)
13	28.2	28.4	-	140.2	140.2	-	139.6	138.6	-
14	33.0	33.8	-	42.2	42.7	-	42.3	41.6	-
15	22.8	22.7	1.49 (m) 2.09 (m)	29.4	29.7	1.00 (m) 1.80 (m)	29.1	28.0	0.88 (m) 1.68 (m)
16	26.9	26.7	1.52 (m) 2.14 (m)	26.9	26.7	1.52 (m) 2.56 (m)	26.3	25.2	1.39 (m) 2.50 (m)
17	47.9	48.1	-	48.2	49.0	-	48.3	46.9	-
18	47.8	47.8	2.34 (s)	54.7	55.2	2.55 (s)	54.4	53.2	2.37 (br s)
19	75.9	76.0	-	72.7	73.7	-	73.2	71.6	-
20	42.9	43.2	1.35 (m)	42.4	43.1	1.37 (m)	42.5	41.2	1.25 (m)
21	27.4	27.2	1.25 (m) 1.56 (m)	27.0	27.4	1.25 (m) 1.73 (m)	27.0	25.9	0.89 (m) 1.68 (m)
22	38.0	37.8	1.28 (m) 1.80 (m)	38.9	39.1	1.63 (m) 1.74 (m)	38.4	37.3	1.50 (m) 1.59 (m)
23	71.7	71.2	3.54 (d, 10.8) 3.41 (d, 10.8)	68.2	67.7	3.56 (d, 10.8) 3.33 (d, 10.8)	29.5	28.9	0.88 (s)
24	17.0	17.0	0.79 (s)	13.2	12.7	0.74 (s)	22.3	21.8	0.78 (s)
25	19.4	19.0	0.98 (s)	16.8	16.2	1.00 (s)	16.7	16.1	0.88 (s)
26	16.9	16.8	0.82 (s)	16.8	17.7	0.84 (s)	17.4	16.6	0.68 (s)
27	16.9	16.9	NA	24.9	24.9	1.37 (s)	24.7	24.1	1.29 (s)
28	181.2	[#]182.9	-	180.7	183.2	-	179.4	179.0	-
29	27.4	27.1	1.38 (s)	27.2	27.2	1.29 (s)	27.2	26.4	1.08 (s)
30	16.2	15.7	0.92 (d, 6.6)	16.0	16.0	0.97 (d 6.6)	16.6	16.3	0.84 (d, 7.8)

[a] recorded in CD_3OD, [b]150 MHz, [c]600 MHz; [#]signal identified by HMBC spectrum
**recorded in pyridine-d_5, [d] 125 MHz, [e]90 MHz; **recorded in DMSO-d_6; NA: Not appeared*

Compound **4** was isolated as a white powder. The NMR spectra of **4** (Table 2) indicated similar signals to those of **2**, except for the absence of the 19-OH group and the 23-oxymethylene was replaced by a methyl group. The NMR assignments of **4** based on the analysis of ^{13}C-NMR, DEPT, HSQC and HMBC spectra in comparison with literature [9] exhibiting signals of a hydroxyurs-12-en-28-oic acid with 30 carbons. In the NMR spectra, one oxymethine at δ_H 3.06 (1H, dd, J = 11.5, 4.2 Hz, Hax-3)/δ_C 79.7 (C-3), one olefinic methine at δ_H 5.13 (1H, t, J = 1.5 Hz, H-12)/δ_C 126.9 (C-12), two doublet methyls at δ_H 0.79 (3H, d, J = 6.6 H, H_3-29), and 0.87 (3H, d, J = 6.6 Hz, H_3-30) were determined. Furthermore, the large proton coupling constant of H-3 (J = 11.4 Hz) confirmed its α/axial position, and the HMBC correlation of H-18 (δ_H 2.34) to C-28 indicated a carbonyl group at δ_C 182.5 (C-28). The ESI-MS spectrum of compound **4** gave the ion peak at *m/z* 457 $[M+H]^+$, corresponding to the molecular formula of $C_{30}H_{48}O_3$. Hence, **4** was deduced as ursolic acid (3β-hydroxyurs-12-en-28-oic acid) [9].

Table 2. ^{13}C-NMR spectral data of compounds **4**, **5**, **6** and the references.

C	**4**		**5**		**6**				
	δ_C *b [9]	δ_C a,b	δ_C *c [5]	δ_C a,b	δ_C *d [10]	δ_C a,b	**C**	δ_C *d [10]	δ_C a,b
1	39.2	40.0	39.4	38.7	48.6	47.7	1'	126.2	126.0
2	28.2	27.9	28.1	27.2	66.4	67.2	2', 6'	130.6	130.0
3	78.2	79.7	78.2	79.1	85.0	84.7	3', 5'	116.8	115.8
4	39.6	39.8	39.4	38.9	39.5	39.5	4'	161.6	159.3
5	55.9	56.8	55.9	53.5	55.6	55.0	7'	144.8	145.4
6	18.8	19.5	18.9	18.6	18.9	18.3	8'	116.1	114.5
7	33.7	34.4	33.6	33.1	33.3	32.8	9'	167.9	168.9
8	40.1	40.8	40.3	40.1	39.8	39.0			
9	48.1	49.0	47.8	47.4	48.0	47.4			
10	37.5	38.2	37.3	37.2	38.3	38.0			
11	23.7	24.4	24.0	23.9	23.7	23.3			
12	125.7	126.9	128.0	129.2	125.4	125.1			
13	139.3	139.7	140.0	138.4	139.4	138.3			
14	42.6	43.3	42.1	41.4	40.0	39.5			
15	28.8	29.3	29.3	28.4	28.7	27.9			
16	25.0	25.4	26.4	25.7	24.9	24.1			
17	48.1	48.9	48.4	47.8	42.6	42.1			
18	53.6	54.4	54.6	55.4	53.5	52.7			
19	39.5	40.5	72.7	73.3	39.5	39.0			
20	39.4	40.4	42.4	41.4	39.4	38.8			
21	31.1	31.8	26.9	26.2	31.1	30.6			
22	37.4	38.1	37.3	37.8	37.5	36.7			
23	28.8	28.8	28.8	28.2	29.0	28.5			
24	16.5	16.4	16.8	16.2	18.3	17.7			
25	15.7	16.0	16.5	15.4	17.4	16.5			
26	17.5	17.9	17.4	16.7	17.5	16.8			
27	24.0	24.1	24.7	24.5	24.0	23.5			
28	179.7	#181.5	180.8	181.1	179.9	180.7			
29	17.5	17.7	27.1	27.0	21.4	16.9			
30	21.4	21.6	16.5	15.7	16.9	21.1			

[a] recorded in CD_3OD, [b]150 MHz; [#]signal identified by HMBC spectrum
**recorded in pyridine-d_5, [c] 100 MHz, [c] 75.4 MHz.*

Compound **5** was isolated as a white powder. The ^{1}H and ^{13}C NMR data of **5** (Table 2) were similar to those of **4**, except for the addition of a hydroxyl group at C-19. The signals of an urs-12-ene-28-oic acid were indicated, including one carbonyl, one oxymethine, one olefinic methine, and seven methyls. The large coupling constant of H-3 (J = 10.8) suggested its α/axial position. Furthermore, the singlet proton signals of CH_3-29 and H-18, together with the downfield carbon chemical shift of C-19 at δ_C 73.3, confirmed the group 19α-OH. The ESI-MS spectrum of **5** gave the ion peak at *m/z* 473 $[M+H]^+$, corresponding to the molecular formula of $C_{30}H_{48}O_4$. Accordingly, **5** was determined to be pomolic acid (3β,19α-dihydroxyurs-12-en-28-oic acid) [5].

Compound **6** was isolated as a white powder. The ^{1}H and ^{13}C NMR spectra of **6** gave signals similar to those of **4**, except for the addition of a hydroxyl group at C-2 and a *trans-p*-coumaroyloxy unit (Table 2). The signals of an urs-12-ene skeleton were revealed with one carbonyl; seven methines, including two oxymethines, one olefinic methine, and four methines; eight methylenes; seven methyls, including five singlet methyls and two doublet methyls; one oxygenated tertiary carbon; and six quarnatery carbons; The signals of the coumaroyloxy unit were also indicated at δ_H 7.65 (1H, d, 16.0 Hz, H-7')/δ_C 145.4 (C-8'), δ_H 6.33 (1H, d, 16.0, H-8')/δ_C 114.5 (C-8'), δ_H 7.41 (2H, dd, 8.4, 1.8, H-2', H-6')/δ_C 130.0 (C-2', 6'), δ_H 6.83 (2H, dd, 8.4, 1.8, H-3', H-5')/δ_C 115.8 (C-3', 5'), δ_C 126 (C-1'), 159.3 (C-4') and 168.9 (C-9'). The coupling constants of H-7' and H-8' (J = 16.0 Hz) suggested a *trans*-configuration of C-7'/C-8' double bond in the coumaroyloxy unit. The downfield chemical shift of H-2 at δ_H 3.86 (ddd, 13.2, 10.2, 3.0)/C-2 (δ_C 67.2), H-3 at δ_H 4.61 (dd, 10.2, 1.8 Hz)/C-3 (δ_C 84.7), along with the HMBC correlations between H-2 and C-3, H-3 and C-2 (δ_C 67.2)/C-1(δ_C 47.7)/C-4 (δ_C 39.5)/C-23 (δ_C 28.5)/C-24 (δ_C 17.7)/C-9' (δ_C 168.9) confirmed the positions of the hydroxyl group at C-2 and the *trans-p*-coumaroyloxy unit at C-3 [10]. The ESI-MS spectrum of **6** gave the ion peak at *m/z* 641 $[M+Na]^+$, corresponding to the molecular formula of $C_{39}H_{54}O_6$. Consequently, **6** was established as jacoumaric acid [10].

Table 3. The NMR spectral data for compounds **7**, **8** and the references.

C.	**7**			**8**		
	δ_C [12]	δ_C [a,b]	δ_H [a,c] (mult., J in Hz)	δ_C [12]	δ_C [a,b]	δ_H [a,c] (mult., J in Hz)
2	2.88	82.9	4.59 d (7.2)	79.5	79.9	4.81 (s)
3	68.4	68.8	3.99 m	67.0	67.5	4.17 (br s)
4	28.8	28.5	2.53 dd (16.2, 8.4)	29.0	29.2	2.86 (dd, 16.8, 4.8) Heq
			2.87 dd (16.2, 5.4)			2.73 (dd, 16.8, 2.4) Hax
5	157.1	157.6	-	157.6	157.4	-
6	96.2	96.3	5.88 d (2.4)	96.2	96.4	5.94 (d, 2.4)
7	157.6	157.8	-	157.6	158.0	-
8	95.5	95.5	5.95 d (2.4)	95.7	95.9	5.92 (d, 2.4)
9	156.9	156.9	-	157.2	157.7	-
10	100.7	100.8	-	100.0	100.1	-
1′	132.3	132.2	-	132.3	132.3	-
2′	115.3	115.3	6.86 d (1.8)	115.3	115.3	6.97 (d, 1.8)
3′	145.6	146.2	-	145.4	146.0	-
4′	145.7	146.3	-	145.3	145.8	-
5′	115.7	116.1	6.78 d (8.4)	115.5	115.9	6.75 (d, 8.4)
6′	120.1	120.0	6.74 dd (8.4, 1.8)	119.4	119.4	6.80 (dd, 8.4, 1.8)

[a]recorded in CD_3OD, [b]150 MHz, [c]600 MHz, [#]δ_C of (−)-catechin and

[##]δ_C of (−)-epi-catechin (in acetone-d_6 at 125 MHz).

Compounds **7** and **8** were isolated as yellow powders. Using TLC on a silica gel plate, they were identified by comparison with the authentic samples of (−)-catechin and (−)-*epi*-catechin, which were previously isolated from the flowers of *Amesiodendron chinense* (Sapindaceae) in our laboratory [11]. Their ^{1}H, ^{13}C NMR spectra (Table 3) indicated signals of a flavanol skeleton, including five aromatic methines, five oxygenated aromatic carbons, two non-protonated aromatic carbons of the A and B rings, and two oxymethines and one methylene of the C ring. The large proton coupling constant of H-2 at δ_H 4.59 (J = 7.2 Hz), together with the resonance of C-2 at δ_C 82.9, suggested the *trans*-2,3 stereochemistry of **7**. Furthermore, the optical rotation of **7** with $[\alpha]_D^{25}$ = +17 (*c* 0.1, MeOH) confirmed **7** as (+)-catechin [11, 12]. On the contrary, the small proton coupling constant of H-2 at δ_H 4.81 (s), along with the resonance of C-2 at δ_C 79.9, suggested the *cis*-2,3 stereochemistry of **8**. In addition, the optical rotation of **8** with $[\alpha]_D^{25}$ = −39 (*c* 0.1, MeOH) determined **8** as (−)-*epi*-catechin [11, 12].

The ursane-type triterpenes (**1**-**6**) were evaluated for their cytotoxic activity towards three cancer cell lines, human carcinoma (KB), human hepatocellular carcinoma (Hep-G2), and human lung carcinoma (LU-1) using the method of Alley [13]. All the compounds (**1**-**6**) (except compound 4, its cytotoxic results were reported in ref. 4) showed no cytotoxic activity with the IC_{50} values over 100 µM compared to the positive control compound, ellipticine (IC_{50} ~ 0.33 - 0.39 µM).

4. CONCLUSION

From the leaves of *S. kontumense*, eight known compounds were isolated, including the six ursane-type triterpenes 2*α*,3*α*,19*α*,23-tetrahydroxy-13,27-cyclours-11-en-28-oic acid (**1**), rotundic acid (**2**), euscaphic acid (**3**), ursolic acid (**4**), pomolic acid (**5**), and jacoumaric acid (**6**); and two flavonoids, (+)-catechin (**7**) and (-)-*epi*-catechin (**8**). Their structures were elucidated by spectroscopic analysis, including ESI-MS, 1D-, 2D-NMR spectra, and comparison with the previously reported data. To the best of our knowledge, compounds **1**, **2**, **4**, **6**, and **7** were isolated from the genus *Sarcosperma* for the first time in our recent studies. In addition, the ursane-type triterpene **1** with the 13,17-cyclo ring is very rare in nature.

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