Fitoterapia 140 (2020) 104441

Contents lists available at ScienceDirect

Fitoterapia

journal homepage: www.elsevier.com/locate/fitote

Triterpenoids from stems of *Kadsura heteroclita*

Liang Cao[a,b,1], Bin Li[a,1], Nuzhat Shehla[a,c], Li-min Gong[a], Yu-Qing Jian[a], Cai-Yun Peng[a,*], Wen-Bing Sheng[a], Le-Ping Liu[a], Xiong Cai[a], Rong-Yong Man[d], Duan-Fang Liao[a], Xiao-Qi Zhu[b], M. Iqbal Choudhary[c], Atta-ur Rahman[c], Wei Wang[a,*]

[a] TCM and Ethnomedicine Innovation & Development International Laboratory, Academician Atta-ur-Rahman Belt and Road Traditional Medicine Research Center, Innovative Materia Medica Research Institute, School of Pharmacy, Hunan University of Chinese Medicine, Changsha 410208, People's Republic of China
[b] Institute of Agriculture Environment and Agroecology, Hunan Academy of Agriculture Sciences, Changsha 410125, People's Republic of China
[c] H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi 75270, Pakistan
[d] Clinic Experimental Research Center, The First People's Hospital of Huaihua, Huihua 418000, People's Republic of China.

ARTICLE INFO

Keywords:
Kadsura heteroclita
Schisandraceae
Xuetong
Tujia ethnomedicine
Triterpenoids

ABSTRACT

In the present work, we reported the triterpenoids isolated from *n*-butanol fraction of *Kadsura heteroclita* which is a Tujia ethnomedicine with trivial name "Xuetong". This effort resulted in the isolation of six unpresented triterpenoids xuetongsu A-F (**1**–**6**), along with five known triterpenoids (**7**–**11**). The structures of the reported compounds were established on the 1D, and 2D NMR and HRESIMS spectra, along with CD spectroscopic analysis. Moreover, the absolute stereochemistry of compound **7** was determined by X-ray diffraction analysis. Antioxidant and cytotoxic activities were evaluated for all isolated compounds, compound **7** shown weak cytotoxic activity against HL-60 with IC_{50} value of 50.0 μM.

1. Introduction

Stems and roots of the plants from genus *Kadsura* (Schisandraceae) were commonly applied in traditional Chinese medicines (TCMs), especially in ethnomedicine for the treatment of traumatic injury, arthralgia with wind-dampness, fracture, irregular menstruation, and wound bleeding [1].

The stem of *Kadsura heteroclita* (Roxb.) Craib is a kind of Tujia ethnomedicine called "Xuetong" in local trivial name, traditionally used to treat rheumatism arthritis, gastric and duodenal ulcers, gastroenteritis, chronic hepatopathy, dysmenorrhea, postpartum abdominal pain and traumatic injury by the "Tujia" people lived in the Wulin mountain area of China [2]. As an important local herb medicine, the stem of *K. heteroclita* is critical in protecting people from the humiliated living atmosphere. The current pharmacology work revealed that the plant has good bioactivity in treating rheumatism arthritis [3,4].

Previous chemical investigations on this species collected from Shimen County of Hunan Province, People's Republic of China, resulted in the isolation of triterpenoids [5–7], sesquiterpenes [8,9], and lignans [10]. In the purpose of a systematic research for chemically novel and biologically potential active compounds from *K. heteroclita*, an *n*-BuOH fraction of 95% ethanol extract of the stems of *K. heteroclita* was investigated, mimic the traditional usage of decoction to explore the more polarity constituents in the plant. The present work resulted in the isolation of six undescribed triterpenoids, namely xuetongsu A-F (**1**–**6**), together with five known triterpenoids (Fig. 1). All the isolated compounds were screened for their cytotoxicity and antioxidant activities.

2. Experimental

2.1. General experimental procedures

Optical rotations were measured on a Perking-Elmer 341-MC digital polarimeter. UV spectra were recorded on a TU-1900 spectrophotometer. Experimental CD spectra were recorded on a JASCO J-815 Circular Dichroism (CD) Spectropolarimeter. A Hitachi 260–30 spectrometer was used for scanning IR spectroscopy. ID and 2D NMR spectra were performed on Bruker ARX-600 spectrometer using TMS as the internal standard, with chemical shifts (δ) expressed in ppm and referenced to the solvent signals. HRESIMS were performed on a UPLC/xevo G2 Qtof spectrometer. Column chromatography (CC) was performed with silica gel (100–200 mesh and 200–300 mesh, Qingdao

* Corresponding authors.
E-mail addresses: paudy@126.com (C.-Y. Peng), caixiong@hnucm.edu.cn (X. Cai), dfliao@hnucm.edu.cn (D.-F. Liao), iqbal.choudhary@iccs.edu (M.I. Choudhary), wangwei402@hotmail.com (W. Wang).
[1] They contributed equally to the work.

https://doi.org/10.1016/j.fitote.2019.104441
Received 30 August 2019; Received in revised form 24 November 2019; Accepted 24 November 2019
Available online 26 November 2019
0367-326X/

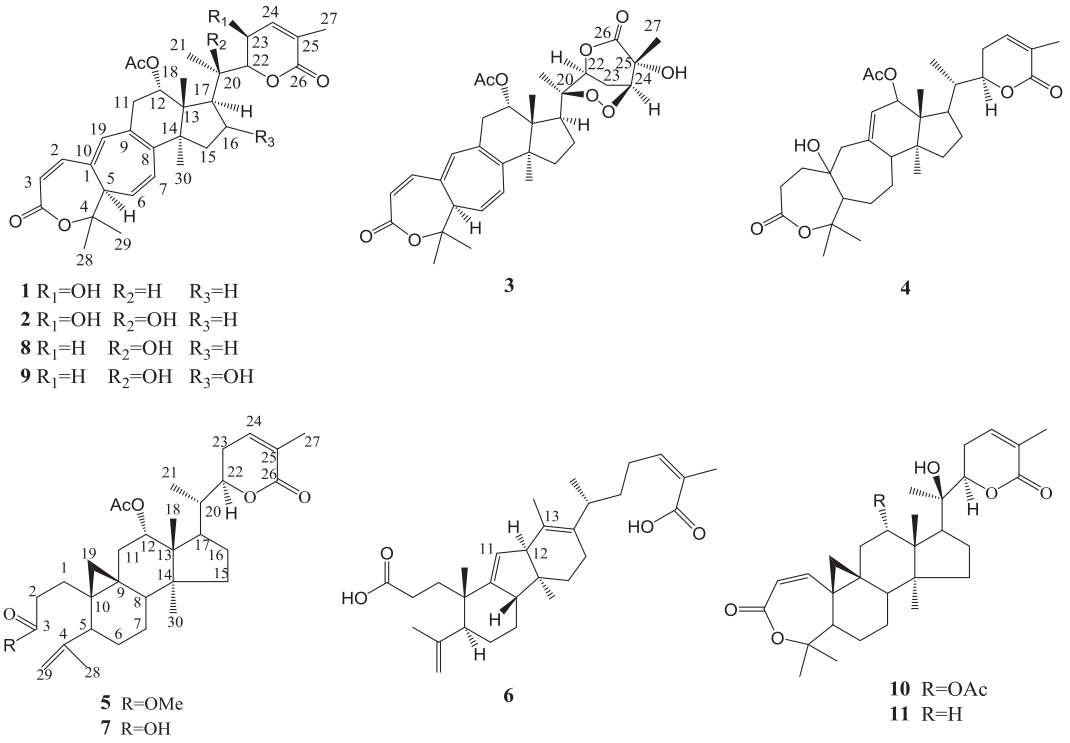

Fig. 1. Structures of **1–11.**

Marine Chemical, Inc., Qingdao, People's Republic of China), and Sephadex LH-20 (Pharmacia). Semipreparative HPLC was performed on an Agilent 1100 liquid chromatograph with an Alltima C_{18} (5 μ ODS 10 mm × 250 mm) column. Fractions were monitored by thin-layer chromatography (TLC) (Qingdao Marine Chemical Inc.), and spots were visualized by heating the silica gel plates after sprayed with 10% H_2SO_4 in EtOH (10:90, *v*/v). Petroleum ether (PE), ethyl acetate (EtOAc), *n*-butanol (*n*-BuOH), dichloromethane (CH_2Cl_2) and methanol (MeOH) were purchased from Shanghai Titan Scientific Co., Ltd., Shanghai, China. MeOH (HPLC grade) and Water (HPLC grade) were purchased from Merck KGaA, 64,271 Darmstadt, Germany.

2.2. Plant material

The stems of *Kadsura heteroclita* were collected in Shimen, Hunan, People's Republic of China, in September 2014. The plant was identified by Prof. Wei Wang, and a voucher specimen (KH-shimen-201,409) has been deposited in the TCM and Ethnomedicine Innovation & Development International Laboratory, School of Pharmacy, Hunan University of Chinese Medicine.

2.3. Extraction and isolation

The air-dried stems of *K. heteroclita* (400 kg) were powdered and extracted with 95% aqueous ethanol (3 × 400 L, 3 days each) at room temperature and concentrated at reduced pressure to afford a crude extract (8 kg). 1 kg of extract was suspended in H_2O (10*L*) and was partitioned between water and *n*-BuOH (10 L, repeated for 3 times). Totally 251.5 g of *n*-BuOH extract was harvested, and then 240.0 g extract was chromatographed on a silica gel (3.0 kg, 100–200 mesh) column using dichloromethane/methanol (from 99.5:0.5 to 0:100 gradient system) as elution solvents to afford fraction A-J. Fraction C (35.1 g) was chromatographed using silica gel CC (0.9 kg, 200–300 mesh), eluted with DCM/MeOH gradient system (99.8:0.2 to 0:100) to obtain ten fractions (Fr·C-1 to Fr. C-10).

Fraction C-7 (1.5 g) was separated over silica gel CC (0.1 kg, 200–300 mesh), eluting with petroleum ether/ethyl acetate (95:5 to 0:100) to afford eight fractions (Fr. C-7-1 to C-7-8). Fr. C-7-4 (350.1 mg) was subjected to sephadex LH 20 eluted with (PE/$CHCl_3$/MeOH = 4:5:1) to get seven subfractions, then Fr. C-7-4-6 (82.3 mg) was separated by semipreparative HPLC (eluted with gradient solvent system of MeOH/H_2O, 3 ml/min) to yield compound **11** (t_R = 15.1 min, 7.8 mg). Fraction C-8 (3.5 g) was chromatographed by silica gel CC (0.3 kg, 200–300 mesh), eluting with PE/EtOAc (90:10 to 0:100) to afford Fr. C-8-1 to C-8-14. Compounds **7** (220.6 mg) and **5** (24.9 mg) were recrystallized and purified from C-8-1 and C-8-2 respectively.

Fr C-10 (2.0 g) was subjected to silica gel CC (0.15 kg, 200–300 mesh) eluted with PE/EtOAc (80:20 to 0:100) to give twelve fractions (Fr. C-10-1 to Fr. C-10-12). Fr. C-10-10 (323.3 mg) was separated over silica gel CC (0.03 kg, 200–300 mesh), eluting with PE/EtOAc (80:20 to 0:100) to afford eight fractions (Fr. C-10-10-1 to C-10-10-8). Fr. C-10-10-4 (66.4 mg) was subjected to semipreparative HPLC (3 ml/min, eluted with 80% MeOH/H_2O) to yield compound **8** (t_R = 7.2 min, 7.1 mg), **10** (t_R = 21.4 min, 13.3 mg). Fr. C-10-10-6 (58.8 mg) was subjected to semipreparative HPLC (3 ml/min, eluent 80% MeOH/H_2O) to yield compound **1** (t_R = 12.9 min, 14.9 mg), **2** (t_R = 15.8 min, 2.5 mg). Fr. C-10-10-7 (32.7 mg) was also subjected to semipreparative HPLC (3 ml/min, eluent 80% MeOH/H_2O) to yield compound **2** (t_R = 16.0 min, 0.7 mg) and **3** (t_R = 13.7 min, 2.5 mg). Fr. C-10-11 (420.0 mg) was separated over silica gel CC (0.05 kg, 200–300 mesh) with a gradient elution solvent system of DCM/MeOH (99.5:0.5 to 0:100) to give eleven fractions (Fr. C-10-11-1 to Fr. C-10-11-11). The subfraction Fr. C-10-11-4 (63.1 mg) was subjected to semipreparative HPLC, with a gradient solvent system of MeOH/H_2O (3 ml/min, from 70% to 100% methanol), to yield compounds **9** (t_R = 8.3 min, 17.4 mg) and **4** (t_R = 24.5 min, 3.0 mg). Compound **6** (35.0 mg) was isolated from Fr. C-10-11-7.

Table 1
^{1}H NMR (600 MHz) spectroscopic data of compounds **1**–**6** (δ in *ppm*, *J* in Hz).

Num.	1[a]	2[b]	3[a]	4[c]	5[c]	6[c]
1α				2.54 (m)	2.09 (m)	1.96 (m)
1β	6.73 (d, 12.2)	6.77 (d, 12.2)	6.70 (d, 12.3)	1.82 (m)	1.35 (m)	1.96 (m)
2α	6.07 (d, 12.1)	5.75 (d, 12.1)	6.06 (d, 12.1)	2.60 (m)	2.45 (m)	2.47 (m)
2β				2.60 (m)	2.23 (m)	2.35 (m)
5	1.92 (d, 6.9)	1.79 (d, 6.9)	2.0 (overlap)	2.00 (dd, 12.7 3.4)	2.43 (m)	1.85 (dd, 2.9, 12.6)
6α	5.92 (dd, 9.5, 7.0)	5.80 (dd, 9.5, 6.9)	5.94 (dd, 9.3 7.0)	2.60 (m)	1.58 (m)	1.78 (m)
6β				2.23 (m)	1.02 (m)	1.55 (m)
7α	6,23 (d 8.6)	6.25 (d, 9.5)	6.21 (d, 10)	1.63 (m)	1.29 (m)	1.75 (m)
7β				1.40 (m)	1.09 (dd, 23.5, 9.5)	1.14 (m)
8				1.94 (m)	1.42 (m)	2.04 (dd, 12.3, 1.7)
11α	2.60 (d, 19.6)	2.54 (d, 19.7)	2.80 (d, 19.8)	5.66 (m)	1.95 (m)	5.28 (d, 1.5)
11β	3.26 (dd, 19.6, 8.2)	3.19 (dd, 19.5, 7.8)	3.20 (dd, 19.7, 7.2)		2.00 (m)	
12	5.25 (d, 7.9)	5.26 (d, 7.5)	5.42 (d, 7.0)	4.95 (d, 4.2)	4.86 (dd, 9.2, 6.2)	2.51 (s)
15α	1.96 (m)	2.08 (m)	2.0 (m)	1.47 (m)	1.39 (m)	1.49 (m)
15β	1.44 (m)	1.58 (m)	1.57 (m)	1.47 (m)	1.39 (m)	1.15 (m)
16α	2.41 (m)	2.07 (m)	1.87 (m)	1.85 (m)	1.85 (m)	1.69 (m)
16β	1.63 (m)	2.06 (m)	1.59 (m)	1.46(m)	1.45 (m)	0.89 (m)
17	3.15 (dd, 20.2, 9.3)	2.88 (t, 9.2)	3.59 (t, 9.6)	2.18 (m)	2.23 (m)	
18	0.79 (s)	1.10 (s)	1.03 (s)	0.84 (s)	1.05 (s)	1.62 (s)
19a	6.25 (s)	6.30 (s)	6.22 (s)	2.90 (d 15.7)	0.60 (d, 4.6)	1.06 (s)
19b				2.56 (m)	0.69 (d, 4.6)	
20	2.18 (m)			2.04 (m)	2.01 (m)	2.66 (m)
21	1.22 (d, 6.8)	1.28 (s)	1.38 (s)	0.88 (d 6.6)	0.85 (d, 6.7)	0.91 (d, 6.9)
22	4.68 (dd, 9.8, 2.2)	4.37 (d, 8.1)	4.49 (t, 1.5)	4.47 (dt 13.2 3.4)	4.51 (dt, 13.1, 3.5)	1.54 (m); 1.32 (m)
23α	4.82 (br s)	4.63 (d, 8.2)	2.60 (dd, 14.2, 2.3)	2.37 (m)	2.38 (m)	2.60 (m)
23β			2.89 (dd, 14.2, 3.1)	2.13 (m)	2.15 (m)	2.18 (m)
24	6.81 (s)	6.55 (s)	4.41 (d, 3.2)	6.61 (d 6.4)	6.62 (d, 6.5)	5.95 (dd, 1.26, 5.26)
27	1.96 (s)	1.89 (s)	2.00 (s)	1.93 (s)	1.92 (s)	1.90 (s)
28	1.61 (s)	1.62 (s)	1.59 (s)	1.31 (s)	1.68 (s)	1.73 (s)
29a	1.55 (s)	1.51 (s)	1.57 (s)	1.25 (s)	4.47 (d, 1.5)	4.86 (s)
29b					4.83 (d, 1.7)	4.67 (d, 1.6)
30	1.14 (s)	1.28 (s)	1.32 (s)	0.81 (s)	1.02 (s)	0.87 (s)
OAc	2.02 (s)	2.07 (s)	2.09 (s)	2.03 (s)		
OMe					3.64 (s)	

[a] Recorded in C_5D_5N
[b] Recorded in CD_3OD.
[c] Recorded in $CDCl_3$.

2.4. Spectral data of new compounds

2.4.1. Xuetongsu A (1)

White amorphous solid (MeOH); $[\alpha]_D^{20}$ -63.66 (*c* 1.78, MeOH); UV (MeOH) λ_{max} (log ε) 240 (3.78) nm, 329 (3.67) nm; ECD (*c* 0.41, MeOH) λ_{max} ($\Delta\varepsilon$) 208 (+3.37), 223 (−1.59), 247 (−2.40), 303 (−0.58) nm, 342 (+0.16) nm; IR ν_{max} 3433, 2969, 2362, 1727, 1690, 1668, 1379, 1294, 1249, 1134, 1027, 977 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive HRESIMS *m/z* 559.2671, [M + Na] $^+$ (calcd for $C_{32}H_{40}O_7Na$, 559.2666).

2.4.2. Xuetongsu B (2)

White amorphous solid; $[\alpha]_D^{20}$ -46.40 (*c* 0.63, MeOH); UV(MeOH) λ_{max} (log ε) 240 (3.71) nm, 329 (3.59) nm; ECD (*c* 0.68, MeOH) λ_{max} ($\Delta\varepsilon$) 208 (+2.25), 223 (−3.63) nm, 247 (−1.68) nm, 273 (+0.08) nm, 296 (−0.29) nm, 338 (+0.38) nm; IR ν_{max} 3433, 2963, 2930, 2854, 1733, 1688, 1665, 1381, 1297, 1249, 1134, 1053 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive HRESIMS *m/z* 575.2617 [M + Na] $^+$ (calcd for $C_{32}H_{40}O_8Na$, 575.2615).

2.4.3. Xuetongsu C (3)

White amorphous solid; $[\alpha]_D^{20}$ -53.3 (*c* 0.45, MeOH); UV(MeOH) λ_{max} (log ε) 239 (3.90) nm, 326 (3.68) nm; ECD (*c* 0.31, MeOH) λ_{max} ($\Delta\varepsilon$) 205 (+3.61), 221 (−2.87), 233 (−0.81), 247 (−2.88), 279 (−0.49) nm, 305 (−0.88); IR ν_{max} 3447, 2989, 2963, 2935, 1733, 1682, 1668, 1376, 1251, 1237, 1109, 1067, 1031 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive HRESIMS *m/z* 591.2568 [M + Na] $^+$ (calcd for $C_{32}H_{40}O_9Na$, 591.2565); positive HRESIMS *m/z* 569.2750 [M + H] $^+$ (calcd for $C_{32}H_{41}O_9$, 569.2745).

2.4.4. Xuetongsu D (4)

White amorphous solid; ECD (*c* 0.21, MeOH) λ_{max} ($\Delta\varepsilon$) 234 (−1.53), 256 (+8.89); ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive HRESIMS *m/z* 565.3159 [M + Na] $^+$ (calcd for $C_{32}H_{46}O_7Na$, 565.3141).

2.4.5. Xuetongsu E (5)

Colorless needle crystal (PE/EtOAc); M.p. 135–138° $[\alpha]_D^{20}$ +90.5 (*c* 0.20, MeOH); ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive HRESIMS *m/z* 563.3354, [M + Na] $^+$ (calcd for $C_{33}H_{48}O_6Na$, 563.3338).

2.4.6. Xuetongsu F (6)

White amorphous solid; ^{1}H and ^{13}C NMR data, see Tables 1 and 2; positive HRESIMS *m/z* 491.3135, [M + Na] $^+$ (calcd for $C_{30}H_{44}O_4Na$, 491.3132).

2.5. X-ray crystallography

The crystallographic data for compound **7** was recorded on a Bruker APEX DUO diffractometer equipped with an APEX II CCD, using Cu Kα radiation. Intensity data was collected at 100 K Cell refinement and data reduction was performed with Bruker SAINT. The structure was solved by direct methods using SHELXS-97. Refinements were performed with SHELXS-97 using full-matrix least-squares, with anisotropic displacement parameters for all the non-hydrogen atoms. The hydrogen atoms were fixed at the calculated positions. Molecular graphics were computed with PLATON. Crystallographic data for the structure reported for **7** had been deposited in the Cambridge

Table 2
^{13}C NMR (150 MHz) spectroscopic data of compounds **1–6** (δ in *ppm*).

Num.	1[a]	2[b]	3[a]	4[c]	5[c]	6[c]
1	142.1	144.1	142.1	25.6	29.1	32.8
2	120.1	118.8	120.2	28.8	31.4	30.1
3	166.6	169.1	166.6	176.1	174.0	182.1
4	79.0	80.6	79.0	74.2	148.9	147.0
5	52.1	52.7	52.2	58.2	45.9	56.6
6	123.7	124.1	124.0	28.7	28.1	28.6
7	125.7	126.2	125.8	26.8	25.3	29.2
8	150.4	151.2	149.7	48.3	48.9	53.8
9	132.4	132.6	132.1	140.1	20.6	150.8
10	128.2	129.0	128.4	91.2	26.7	41.1
11	38.1	38.3	38.0	123.5	36.0	119.9
12	74.4	75.3	73.6	74.2	75.4	57.8
13	48.5	49.6	49.3	46.9	48.7	126.8
14	52.0	53.0	51.9	47.1	48.6	40.0
15	32.7	33.0	32.8	33.7	36.6	33.1
16	27.8	23.2	23.3	26.1	27.0	19.4
17	39.9	43.6	38.1	40.3	40.0	132.2
18	16.8	19.0	20.0	15.2	16.8	18.3
19	135.9	137.0	136.0	51.9	30.2	18.1
20	40.9	78.0	87.7	39.0	39.1	33.6
21	14.2	23.5	18.1	12.2	12.0	19.9
22	85.7	86.0	76.1	80.2	80.3	34.4
23	64.1	64.0	23.0	23.3	23.3	27.8
24	147.5	144.1	81.3	139.1	139.2	146.4
25	126.8	127.8	72.7	128.5	128.5	126.7
26	166.0	165.5	174.1	166.3	166.4	173.6
27	17.3	16.8	22.9	17.0	17.0	20.4
28	25.5	25.2	25.5	32.4	19.9	23.6
29	29.6	29.3	29.6	29.1	112.0	113.8
30	27.1	28.0	28.5	18.3	20.5	19.2
OAc	170.4	172.9	170.4	169.9	169.7	
OAc	21.6	22.0	21.9	21.3	21.5	
OMe					51.6	

[a] Recorded in C_5D_5N
[b] Recorded in CD_3OD.
[c] Recorded in $CDCl_3$.

Crystallographic Data Centre as CCDC 1859556. Copies of the data can be obtained free of charge from the CCDC via www.ccdc.cam.ac.uk.

2.5.1. Crystallographic data for polysperlactone B (7)

$C_{32}H_{46}O_6$, M_W = 526.69, orthorhombic, a = 10.1409(2) Å, b = 10.5383(3) Å, c = 55.2789(13) Å, α = 90.00°, β = 90.00°, γ = 90.00°, V = 5907.5(3) Å^3, T = 100.00(10) K, space group $P2_12_12_1$, Z = 8, ρ_{calc} = 1.184 g/cm^3, μ(Cu Kα) = 0.641 mm^{-1}, 53,938 reflections measured, 11,749 independent reflections (R_{int} = 0.0709, R_{sigma} = 0.0698). The final R_1 indexes were 0.0733 [$I \geq 2\sigma$ (I)]. The final wR_2 indexes were 0.1777 [$I \geq 2\sigma$ (I)]. The final R_1 indexes were 0.0787 (all data). The final wR_2 indexes were 0.1810 (all data). The goodness of fit on F^2 was 1.053. Flack parameter = 0.07(10). The Hooft parameter was 0.05(10).

2.6. Cytotoxicity assays

The cytotoxicity assay was performed using an MTT method [16,17], and the following human tumor cell lines were selected for the bioassay: HL-60 (acute leukemia), HepG-2 (liver hepatocellular carcinoma), HCT-16 (colorectal carcinoma), BGC-823 (gastric carcinoma cell). Each cell line was exposed to test compounds at various concentrations in triplicate for 48 h, and Taxol was used as positive control substances.

2.7. Antioxidant assay

The antioxidant activity was measure by the Chemiluminescence assay which is a sensitive method to measure the inhibition of ROS production [18]. The method referred to the reported literature [17].

3. Results and discussion

Eleven triterpenoids were isolated from a *n*-butanol fraction of 95% ethanol extract of *K. heteroclita* (Fig. 1). The structure elucidation proved that **1–6** were new compounds. Compound **7–11** were identified by comparing its spectral data with the literatures.

Xuetongsu A (**1**) was obtained as a white solid from MeOH. The molecular formula of $C_{32}H_{40}O_7$ was determined by HRESIMS ([M + Na]$^+$ *m/z* 559.2671, calc. 559.2666), indicating 13 degrees of unsaturation. The IR spectrum exhibited absorption bands for hydroxyl (3432 cm^{-1}) and carbonyls (1727, 1680, 1668 cm^{-1}). The ^{1}H NMR spectrum (Table 1) showed six quaternary methyls (δ_H 2.02, 1.96, 1.61, 1.55, 1.14, 0.79,) and one secondary methyl (δ_H 1.22, d, J = 6.8 Hz). The ^{13}C NMR and DEPT spectrum (Table 2) represented 32 carbons, consisting of three carbonyls (δ_C 170.4, 166.6, 166.0), ten olefinic carbons (six methine, δ_C 147.5, 142.1, 135.9, 125.7, 123.7, 120.1, four quaternary carbons, δ_C 150.4, 132.1, 128.2, 126.8) and four oxygenated carbons (three methine, δ_C 74.4, 85.7, 64.1, one quaternary carbon δ_C 79.0) in the low field region, and seven methyls, three methylenes, three methines and two quaternary carbons in the high field region. These data are matched with the HRESIMS calculated formula well. According to the chemical shifts of five double bonds and three carbonyls, the remaining five degree of unsaturation suggested **1** to be a pentacyclic compound.

The similarity of the chemical shifts of **1** to those of heteroclitalactone G [5] suggested that the two compounds were analogues. The major difference between them was the methylene C-23 (δ_C 23.3, δ_H 2.14, 2.37) in heteroclitalactone G was replaced by an oxygenated carbon C-23 (δ_C 64.1, δ_H 4.82) in **1**. It was confirmed by HMBC cross peaks of H-20 (δ_H 2.18), H-22 (δ_H 4.68) to C-23 and ^{1}H−^{1}H COSY correlation of H-24 (δ_H 6.81) to H-23. The acetoxyl group (δ_C 170.4, 21.6; δ_H 2.02) was located at C-12 which was proved by the HMBC cross peak of H-12 (δ_H 5.25) to the carbonyl carbon (δ_C 170.4) of the acetoxyl group (Fig. 2). Thus, the planar structure of compound **1** was assigned.

The CD spectrum of **1** showed a negative Cotton effect at 247 nm (Fig. 3) which was similar to that of heteroclitalactone G and other reported cases [5,11,12], indicating the absolute (*S*)-configuration at C-22. In the ROESY experiment, CH_3–30 (δ_H 1.14) correlated to H-17 (δ_H 3.15), H-17 further correlated to H-23 (δ_H 4.82), indicating H-23 and H-17 should be in α-orientation like CH_3–30. While H-12 (δ_H 5.25) showed a cross peak with CH_3–18 (δ_H 0.79), indicating H-12 was β-oriented, the acetyl group should be in α-orientation (Fig. 2). The other chiral centers have the same character as heteroclitalactone G. Thus, the structure of **1** was established as shown in Fig. 1.

Xuetongsu B (**2**) was obtained as a white solid from MeOH, and has the molecular formula of $C_{32}H_{40}O_8$ as calculated from HRESIMS ([M + Na]$^+$, *m/z* 575.2617, calc. 575.2615). The similarity of the ^{1}H and ^{13}C NMR data of **2** (Tables 1 and 2) to those of **1** suggested that they possess the same core structure. The two compounds only have different chemical shift at C-20, which is a methine in **1** (δ_C 40.9, δ_H 2.18), while turn to be a quaternary oxygenated carbon (δ_C 78.0) in **2**. This was supported by the HMBC cross peaks of CH_3–21, H-22 to C-20. The ROESY correlations of H-17 with CH_3–30, and H-17, CH_3–21 with H-23 suggested that H-17, CH_3–21 and H-23 were in α-orientation while the 20-OH group and 23-OH was in β-orientation (Fig. 2). From the CD spectrum (Fig. 3), **2** showed a negative Cotton effect at 247 nm, hence C-22 was assigned as the (*S*)-configuration. Based on these data, the structure of compound **2** was elucidated.

Xuetongsu C (**3**) had a molecular formula of $C_{32}H_{40}O_9$, as determined by HRESIMS ([M + Na]$^+$, *m/z* 591.2568 , calc. 591.2565; [M + H]$^+$, *m/z* 569.2750, calc. 569.2745), suggesting thirteen degrees of unsaturation. Comparison of the spectroscopic data of **3** with those of **2** revealed that they were quite similar in carbon and proton chemical shifts (Tables 1, 2) except in the moiety of side chain (C-20 to C-27). The HMBC cross peaks of CH_3–27 (δ_H 2.00) with C-24 (δ_C 81.3), C-25

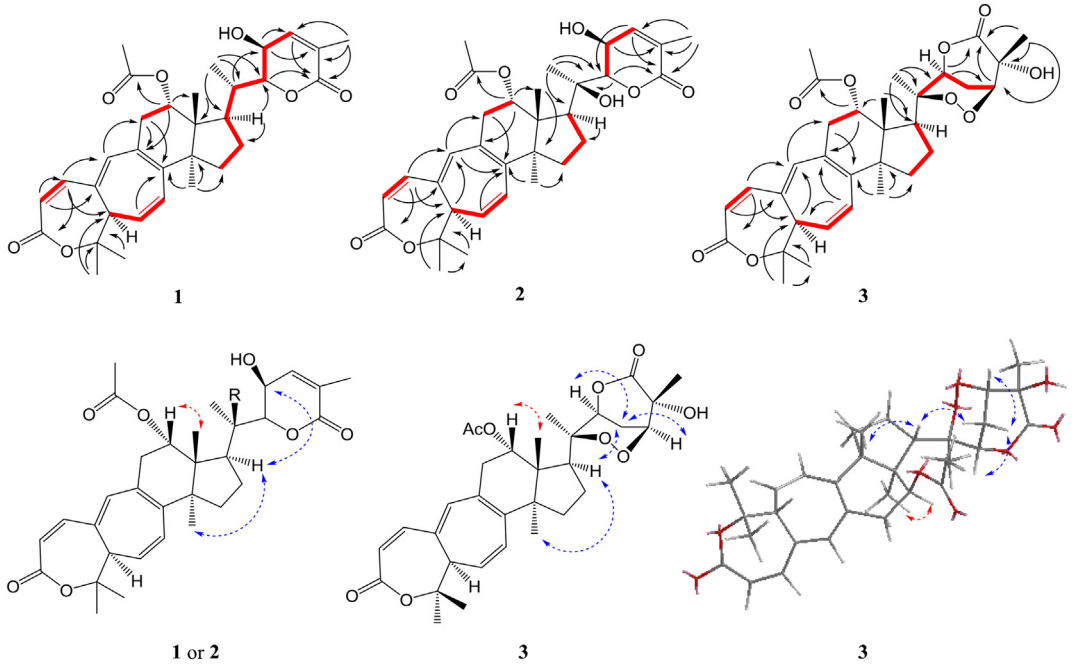

Fig. 2. Key $^{1}H - {}^{1}H$ COSY (bold —), and HMBC (→) correlations of compounds **1–3**; Key NOESY (↷) correlations of compounds **1–3**.

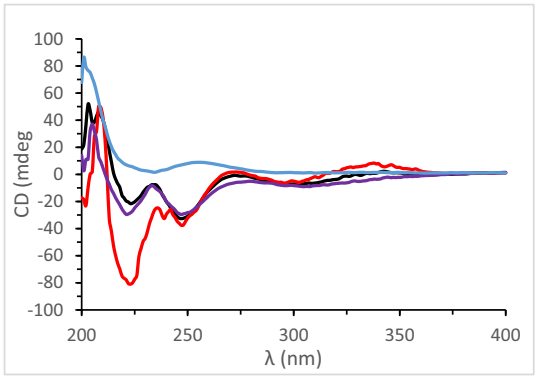

Fig. 3. CD spectra of **1–4**. ECD spectrum of **1** (black), **2** (red), **3** (purple), and **4** (blue) in MeOH. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(δ_C 72.7), C-26 (δ_C 174.1), and H-24 (δ_H 4.41) with C-22 and C-26, H-22 with C-26 and C-24 indicated that compound **3** had a six-member lactone moiety (Fig. 2). While the two oxygenated carbons (C-24, C-25) in **3** shown obvious difference to the double bond between C-24 and C-25 in **2**. The $^{1}H-{}^{1}H$ COSY correlations of H-22 (δ_H 4.49) / H_2–23 (δ_H 2.89, 2.60) / H-24 suggested that C-23 (δ_C 23.0) was a methylene in **3** rather than oxygenated methine (δ_C 64.0) in **2** (Fig. 2). Considering the same unsaturation degree of compounds **2** and **3**, there should be one more cyclic moiety in **3** to replace the lack of one double bond between C-24 and C-25. While one more oxygen atom in the molecular formula of **3** than those of **2** suggested that there may be one peroxide group in **3**. The peroxide group was deduced to be located between C-20 and C-24 by considering the downfield chemical shifts of C-20 (δ_C 87.7) in **3** than those of **2** (C-20, δ_C 78.0). The deduced C-20, C-24 peroxide bridge moiety in **3** is much like the structure of 20, 24-epoxy group in literature [19], the later one was established based on NMR elucidation and X-ray crystallographic analysis. Thus, the planar structure of **3** was established. The ROESY correlations of CH_3–18 with H-12, indicated that H-12 was in *β*-orientation, while the acetyl group should be *α*-oriented. The ROESY correlations of CH_3–30 (δ_H 1.32) / H-17 (δ_H 3.59) suggested that CH_3–30 and H-17 were co-facial in *α*-orientation, while the C-17 side chain was in *β*-orientation.

The obvious ROESY correlations of H-17 with H-23a (δ_H 2.60) and H-22, along with the ROESY correlations of H-22 with H_2–23, and H_2–23 with H-24, indicating the *α*-orientation of H-22, H_2–23 and H-24. The broad singlet of H-22 (δ_H 4.49, s), and ^{1}H coupling constant between H-24 (δ_H 4.41, d J = 3.2 Hz) /H_2–23 (δ_H 2.60, dd, J = 14.2, 2.3; 2.89 Hz, dd, J = 14.2, 3.1 Hz), indicating that H-23a (δ_H 2.60), H-23b (δ_H 2.89) are in *ee* and *ae* formation, which was shown in 3D molecule mode (Fig. 2). The absent ROESY correlation of CH_3–27 (δ_H 2.00) with H-24 and other protons suggested the *β*-orientation *of* CH_3–27, while 25-OH was in *α*-orientation. *S*-configuration of C-22 in **3** was deduced from CD spectrum by showing the same negative Cotton effect at 247 nm (Fig. 3), similar to those of **1** and **2**. Thus, the structure of **3** was established.

Xuetongsu D (**4**) had a molecular formula of $C_{32}H_{46}O_7$, as determined by HRESIMS ($[M + Na]^+$, *m/z* 565.3159, calc. 565.3141), suggesting ten degrees of unsaturation. The ^{1}H and ^{13}C NMR along with DEPT spectrum indicated seven methyl (one doublet), eight methylene, eight methine (two olefinic, two oxygenated) and nine quaternary carbons (three carbonyls, two olefinic and two oxygenated). The chemical shift of (δ_C 169.9, 21.3; δ_H 2.03) probably attributed to an acetoxyl group (Tables 1, 2). The chemical shift pattern and the remaining five degrees of unsaturation (except two double bonds and three carbonyl) suggesting a pentacyclic skeleton triterpenoid for **4**.

The chemical shifts and 2D NMR correlations (Fig. 4) indicated that compound **4** was in similar structure skeleton to heteroclitalactone D [6,13]. While there were only two double bonds in the structure of **4**, one between C-24 (δ_C 139.1, δ_H 6.61) and C-25 (δ_C 128.5), which were proved by the HMBC cross peaks of CH_3–27 (δ_H 1.93) to C-24, C-25, C-26, and of H-24 to C-26 (δ_C 166.3), C-27 (δ_C 17.0). Another double bond was between C-11 (δ_C 123.5, δ_H 5.66) and C-9 (δ_C 140.1), which was demonstrated by the HMBC correlations of H_2–19 (δ_H 2.90, 2.56), H-12 (δ_H 4.95) to C-11, and C-9 respectively, and COSY correlation of H-11 (δ_H 5.66) to H-12. The HMBC correlations of H_2–1 (δ_H 2.54, 1.82) to C-2 (δ_C 28.8), C-3 (δ_C 176.0), and H_2–2(δ_{2H} 2.60) to C-1 (δ_C 25.6), C-3, along with $^{1}H-{}^{1}H$ COSY correlation of H_2–1 to H_2–2 demonstrated that the double bond between C-1 and C-2 in heteroclitalactone D [6,13] was replaced by two methylenes in compound **4**. The oxygenated C-10 (δ_C 91.2) was verified by the HMBC correlations of H_2–1,

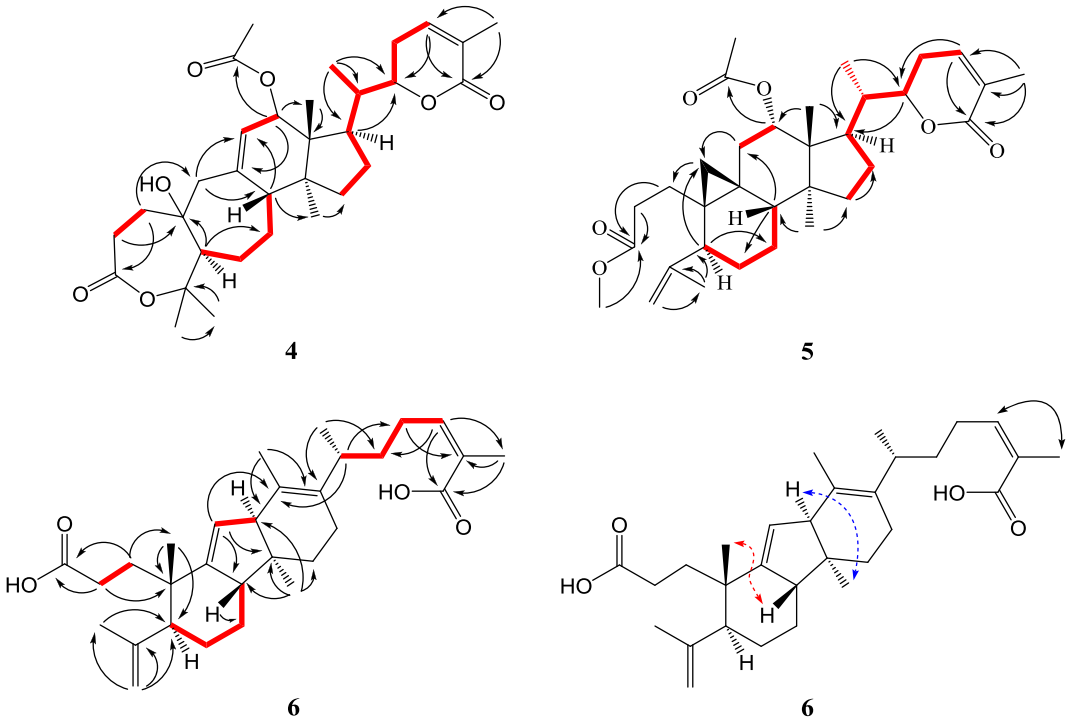

Fig. 4. Key $^{1}H - {}^{1}H$ COSY (bold —), and HMBC (→) correlations of **4–6**; Key NOESY (↷) correlations of **6**.

H_2–19 (δ_H 2.90, 2.56), H-5 (δ_H 2.00) to C-10. Referred to the molecular formula, there was a hydroxy group attached to C-10, thus the planar structure of **4** was determined. The CD spectrum (Fig. 3) showed positive Cotton effect at 256 nm, which was different from those of compounds **1–3**, while similar to that of heteroclitalactone D [6,13], therefore the stereochemistry of C-22 was assigned as the (*R*)-configuration.

Xuetongsu E (**5**), obtained as colorless needles, was assigned the molecular formula $C_{33}H_{48}O_6$ on the basis of HRESIMS ($[M + Na]^+$, *m/z* 563.3354, calc. 563.3338). The ^{1}H and ^{13}C chemical shift of **5** (Tables 1, 2) are almost the same by compared to those of polysperlactone B (**7**) [13], while one more methoxy group (δ_C 51.6, δ_H 3.64) was observed in **5**. The methoxy group attached to C-3 was revealed by the clear HMBC correlation of OCH_3 (δ_H 3.64) to C-3 (δ_C 174.0) (Fig. 4). The configuration of **5** was deduced to be the same as **7** by referring to the chemical shift, and coupling constants. Thus, the structure of compound **5** was established.

Xuetongsu F (**6**) obtained as colorless granules, had the molecular formula of $C_{30}H_{44}O_4$ revealed by HRESIMS ($[M + Na]^+$, *m/z* 491.3135, calc. 491.3132), nine degrees of unsaturation were calculated from the formula. The ^{1}H NMR spectrum (Table 1) showed signals for one doublet methyl (δ_H 0.91, d, J = 6.9 Hz) and five singlets methyl (δ_H 0.87, 1.06, 1.62, 1.73, 1.90), two olefinic protons (δ_H 5.95, ddd, J = 1.26, 5.26, 9.18 Hz; δ_H 5.28, d, J = 1.5 Hz) and one extracellular methylene (δ_H 4.67, 4.86, d, J = 1.6 Hz). The ^{13}C NMR (Table 2) and DEPT showed six methyls, nine methylenes (one olefinic), six methines (two olefinic), and nine quaternary carbons (five olefinic, two carbonyl). Beyond the four double bond and two carbonyl moiety, the remaining three unsaturation degrees suggested that **6** was a tricyclic triterpenoid skeleton. The chemical shifts of **6** were comparable with kadcoccinic acid D [14]. It was clear that a carboxyl group (δ_C 182.1) and an isopropenyl group (C-30, δ_C 113.8, δ_H 4.67, 4.86; C-29, δ_C 23.6, δ_H 1.63; C-4, δ_C 147.0) presented a 3,4-seco ring A in compound **6** [14], which were further proved by the $^{1}H-{}^{1}H$ COSY correlations of H_2–1 (δ_H 1.96) / H_2–2 (δ_H 2.35, 2.47), and HMBC correlation of H_2–1, H_2–2 to C-3 (δ_C 182.1), and CH_3–29 (δ_H 1.63) to C-30 (δ_C 113.8), C-4 (δ_C 147.0), and C-5 (δ_C 56.6) (Fig. 4). The planar structure of **6** was therefore assigned. The ROESY correlation (Fig. 4) between H-12 (δ_H 2.51) and CH_3–30 (δ_H 0.87) suggested that H-12 was in *α*-orientation, while CH_3–19 (δ_H 1.06) correlated with H-8 (δ_H 2.04) implied that H-8 was in *β*-orientation. The ROESY correlation between H-24 (δ_H 5.95) and CH_3–27 (δ_H 1.90) indicated that the geometry of the double bond between C-24 (δ_C 146.4) and C-25 (δ_C 126.7) was in the *Z* configuration. Thus, the structure of **6** was established.

Polysperlactone B (**7**) was identified by comparison of its spectroscopic data with those of reported previously [13]. The absolute structure of **7** was established as C5 *S*; C8 *R*; C9 *R*; C10 *R*; C12 *S*; C13 *R*; C14 *S*; C17 *R*; C20 *S*; C22 *R* by single-crystal X-ray diffraction using Cu K*α* radiation, which resulted in a Flack/Hooft parameter of 0.07(10)/0.05(10) (Fig. 5). The other four known triterpenoids were identified as heteroclitalactone J (**8**) [5], heteroclitalactone K (**9**) [5], heteroclitalactone P (**10**) [5], and kadsuphilactone B (**11**) [15] by referenced to the literatures.

All the isolated compounds were screened for their cytotoxic activities against four cell lines HL-60, HepG-2, HCT-16, and BGC-823. Compound **7** displayed weak inhibition effects against HL-60 with IC_{50} value of 50 μM. No antioxidant activity of the screened compounds was observed. In the present work, six new and five known 3,4-seco triterpenoids were isolated from the stems of *K. heteroclita*. It was clear that this species is abundance in the novel structure of triterpenoids along with the previous reported work [5–7]. 3,4-seco triterpenoids have been proved to have good activity in anti-HIV, anti-cancer cell lines and anti-inflammation [1,14]. It was interesting that one reported anti-HBV active compound kadsuphilactone B (**11**) [15] was also isolated from the plant, which may be the substance of the hepatoprotective effect of “Xuetong”.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was supported in part by National Natural Science

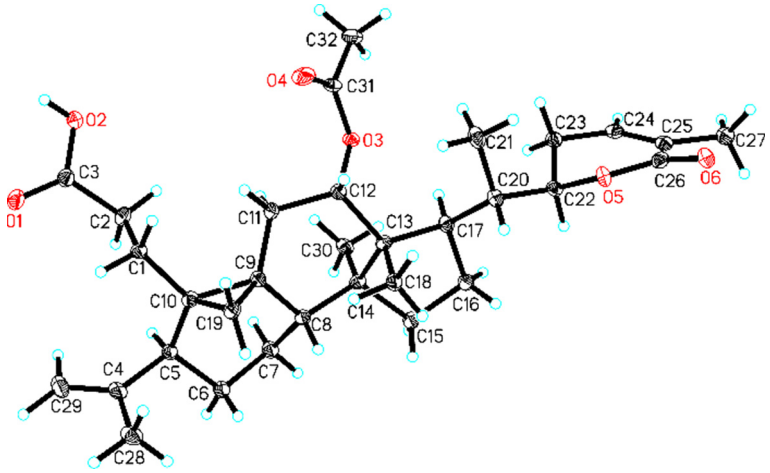

Fig. 5. X-ray crystallographic structure of **7**.

Foundation of China (81673579, 81874369 and 81374062), Ministry of Science and Technology of P.R.China (2018FY100703) and Hunan Science and Technology Department (2019JJ50315, 2018SK2110, 2018SK2081 and 2016NK2133), the authors would like to express the acknowledgment to Dr. Zulfiqar Ali and Dr. Xing-cong Li from University of Mississippi for the suggestions in the structure elucidation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fitote.2019.104441.

References

[1] J.S. Liu, Y.D. Qi, H.W. Lai, J. Zhang, X.G. Jia, H.T. Liu, B.G. Zhang, P.G. Xiao, Genus *Kadsura*, a good source with considerable characteristic chemical constituents and potential bioactivities, Phytomedicine 21 (2014) 1092–1097.

[2] W. Wang, The research and development of Tujia ethnomedicine, Planta Med. 79 (2013), https://doi.org/10.1055/s −0033 -1336436 OP22, (Abstracts of the 12th International Conference on the Science of Botanicals, Oxford, Mississippi, USA, 15–18, April 2013; Smillie, T.J. Parcher, J.F.;).

[3] H.H. Yu, R. Zeng, Y. Lin, X. Li, S. Tasneem, Z. Yang, Y.X. Qiu, B. Li, Y.H. Wang, X. Cai, W. Wang, *Kadsura heteroclita* stem suppresses the onset and progression of adjuvant induced arthritis in rats, Phytomedicine 58 (2019) 152876.

[4] H.H. Yu, Y. Lin, R. Zeng, X. Li, T. Zhang, S. Tasneem, C. Chen, Y.X. Qiu, B. Li, J. Liao, Y.H. Wang, X. Cai, W. Wang, Analgesic and anti-inflammatory effects and molecular mechanisms of *Kadsura heteroclita* stems, an anti-arthritic Chinese Tujia ethnomedicinal herb, J. Ethnopharmacol. 238 (2019) 111902.

[5] W. Wang, Z.R. Xu, M. Yang, R.X. Liu, W.X. Wang, P. Liu, D.A. Guo, Structural determination of seven new triterpenoids from *Kadsura heteroclita* by NMR techniques, Magn. Reson. Chem. 45 (2007) 522–526.

[6] W. Wang, J.Z. Liu, J. Han, Z.R. Xu, R.X. Liu, P. Liu, W.X. Wang, X.C. Ma, S.H. Guan, D.A. Guo, New triterpenoids from *Kadsura heteroclita* and their cytotoxic activity, Planta Med. 72 (2006) 450–457.

[7] W. Wang, J.Z. Liu, X.C. Ma, M. Yang, W.X. Wang, P. Liu, D.A. Guo, Three new cyclolanostane triterpenoids from the ethanol extract of the stems of *Kadsura heteroclta*, Helv. Chim. Acta. 89 (2006) 1888–1893.

[8] L. Cao, N. Shehla, S. Tasneem, M.R. Cao, W.B. Sheng, Y.Q. Jian, B. Li, C.Y. Peng, M.I. Choudhary, D.F. Atta-ur-Rahman, W. Wang Liao, New cadinane sesquiterpenes from the stems of *Kadsura heteroclita*, Molecules 24 (2019) 1664.

[9] W. Su, J.P. Zhao, J. Hu, M. Yang, M. Jacob, X. Cai, R. Zeng, S.H. Chen, H.Y. Huang, I.A. Khan, D.A. Guo, W. Wang, Two new bicyclic sesquiterpenes from the stems of *Kadsura heteroclita*, Nat. Prod. Res. 28 (2014) 11971201.

[10] W. Wang, X.C. Ma, P. Liu, R.X. Liu, S.H. Guan, D.A. Guo, Two new dibenzylbutyrolactone type lignans from *Kadsura heteroclita*, Nat. Prod. Commun. 1 (2006) 109–112.

[11] Y.P. Chen, Z.W. Lin, H.J. Zhang, H.D. Sun, A triterpenoid from *Katsura heteroclita*, Phytochemistry 29 (1990) 3358–3359.

[12] J.S. Liu, M.F. Huang, G.F. Arnold, E. Arnold, J. Clardy, W.A. Ayer, A. Schisanlactone, A new type of triterpenoid from a *Schisandra sp*, Tetrahedron Lett. 24 (1983) 2351–2354.

[13] Z.W. Jia, Y. Lu, Z.X. Liao, D.F. Chen, Two new triterpene lactones from the stems of *Kadsura polysperma*, Helv. Chim. Acta. 90 (2007) 1236–1243.

[14] C.Q. Liang, Y.M. Shi, W.G. Wang, Z.X. Hu, Y. Li, Y.T. Zheng, X.N. Li, X. Du, J.X. Pu, W.L. Xiao, H.B. Zhang, H.D. Sun, Kadcoccinic acids A−J, triterpene acids from *Kadsura coccinea*, J. Nat. Prod. 78 (2015) 2067–2073.

[15] Y.C. Shen, Y.C. Lin, M.Y. Chiang, S.F. Yeh, Y.B. Cheng, C.C. Liao, Kadsuphilactones A and B, two new triterpene dilactones from *Kadsura philippinensis*, Org. Lett. 7 (2005) 3307–3310.

[16] U. Schauer, I. Krolikowski, C.H. Rieger, Detection of activated lymphocyte subsets by fluorescence and MTT staining, J. Immunol. Methods 116 (1989) 221–227.

[17] Y.B. Liu, Y.P. Yang, S. Tasneem, N. Hussain, M. Daniyal, H.W. Yuan, Q.L. Xie, B. Liu, J. Sun, Y.Q. Jian, B. Li, S.H. Chen, W. Wang, Lignans from Tujia ethnomedicine Heilaohu: chemical characterization and evaluation of their cytotoxicity and antioxidant activities, Molecules 23 (2018) 2147.

[18] M.M. Tarpey, I. Fridovich, Methods of detection of vascular reactive species: nitric oxide, superoxide, hydrogen peroxide, and peroxynitrite, Circ. Res. 89 (2001) 224–236.

[19] E. Maldonado, N.E. Hurtado, A.L. Pérez-Castorena, M. Martínez, Cytotoxic 20, 24-epoxywithanolides from *physalis angulata*, Steroids 104 (2015) 72–78.