Chemistry of Natural Compounds, Vol. 43, No. 6, 2007

SEPARATION AND IDENTIFICATION OF STEROIDAL COMPOUNDS WITH CYTOTOXIC ACTIVITY AGAINST HUMAN GASTRIC CANCER CELL LINES *IN VITRO* FROM THE RHIZOMES OF *Paris polyphylla* VAR. *chinensis*

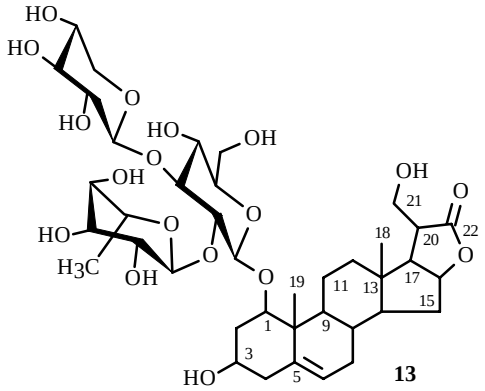
Huang Yun,[1] Cui Lijian,[2] Zhan Wenhong,[1] Dou Yuhong,[2] Wang Yongli,[1] Wang Qiang,[3] and Zhao Ding[1]

UDC 547.92+547.59

A novel steroidal saponin, along with 12 known steroidal compounds, was isolated from the rhizomes of Paris polyphylla *var.* chinensis*. Spectral data, including two-dimensional NMR, showed that the structure of the novel saponin was 3*β*,21-dihydroxypregnane-5-en-20S-(22,16)-lactone-1*-O-α-*L-rhamnopyranosyl(1→2)-[*β-*D-xylopyranosyl(1→3)]-*β-*D-glucopyranoside. The isolated steroidal compounds were evaluated for their cytotoxic activity on human gastric cancer cell line HepG$_2$, SGC7901, BxPC3. Diosgenin-3-*O-α-*L-rhamnopyranosyl(1→2)[*α-*L-arabinofuranosyl(1→4)]-*β-*D-glucopyranoside exhibited the most potent cytotoxic activity among the isolated steroids.*

Key words: *Paris polyphylla* var. *chinensis*, *Paris*, steroidal compound, cytotoxic activity, human gastric cancer cell line, HepG$_2$, SGC7901, BxPC3.

The genus *Paris* is a complex genus with approximately 24 species [1] that grows primarily in the temperate zones and tropical regions of Europe and the Asian continent. The rhizomes of several species of genus *Paris* have been used as hemostatic and anti- inflammatory agent to treat traumatic injuries, snake bite, abscess, parotitis, and mastitis. Steroidal saponins in the genus *Paris* are active components having hemostatic and antitumor activities as reported [2].



13

Paris polyphylla var. chinensis, distributed in southwest China, is an important crude drug in the famous traditional Chinese medicine "Yunnan Baiyao".

Our ongoing research [3–6] for steroids from *Paris* plants has so far resulted in the isolation and analysis of cytotoxic compounds against several tumor cell lines.

1) Department of Pharmacognosy, Pharmaceutical College, Hebei Medical University, 050017, China, tel: +86-311-86265628, hy9317536@126.com; 2) Chinese Traditional Medicine College, Hebei Medical University, 050019, China; 3) China Pharmaceutical University, 210038, China. Published in Khimiya Prirodnykh Soedinenii, No. 6, pp. 556-560, November-December, 2007. Original article submitted October 4, 2006.

 0009-3130/07/4306-0672

TABLE 1. ^{13}C NMR Spectral Data of Compounds **1-12** in C_5D_5N (125 MHz)

C atom	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
1	36.8	37.4	37.3	37.5	38.1	37.5	37.2	37.2	37.3	37.1	39.0	38.268.3
2	31.2	32.4	30.0	29.9	30.7	30.1	29.8	29.9	30.2	29.9	69.1	68.4
3	70.2	71.2	77.6	78.2	77.7	77.7	77.6	77.5	77.6	77.7	69.0	32.1
4	42.5	43.4	38.8	39.0	39.6	38.9	38.5	38.7	38.7	38.7	33.4	51.7
5	141.0	142.0	140.7	140.8	141.4	140.8	140.5	140.6	140.5	140.8	52.3	203.8
6	120.0	121.0	121.5	121.7	122.4	121.8	121.6	121.5	121.5	121.5	204.2	121.9
7	31.6	32.2	32.1	32.4	32.9	32.4	32.0	32.0	31.8	32.1	122.6	166.9
8	30.8	32.4	31.5	30.4	33.3	31.8	31.4	31.6	31.5	30.7	166.9	34.7
9	49.4	50.4	50.1	50.2	50.9	50.2	50.0	50.1	50.0	50.3	35.4	38.9
10	36.0	37.0	36.9	37.1	37.7	37.1	36.9	36.9	36.9	36.9	39.6	21.3
11	20.2	21.0	20.9	20.9	21.7	20.9	20.8	20.8	20.7	21.0	22.1	31.8
12	39.5	32.4	39.6	32.1	40.5	32.3	39.6	39.6	32.2	36.7	32.7	47.8
13	41.0	44.8	40.2	45.1	41.1	45.1	40.2	40.2	44.9	46.9	49.1	84.7
14	55.8	53.2	56.4	53.0	57.2	53.0	56.4	56.4	52.8	57.4	85.1	32.8
15	31.3	31.8	32.0	32.3	32.8	32.1	32.1	32.1	32.1	31.0	33.0	22.3
16	80.1	90.0	80.9	90.0	81.7	90.1	80.8	80.8	89.9	140.4	22.6	54.0
17	61.9	90.0	62.5	90.1	63.5	90.1	62.9	62.7	89.9	151.5	51.1	18.2
18	15.4	17.2	16.1	17.1	16.9	17.3	16.1	16.1	16.9	16.3	18.8	24.7
19	18.6	19.0	19.2	19.4	20.0	19.4	19.1	19.1	19.2	19.1	25.4	74.9
20	39.0	45.6	41.7	44.8	42.6	44.8	41.7	41.7	44.5	131.0	77.8	27.4
21	14.0	9.5	14.8	9.7	15.9	9.6	14.7	14.7	9.4	14.4	22.4	42.8
22	108.2	109.6	109.0	109.8	109.9	109.8	109.0	109.3	109.6	139.5	78.5	27.1
23	30.8	32.6	31.6	31.8	32.4	32.1	31.6	31.4	31.8	31.7	28.4	80.2
24	28.3	29.3	29.0	28.8	29.9	28.8	29.0	29.0	28.5	35.2	43.6	73.0
25	29.6	30.4	30.4	30.2	31.2	30.4	30.3	30.3	29.9	33.8	70.5	26.2
26	65.9	66.7	66.6	66.7	67.5	66.7	66.6	66.6	66.4	74.7	31.0	26.2
27	16.3	17.3	17.1	17.2	17.9	17.1	17.0	17.0	17.0	17.1	31.0	
16′										122.6		
22′										127.1		
3-*O*-*β*-D-Glc*p*												
1			100.2	100.3	100.8	100.1	99.7	100.1	100.1	100.0		
2			79.4	79.6	78.5	77.9	78.4	77.8	77.8	77.0		
3			78.0	77.9	78.3	77.1	89.2	76.7	76.7	77.4		
4			71.6	71.8	77.3	76.7	69.3	77.6	77.5	76.4		
5			77.7	77.8	78.0	77.4	78.2	77.7	77.7	77.2		
6			62.7	62.7	63.1	62.5	62.2	61.0	61.0	62.3		
2-*α*-L-Rha*p*												
1			101.8	102.0	102.5	101.9	101.9	102.0	101.9	101.6		
2			72.3	72.6	73.4	72.8	72.2	72.4	73.0	72.5		
3			72.6	72.8	73.0	72.4	72.5	70.1	70.1	72.1		
4			74.0	74.2	74.7	74.1	73.9	80.1	80.1	73.9		
5			69.2	69.4	70.0	69.4	71.3	68.1	68.1	69.1		
6			18.4	18.6	19.2	18.6	18.4	18.1	18.6	18.3		
4-*α*-L-Ara*p*												
1					110.2					109.4		
2					83.2					82.4		
3					78.7					77.9		
4					87.3					86.5		
5					62.0					61.2		

TABLE 1. (continued)

C atom	1	2	3	4	5	6	7	8	9	10	11	12
							3-*β*-D-Glc*p*	4-*α*-L-Rha*p*		26-*O*-*β*-D-Glc*p*		
1′							104.2	102.0	102.0	104.6		
2′							74.7	73.0	72.4	75.0		
3′							77.5	72.2	72.6	78.2		
4′							71.3	73.9	73.9	71.6		
5′							76.8	69.3	69.3	78.4		
6′							62.2	18.6	18.6	62.7		
4-*α*-L-Rha*p*												
1″								103.0	103.0			
2″								72.6	72.4			
3″								72.2	72.2			
4″								73.8	73.8			
5″								69.3	69.2			
6″								18.4	18.4			

In the present study, we have investigated the steroidal compounds from *P. polyphylla* rhizomes and their cytotoxic activity against gastric cancer cell lines *in vitro*.

The 95% alcohol extract of *P. polyphylla* rhizomes was passed through a macroreticular resin (D101) column, successively eluting with water and alcohol. The 40% and 80% alcohol fractions were repeatedly subjected to column chromatography on silica-gel, Sephadex LH-20, and octadecylsilanized (ODS) silica-gel to give compounds **1–13**.

Counpounds **1–12** are known steroids and their structures were identified by ^{13}C NMR spectrum (in pyridine-d_5) as diosgenin (**1**) [7], pennogenin (**2**) [7], diosgenin-3-*O*-*α*-L-rhamnopyranosyl(1→2)-*β*-D-glucopyranoside (**3**) [8], pennogenin-3-*O*-*α*-L-rhamnopyranosyl(1→2)-*β*-D-glucopyranoside (**4**) [9], diosgenin-3-*O*-*α*-L-rhamnopyranosyl(1→2)[*α*-L-arabinofuranosyl(1→4)]-*β*-D-glucopyranoside (**5**) [10], pennogenin-3-*O*-*α*-L-rhamnopyranosyl(1→2)-[*α*-L-arabinofuranosyl(1→4)]-*β*-D-glucopyranoside (6) [10], diosgenin-3-*O*-*α*-L-rhamnopyranosyl(1→2)[*β*-D-glucopyranoside(1→3)]-*β*-D-glucopyranoside (**7**) [9], diosgenin-3-*O*-*α*-L-rhamnopyranosyl(1→4)-*α*-L-rhamnopyranosyl(1→4)[*α*-L-rhamnopyranosyl(1→2)]-*β*-D-glucopyranoside (**8**) [8], pennogenin-3-*O*-*α*-L-rhamnopyranosyl(1→4)-*α*-L-rhamnopyranosyl(1→4)[*α*-L-rhamnopyranosyl(1→2)]-*β*-D-glucopyranoside (**9**) [8], 3-*O*-*α*-L-arabinofuranosyl(1→4)[*α*-L-rhamnopyranosyl(1→2)]-*β*-D-glucopyranoside-*β*-D-chacotriosyl-26-*O*-*β*-D-glucopyranoside (**10**) [3], 2*β*,3*β*,14*α*,20*β*,22*α*,25*β*-hexahydroxycholest-7-en-6-one (**11**) [11], and 2*β*,3*β*,14*α*,20*β*,24*β*,25*β*-hexahydroxycholest-7-en-6-one (**12**) [12], respectively (Table 1).

Compound **13** was obtained as an amorphous solid, mp. 272~274°C, $[\alpha]_D^{25}$ –6.0° (*c* 0.012) (MeOH), which was shown to have the quasimolecular ion peak at *m/z* 815 $[M-1]^-$ (815 $[M-1]^- \rightarrow$683$[815-132]^- \rightarrow$537$[683-146]^-$) in the negative ESI-MS. Acid hydrolysis of **13** with 1M HCl liberated glucose, rhamnose, xylose.

The structural assignment of **13** was accomplished by the extensive used of one and two-dimensional NMR spectroscopy.

The ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) spectrum led to the correlation of all the proton resonances with those of corresponding single-bond coupled carbons (Table 2).

The ^{1}H NMR data (Table 2) of **13** in pyridine-d_5 displayed signals for two tertiary methyl groups (H-18, H-19), a secondary methyl (Rha H-6), an olefinic proton (H-6), and three anomeric protons (Glc H-1, Xyl H-1, Rha H-1).

The ^{13}C NMR data (Table 2) of **13** in pyridine-d_5 contained 38 signals that included two tertiary methyls (C-18, C-19), a secondary methyl (Rha C-6), three anomeric carbons (Glc C-1, Rha C-1, Xyl C-1), a $C_{5,6}$-double bond, and a carbonyl group (C-22).

The heteronuclear multiple bond correlation (HMBC) spectrum displayed the correlation of Rha C-1 and Glc H-2; Xyl C-1 and Glc H-3. The ^{13}C NMR shifts of the triglycoside moiety of **13** were in good agreement with those of the reported [13] ophiogenin-3-*O*-*α*-L-rhamnopyranosyl(1→2)[*β*-D-xylopyranosyl(1→3)]-*β*-D- glucopyranoside.

The exact structure of the aglycone moiety of **13** was determined by 2D NMR.

TABLE 2. ^{13}C and ^{1}H NMR Spectral Data of Compound 13 in C_5D_5N

Site	δ_C	δ_H	HMBC	Site	δ_C	δ_H	HMBC
1	85.21	3.81	H-1/Glc C-1	22	181.2		
2	38.8	2.63; 2.36	H-2/C-1	Glc			
3	69.0	3.75		1	101.0	4.80, J = 8	Glc H-1/C-1
4	44.6	2.53; 2.66	H-4/C-2, C-3, C-5, C-6, C-10	2	77.3	4.14	Glc H-2/Rha C-1
5	140.3			3	89.5	4.03	Glc H-3/Glc C-2, Glc C-4, Xyl C-1
6	125.5	5.55; J = 5.5	H-6/C-4, C-7, C-8, C-10	4	71.1	3.81	Glc H-4/Glc C-5, Glc C-6
7	33.7	1.48 (overlap)		5	78.7	3.78	
8	32.6	1.82		6	64.1	4.44, 4.15	
9	51.2	1.63		Xyl			
10	43.6			1	106.2	4.92, J = 7.5	
11	24.7	2.87; 1.82	H-11/C-13	2	75.7	3.96	Xyl H-2/Xyl C-1, Xyl C-3
12	39.7	1.48 (overlap); 1.71		3	79.4	4.05	Xyl H-3/Xyl C-4
13	42.2			4	71.5	4.09	
14	56.0	1.10	H-14/C-7	5	68.2	3.67, 4.23	Xyl H-5/Xyl C-4
15	34.5	2.0; 1.48 (overlap)	H-15/C-13, C-17	Rha			
16	85.28	5.00	H-16/C-13	1	102.7	6.41	Rha H-1/Rha C-5, Rha C-2, Rha C-3
17	56.5	2.45, d, J = 8	H-17/C-21, C-22	2	73.44	4.76	
18	15.4	0.86, s, 3H	H-18/C-12, C-13, C-14, C-17	3	73.47	4.54	
19	16.0	1.38, s, 3H	H-19/C-1, C-5, C-10, C-9	4	75.1	4.29	
20	46.7	2.89, q	H-20/C-22	5	70.5	4.79	
21	64.5	4.02; 4.34	H-21/C-22	6	20.2	1.74; J = 6	Rha H-6/Rha C-4, Rha C-5

TABLE 3. Cytotoxicity of Steroidal Compounds on HepG2, SGC7901, and BxPC3 *in vitro* from the Rhizomes of *Paris polyphylla* var. *chinensis*

Sample	Conc., μg/mL	$HepG_2$, %	SGC7901, %	BxPC3, %	Sample	Conc., μg/mL	$HepG_2$, %	SGC7901, %	BxPC3, %
Epirubicin-HCl	2.5	78.89	70.73	65.78	**7**	2.5	68.35	77.34	76.63
	0.1	75.72	81.93	53.14		0.1	45.87	46.13	37.64
	0.01	64.48	84.36	44.84		0.01	13.61	4.120	24.69
3	2.5	60.59	70.14	76.52	**8**	2.5	61.70	75.81	82.92
	0.1	26.12	19.07	40.49		0.1	36.28	19.32	53.11
	0.01	15.73	11.44	33.04		0.01	28.97	12.84	44.69
4	2.5	44.22	50.13	73.32	**9**	2.5	35.75	47.58	81.77
	0.1	44.20	23.96	7.32		0.1	36.32	19.25	9.71
	0.01	24.85	1.44	12.02		0.01	12.53	10.15	8.74
5	2.5	83.76	88.19	87.06	**11**	2.5	7.21	6.62	12.23
	0.1	54.17	62.88	71.42		0.1	1.71	2.89	12.49
	0.01	47.45	24.37	35.50		0.01	3.65	9.70	23.26
6	2.5	65.22	70.39	79.64	**13**	2.5	3.37	1.58	18.00
	0.1	15.49	24.77	47.92		0.1	2.63	2.98	5.18
	0.01	2.28	13.92	13.83		0.01	8.59	0.25	5.67

In a subsequent inspection of the HMBC spectrum, carbons C-1, C-5, C-9, and C-10 showed correlations with the tertiary methyl group H-18; C-4, C-7, C-8, and C-18 with olefinic protons H-6; and C-13 with one of the methenyl protons H-16.

Furthermore, comparison of the carbon chemical shifts thus assigned with those of the reported [14] 3β,6α,16β-trihydroxy-5α-pregnane-20S-carboxylic acid (22,16)-lactone showed a C-1 substituted *O*-glycosyl, a C-21 substituted hydroxyl, and a substituted double bond between C-5 and C-6.

Thus, from the foregoing spectral studied, the structure of **13** was elucidated as 3β,21-dihydroxy-pregnane-5-en-20S-(22,16)-lactone-1-*O*-α-L-rhamnopyranosyl(1→2)-[β-D-xylopyranosyl (1→3)]-β-D-glucopyranoside.

The cytotoxic effect of steroidal compounds of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltretrazolium bromide (MTT) from the rhizomes of *Paris polyphylla* var. *chinensis* on human gastric cancer cell lines $HepG_2$, SGC7901, and BxPC3 after 48 h is shown in Table 3. The assay involved three different doses of steroidal compounds (2.5, 0.1 and 0.01 μg/mL). Some steroidal saponins showed relatively potent cytotoxic activity, with IC_{50} values ranging from 0.1 ug/mL to 2.5 ug/mL, when epirubicin-HCI was used as positive control. The result indicates a dose-response relationship. Compound **5** exhibited the most potent cytotoxic activity among the isolated steroids (IC_{50} below 0.01 ug/mL).

These results are thought to support the medicinal folkloric background of *P. polyphylla* var. *chinensis*, which has long been used as treatment for a variety of human gastric cancers in China [15].

EXPERIMENTAL

Material and Instruments. The roots of *Paris polyphylla* var. *chinensis* were collected in Nantong Jiangsu province in May 2003 and air-dried. The identity of plant material was verified by Prof. Wang Qiang. A voucher specimen and standard D-Glc, L-Rha, and D-Xyl are kept at the Department of Chinese Materia Medica Analysis, China Pharmaceutical University.

NMR spectra were measured in pyridine-d_5 and recorded on a Bruker AM-500 spectrometer (500 MHz for ^{1}H NMR, 125 MHz for ^{13}C NMR) using standard Bruker pulse programs. Chemical shifts are given as δ values with reference to TMS as internal standard. UV and ESI-MS spectra were recorded on a HP1100 LC/MSD TRAP. Melting point was obtained on a X-4 apparatus and corrected. Optical rotations were measured by using a Jasco DIP-360 automatic digital polarimeter.

Macroreticular resin D101 (Tianjin Pesticide, China), silica gel (Qingdao Marine Chemical, China), ODS silica-gel (Nacalai Tesque, Japan), and Sephadex LH-20 (Amersham Biosciences, USA) were used for column chromatography, and precoated silica-gel F_{254} (0.25mm thick, Qingdao Marine Chemical, China) and Rp-18 F_{254} (0.25 mm thick, Merck, Germany) plates for TLC. The spots on TLC were visualized by spraying on plates with 10% H_2SO_4–EtOH solution, followed by heating. All other Chemicals used were of analytical grade.

The following materials and reagents were used for cell culture and assay of cytotoxic activity: microplate reader (Bio-Tek Instruments Inc.), 96-well flat-bottom plate (Iwaki Glass, Japan), $HepG_2$, SGC7901, BxPC3 cell lines (Nanjing Medical University, China), and RPMI 1640 medium (Gibco. Brl., USA), MTT (Sigma, USA). All other reagents were of biochemical reagent grade.

Extraction and Isolation. The plant material (dry weight 4.0 kg) was extracted with hot 70% ethanol three times (each 12 L, 3 h). The ethanol extract was concentrated under reduced pressure, and the viscous concentrate was passed through a D101 column, successively eluting with H_2O, 40% ethanol, and 80% ethanol. Column chromatography of the 80% ethanol elute portion (80.5g) on silica gel and elution with a stepwise gradient mixture of $CHCl_3$–MeOH (9:1, 17:3, 4:1, 7:3) gave four fractions. The 40% ethanol elute portion (23.6 g) on silica-gel with $CHCl_3$–MeOH (9:1, 4:1) gave two fractions. Fraction (2.2 g) was chromatographed on silica-gel eluting with $CHCl_3$–MeOH (19:1, 9:1) and ODS silica-gel with MeOH–H_2O (17:3, 9:1) to give **1** (23.2 mg) and **2** (9.2 mg). Fraction (4.5 g) on silica-gel eluting with $CHCl_3$–MeOH–H_2O (17:2:0.05, 17:3:0.1) and on ODS silica-gel with MeOH–H_2O (6:1, 5:1) gave **3** (24.7 mg) and **4** (10.7 mg). Fraction (17.8 g) on silica-gel eluting with $CHCl_3$–MeOH–H_2O (33:7:0.1, 4:1:0.2) and ODS silica-gel with MeOH–H_2O (5:1, 4:1) gave **5** (3.26 g), **6** (7.7 mg), and **7** (9.8 mg). Fraction (3.5 g) on silica-gel eluting with $CHCl_3$–MeOH–H_2O (31:9, 3:1, 0.2) and ODS silica-gel with MeOH–H_2O (7:3, 7:4) gave **8** (39.6 mg), **9** (21.2 mg), and **10** (7.1 mg). Fraction (3.2 g) was chromatographed on Sephadex LH-20 eluting with $CHCl_3$-MeOH (1:2) and on silica-gel with $CHCl_3$–MeOH (9:1, 10:1) to give **11** (33.3 mg) and **12** (20.8 mg). Fraction (1.2 g) was chromatographed on ODS silica-gel with MeOH–H_2O (4:1, 5:1) and on Sephadex LH-20 eluting with $CHCl_3$–MeOH (1:2) to give **13** (9.7mg).

Acid Hydrolysis of 13. A solution of **13** (1.5 mg) in 1 M HCl–MeOH (2 mL) was refluxed on a water bath for 4 h as sample solution. We spot 10 μL of sample solution and the standard solution (Glc 0.1 mg/mL, Rha 0.1mg/mL, Xyl 0.1 mg/mL in 1 M HCl–MeOH), respectively, on a plate of silica gel for thin-layer chromatography. We develop the plate with a mixture of *n*-butanol, acetone, and water (4:5:1) to a distance of about 7 cm, and visualized it by spraying on plates with 10% H_2SO_4–EtOH solution. Three spots among several spots from the sample solution are similar to the spots from the standard solution in color tone and R_f value.

Cell Culture Assay. The cytotoxic activity was measured using $HepG_2$, SGC7901, and BxPC3 by MTT. Parental cells stored in liquid nitrogen were thawed by gentle agitation of their containers (vials) for 2 min in a waterbath at 37°C. After

thawing, the content of each vial was transferred to a 75 cm^2 tissue culture flask, diluted with RPMI 1640 supplemented with 10% fetal bovine serum (FBS) and 1% streptomycin and penicillin, and incubated for 24 h at 37°C in a 5% CO_2 incubator to allow the cells to grow and form a monolayer in the flask. Cells grown to 80–95% confluence were washed with phosphate buffer saline (PBS), trypsinized with 3 mL of 0.25% (v) trypsin – 0.3%/v EDTA, diluted, counted, and seeded (5×10^3 cells/well) in 96-well flat-bottom plates. Seeded plates were incubated for 24 h at 37°C in a 5% CO_2 incubator. The old medium was replaced by 180 μL of fresh medium. 20 μL of serial dilutions of samples were added to give final concentrations of 2.5 μg/mL, 0.1 μg/mL, and 0.01 μg/mL, and the medium was added into the control wells and incubated for 48 h. Cell viability assay was performed using the MTT method. The absorbance was read at a wavelength of 550 nm using a microtiter plate reader. A dose response curve was plotted for each compound. Data are mean values of three experiments performed in triplicate.

ACKNOWLEDGMENT

This project was supported by the Hebei Department of Science and Technology of China (No. 06276472), the Hebei Administration of Traditional Chinese Medicine of China (No. 2006191), and the Science Foundation of Hebei Medical University of China (No. 000060).

REFERENCES

1. M. Yoshihiro, K. Minpei, and O. Yuusuke, et al., *Nat. Prod. Lett.*, **14**, 357 (1999).
2. Dequan Yu and Junshan Yang, *Nuclear Magnetic Resonance Spectrum of Analytical Chemistry Manual (volume 7) 2nd ed.*, Chemical Industry Publishers, Beijing, 1999, 901pp.
3. Yun Huang, Qiang Wang, and Wencai Ye, *Chin. J. Nat. Med.*, **3**, 138 (2005).
4. Yun Huang, Lijian Cui, and Qiang Wang, *Acta Pharm. Sin.*, **41**, 361 (2006).
5. Huang Yun, Cui Lijian, and Wang Qiang, *China J. Chin. Mater. Med.*, **31**, 1230 (2006).
6. Hai Liu, Yun Huang, Qing Wang, and Ting Zhang, et al., *Planta Med.*, **72**, 835 (2006).
7. Xu Xuemin and Zhong Chichang, *Chin Trad. Herb Drugs*, **19**, 2 (1988).
8. M. Mitsuhiko, N. Kimiko, N. Toshihiro, T. Toshiaki, and K. Toshio, *Chem. Pharm. Bull.*, **30**, 712 (1982).
9. Chen Changxiang and Zhou Jun, *Acta Bot. Yunnan*, **6**, 111 (1984).
10. Chen Changxiang, Zhou Jun, and Zhang YunTong, *Acta Bot. Yunnan*, **5**, 219 (1983).
11. S. B. Singh and R. S. Thakur, *Tetrahedron*, **38**, 2189 (1982).
12. S. Apichart and S. Chatriwat, *Phytochemistry*, **32**, 303 (1993).
13. Chen Jijun, Zhu Zhenlu, and Luo Shide, *Acta Bot. Yunnan*, **22**, 97 (2000).
14. K. C. Ajit, D. Binayak, and C. P. Satyesh, *Phytochemistry*, **21**, 2083 (1982).
15. Shi Xiaofeng and Du Deji, *Acta Chin. Med. Pharmacol.*, **19**, 42 (1991).