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Triterpenoid saponins from *Impatiens siculifer*

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ABSTRACT

Triterpenoid saponins, impatienosides A–G, together with 12 known saponins, were isolated from the whole plants of *Impatiens siculifer*. Their structures were established on the basis of extensive 1D and 2D NMR and MS analyses coupled with chemical degradation. Cytotoxic activities of the isolated saponins were evaluated against three human cancer cell lines: human myeloid leukemia HL-60 cells, human stomach KATO-III adenocarcinoma, and human lung A549 adenocarcinoma.

1. Introduction

Impatiens siculifer (Balsaminaceae) is an annual herb found in the southwestern region of the People's Republic of China. It has been used in traditional Chinese medicine for treatment of rheumatoid pain and paralysis, burns, scalds, bruises, and fractures (State Administration of Traditional Chinese Medicine, 1999). Pharmacological studies on this plant have demonstrated analgesic and antiphlogistic activities (Du and Xu, 1995). However, only three compounds, coumarin, *N*-phenyl-2-naphthylamine, and α-spinasterol have been reported by chemical investigation (Du and Xu, 1995). As a part of our ongoing investigation on bioactive triterpenoid saponins, we investigated the chemical constituents of *I. siculifer*, which resulted in isolation of 19 triterpenoid saponins, including seven new triterpenoid saponins, impatienosides A (**2**), B (**7**), C (**9**), D (**10**), and E–G (**12**–**14**) (Chart 1). In this paper, we report the isolation and structure elucidation of these new compounds, along with the cytotoxic activities of isolated saponins **1**–**19** against three human cancer cell lines: human myeloid leukemia HL-60 cells, human stomach KATO-III adenocarcinoma, and human lung A549 adenocarcinoma.

2. Results and discussion

The known compounds were identified as 3-*O*-α-L-arabinopyranosyl-(1 → 2)-β-D-glucuronopyranosyl-soyasapogenol E (**1**) (Fang et al., 2004), soyasaponin Bg (**3**) (Tsunoda et al., 2008), dehydrosoyasaponin I (**4**) (Konoshima et al., 1991), sandosaponin A (**5**) (Yoshikawa et al., 1997), 22-*O*-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranosyl-soyasapogenol A (**6**) (Kitagawa et al., 1985a), 3-*O*-α-L-arabinopyranosyl-(1 → 2)-β-D-glucuronopyranosyl-22-*O*-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranosyl-soyasapogenol A (**8**) (Kitagawa, 1989), soyasaponin A_1 (**11**) (Kitagawa et al., 1985b), soyasapogenol B monoglucuronide (**15**) (Udayama et al., 1998), soyasaponin IV (**16**) (Cui et al., 1992), soyasaponin I (**17**) (Rao et al., 1985), soyasaponin I methyl ester (**18**) (Cui et al., 1992), and soyasaponin II (**19**) (Cui et al., 1992) by detailed NMR spectroscopic analysis and comparison with literature data. Although known in other genera, this is the first report of the isolation of these known compounds from *Impatiens* genus. This is the first time that saponin **1** has been isolated in a pure state.

Impatienoside A (**2**) was isolated as an amorphous solid, $[\alpha]_D^{22}$ −10.5 (*c* 1.00, MeOH). Its molecular formula $C_{42}H_{66}O_{14}$ was determined from data of the positive-ion HRESIMS (*m/z* 817.4313, $[M+Na]^+$). On acid hydrolysis, **2** afforded soyasapogenol E (Konoshima et al., 1991) and the monosaccharides D-glucuronic acid and D-galactose. Comparison of the ^{13}C NMR spectroscopic data of **2** and soyasapogenol E showed a glycosylation shift at C-3 (+10.7 ppm) in **2**, suggesting that **2** was a 3-*O*-monodesmoside of soyasapogenol E. The ^1H and ^{13}C NMR spectra (Tables 2 and 4) indicated the presence of β-glucuronopyranosyl and β-galactopyranosyl moieties, with their anomeric proton and carbon resonances at δ_H 4.97 (*d*, *J* = 7.4 Hz, GlcA-H-1) and δ_H 105.6 (GlcA-C-1), and δ_H 5.52 (*d*, *J* = 7.6 Hz, Gal-H-1) and δ_C 105.0 (Gal-C-1), respectively. The HMBC correlations between GlcA-H-1 (δ_H 4.97) and C-3 (δ_C 90.7), and Gal-H-1 (δ_H 5.52) and GlcA-C-2 (δ_C 81.2) established the attachment of the two sugars. Thus, impatienoside A (**2**) was determined as 3-*O*-β-D-galactopyranosyl-(1 → 2)-β-D-glucuronopyranosyl-soyasapogenol E.

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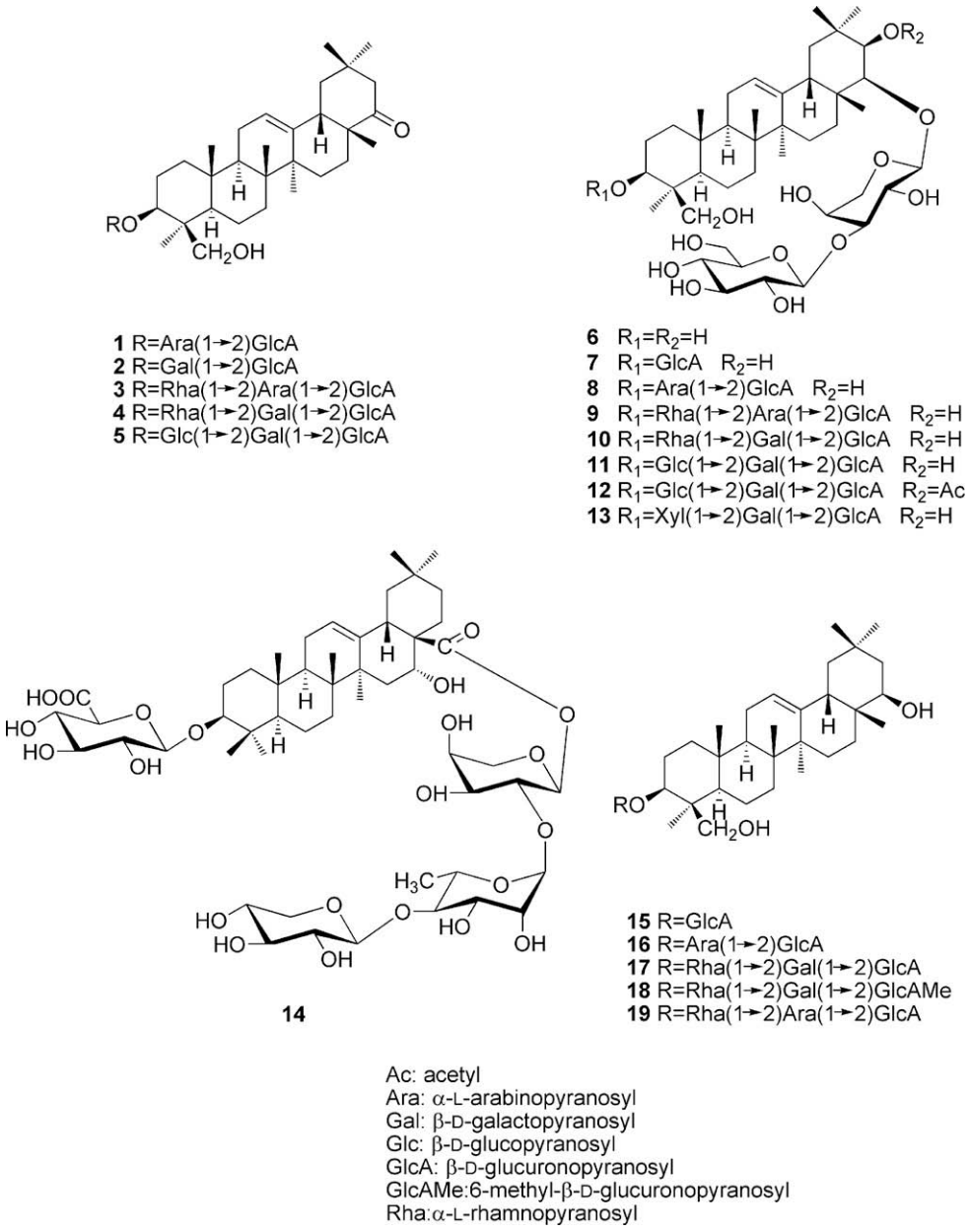

Chart 1.

Impatienosides B (**7**), C (**9**), D (**10**), E (**12**), and F (**13**) were isolated as amorphous solids. On acid hydrolysis, they afforded the same aglycone, soyasapogenol A (Kitagawa et al., 1985a), but different component sugars, suggesting these compounds were structural related analogs.

The molecular formula of impatienosides B (**7**) was determined as $C_{47}H_{76}O_{19}$ from the positive-ion HRESIMS (*m/z* 967.4900, $[M+Na]^+$). On acid hydrolysis, **7** afforded D-glucuronic acid, D-glucose, and L-arabinose as component sugars. Comparison of the ^1H and ^{13}C NMR spectroscopic data of **7** and 22-*O*-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranosyl-soyasapogenol A (**6**) showed superimposable resonances, except for the presence of an additional β-glucuronopyranosyl moiety in **7**. Further comparison of the ^{13}C NMR spectroscopic data of the aglycone parts of **7** and **6** showed a glycosylation shift at C-3 (+8.8 ppm) in **7**, suggesting the β-glucuronopyranosyl was connected to C-3. This was confirmed by the HMBC correlation between GlcA-H-1 (δ_H 5.51) and C-3 (δ_C 89.0). Thus, impatienoside B (**7**) was determined as 22-*O*-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranosyl-3-*O*-β-D-glucuronopyranosyl-soyasapogenol A.

The molecular formula of impatienoside C (**9**) was determined as $C_{58}H_{94}O_{27}$ from the positive-ion HRESIMS (*m/z* 1245.5923, $[M+Na]^+$). On acid hydrolysis, **9** afforded D-glucose, L-rhamnose, and L-arabinose as component sugars. Comparison of the ^1H and ^{13}C NMR spectroscopic data of **9** and **8** showed superimposable resonances, except for the presence of an additional α-arabinopyranosyl moiety in **9**. Further comparison of the ^{13}C NMR spectroscopic data of **9** and **3** showed identical resonances for the sugar chain at C-3, which was determined as a 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 2)-β-D-glucocuronopyranosyl moiety from the HMBC correlations between Rha-H-1 (δ_H 6.23) and δ_C 77.6 (Ara-C-2), Ara-H-1 (δ_H 5.59) and δ_C 76.7 (GlcA-C-2), and GlcA-H-1 (δ_H 4.98) and δ_C 91.0 (C-3). Thus, the structure of impatienoside C (**9**) was elucidated as 22-*O*-β-D-gluco-

Table 1
^{13}C NMR spectroscopic data (δ) of **2**, **7**, **9**, **10**, and **12–14** (125 MHz in pyridine-d_5).[a]

Position	2	7	9	10	12	13	14
1	38.6	38.6	38.7	38.6	38.5	38.6	38.8
2	26.7	26.9	26.7	26.7	26.6	26.7	26.7
3	90.7	89.0	91.0	91.2	90.8	90.6	89.1
4	43.9	44.4	44.0	43.9	43.9	43.8	39.5
5	56.1	56.1	56.0	56.1	56.1	56.1	55.9
6	18.7	18.8	18.6	18.5	18.6	18.6	18.6
7	33.1	33.1	32.9	32.8	32.9	32.9	33.5
8	39.8	40.2	39.3	40.2	40.1	40.1	40.1
9	47.9	47.7	47.8	47.7	47.7	47.7	47.2
10	36.5	36.8	36.5	36.5	36.6	36.4	37.0
11	24.0	24.1	24.1	24.1	24.0	24.1	23.8
12	123.7	123.1	122.6	122.5	122.7	122.6	123.3
13	141.9	144.3	144.3	144.3	144.1	144.2	144.4
14	42.0	41.9	41.8	41.8	41.8	41.8	42.1
15	25.4	26.7	26.7	26.7	26.6	26.6	36.0
16	27.4	27.9	27.8	27.8	28.0	27.8	74.1
17	47.8	39.4	40.2	39.3	39.1	39.2	49.6
18	47.6	44.4	44.4	44.4	44.2	44.4	41.3
19	46.7	47.2	47.2	47.2	47.3	47.2	47.2
20	34.1	36.5	36.8	36.8	36.4	36.8	30.9
21	50.9	75.8	72.8	75.8	75.8	75.8	36.2
22	215.6	92.9	92.9	92.9	85.3	92.9	32.1
23	22.8	23.3	23.1	23.0	22.9	23.1	28.2
24	63.3	63.3	63.5	63.6	63.6	63.6	17.0
25	15.7	15.5	15.8	15.8	15.7	15.7	15.7
26	16.7	16.8	16.7	16.7	16.7	16.7	17.6
27	25.5	26.7	26.7	26.7	26.7	26.7	27.2
28	21.0	23.2	23.0	23.0	22.8	22.8	175.9
29	31.9	31.5	31.5	31.5	31.4	31.4	33.3
30	25.3	21.3	21.3	21.2	21.2	21.2	24.8
$COCH_3$					170.4		
$COCH_3$					21.9		

[a] Assignments were based on DEPT, CHSHF, HMQC, HMQC-TOCSY, and HMBC experiments.

Table 2
^{13}C NMR spectroscopic data (δ) of sugar moieties of **2**, **7**, **9**, **10**, and **12–14** (125 MHz in pyridine-d_5).[a]

Position	2	7	9	10	12	13	14
3-*O*-sugar							
GlcA-1	105.6	106.3	105.5	105.5	104.8	104.6	107.2
2	81.2	75.7	76.7	76.7	80.9	81.0	75.6
3	77.7	78.1	78.3	78.6	77.6	77.9	78.2
4	73.0	73.5	74.0	73.9	72.8	72.7	73.4
5	78.2	78.0	77.8	77.9	77.8	77.8	77.9
6	172.7	172.7	172.6	172.7	172.4	172.7	172.8
	Gal		AraI	Gal	Gal	Gal	28-*O*-Ara
1	105.0		101.9	101.8	103.0	103.0	93.6
2	73.7		77.6	76.8	84.2	84.3	75.1
3	75.5		75.8	76.5	74.7	74.7	70.2
4	71.1		70.6	71.2	70.6	70.5	66.2
5	77.3		67.0	77.6	76.6	76.6	63.3
6	62.8			61.7	62.6	62.4	
			Rha	Rha	GlcI	Xyl	Rha
1			102.5	102.5	106.5	106.7	101.1
2			72.5	72.4	74.5	75.3	71.9
3			72.8	72.8	78.4	78.3	72.7
4			74.4	74.4	71.6	71.0	83.5
5			69.5	69.5	78.5	67.1	68.6
6			19.0	19.0	62.8		18.4
22-*O*-sugar		Ara	AraII	Ara	Ara	Ara	Xyl
1		108.5	108.5	108.5	103.2	108.4	106.8
2		72.8	72.8	72.8	72.5	72.9	76.0
3		85.4	85.4	85.4	81.7	84.8	78.6
4		69.3	69.3	69.3	69.5	69.4	71.0
5		67.5	67.5	67.5	67.0	67.4	67.4
		Glc	Glc	Glc	GlcII	Glc	
1		106.5	106.3	106.3	106.9	106.6	
2		75.4	75.7	75.7	76.7	75.8	
3		78.5	78.4	78.4	78.0	78.3	
4		71.6	71.6	71.6	71.6	71.3	
5		78.7	78.7	78.7	79.1	79.0	
6		62.7	62.7	62.7	62.9	62.6	

[a] Assignments were based on DEPT, CHSHF, HMQC, HMQC-TOCSY, and HMBC experiments.

pyranosyl-(1 → 3)-α-L-arabinopyranosyl-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranosyl-(1 → 2)-β-D-glucocuronopyranosyl-soyasapogenol A.

Impatienoside D (**10**) had the molecular formula $C_{59}H_{96}O_{28}$ determined from the positive-ion HRESIMS (*m/z* 1275.6033, $[M+Na]^+$). On acid hydrolysis, **10** afforded D-glucuronic acid, D-glucose, L-rhamnose, D-galactose, and L-arabinose as component sugars. Comparison of the ^1H and ^{13}C NMR spectroscopic data of **10** and **9** showed superimposable resonances, except that the α-arabinopyranosyl moiety in **9** was replaced by a β-galactopyranosyl moiety in **10**. The C-3 sugar chain was shown to be a 3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 2)-β-D-glucocuronopyranosyl moiety from the HMBC correlations between Rha-H-1 (δ_H 6.24) and δ_C 76.8 (Gal-C-2), Gal-H-1 (δ_H 5.75) and δ_C 76.7 (GlcA-C-2), and GlcA-H-1 (δ_H 4.99) and δ_C 91.2 (C-3). Thus, impatienoside D (**10**) was determiend as 22-*O*-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranosyl-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 2)-β-D-glucocuronopyranosyl-soyasapogenol A.

The molecular formula of impatienoside E (**12**) was $C_{61}H_{98}O_{30}$ from the positive-ion HRESIMS (*m/z* 1333.6007, $[M+Na]^+$). On acid hydrolysis, **12** afforded D-glucuronic acid, D-glucose, D-galactose, and L-arabinose as component sugars. Comparison of the ^1H and ^{13}C NMR spectroscopic data of **12** and soyasaponin A_1 (**11**) showed superimposable resonances, apart from the presence of an acetyl moiety in **12**. The acetyl moiety was located at C-21 from the HMBC correlation between H-21 (δ_H 3.98) and $COCH_3$ (δ_C 170.4). Thus, impatienoside E (**12**) was elucidated as 21-*O*-acetyl-22-*O*-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranosyl-3-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 2)-β-D-glucuronopyranosyl-soyasapogenol A.

The molecular formula of impatienoside F (**13**) was $C_{58}H_{94}O_{28}$ from the positive-ion HRESIMS (*m/z* 1261.5875, $[M+Na]^+$). On acid hydrolysis, **13** afforded D-glucuronic acid, D-glucose, D-galactose, D-xylose, and L-arabinose as component sugars. Comparison of the ^1H and ^{13}C NMR spectroscopic data of **13** and **10** showed superimposable resonances, except that the α-rhamnopyranosyl moiety in **10** was replaced by a β-xylopyranosyl moiety in **13**. The C-3 sugar moiety was 3-*O*-β-D-xylopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 2)-β-D-glucocuronopyranosyl moiety by the HMBC correlations between Xyl-H-1 (δ_H 5.11) and Gal-C-2 (δ_C 84.3), Gal-H-1 (δ_H 5.57) and GlcA-C-2 (δ_C 81.0) and GlcA-H-1 (δ_H 5.40) and C-3 (δ_C 90.6). Thus, impatienoside F (**13**) was determined as 22-*O*-β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranosyl-3-*O*-β-D-xylopyranosyl-(1 → 2)-β-D-galactopyranosyl-(1 → 2)-β-D-glucocuronopyranosyl-soyasapogenol A.

Impatienoside G (**14**) was isolated as an amorphous solid. $[\alpha]_D^{22}$ −31.0 (*c* 1.00, MeOH). Its molecular formula $C_{52}H_{82}O_{22}$ was determined from the positive-ion HRESIMS (*m/z* 1081.5166, $[M+Na]^+$). Acid hydrolysis of **14** afforded echinocystic acid (Zhang et al., 1999) as the aglycone, and D-glucuronic acid, L-arabinose, D-xylose, and L-rhamnose as component sugars. Comparing the ^{13}C NMR spectroscopic data with echinocystic acid, a glycosylation shift was observed at C-3 (+11.0 ppm) and C-28 (−4.0 ppm) in **14**, suggesting that **14** was a bisdesmosidic glycoside. The ^1H and ^{13}C NMR spectra suggested the presence of four sets of sugar moieties, an α-arabinopyranosyl, a D-xylopyranosyl, an α-rhamnopyranosyl, and a β-glucuronopyranosyl moieties. The attachment of the β-glucuronopyranosyl moiety to C-3 was established by the HMBC correlation between GlcA-H-1 (δ_H 5.03) and C-3 (δ_C 89.1). The sugar chain at C-28 was shown to be 28-*O*-β-D-xylopyranosyl-(1 → 4)-rhamnopyr-

Table 3
^{1}H NMR spectroscopic data (δ) of the aglycone moieties of **2**, **7**, **9**, **10**, and **12**–**14** (500 MHz in pyridine-d_5).

Position	**2**	**7**	**9**	**10**	**12**	**13**	**14**
1	0.79 (*td*, 13.4, 3.7)	0.85 (*td*, 14.2, 3.4)	0.84 (*qd*, 11.0, 3.6)	0.77 (*m*)	0.73 (*br d*, 12.3)	0.80 (*m*)	0.91 (*td*, 11.4, 2.3)
	1.34[a]	1.44 (*br d*, 12.9)	1.36 (*br d*, 16.7)	1.33[a]	1.30[a]	1.38[a]	1.44 (*br d*, 13.1)
2	1.91 (*qd*, 13.3, 4.3)	2.00 (*qd*, 13.3, 3.2)	1.96 (*qd*, 12.1, 4.1)	1.83 (*qd*, 13.1, 4.4)	1.89 (*qd*, 9.9, 3.6)	1.92 (*m*)	1.88[a]
	2.29 (*br d* d, 13.7, 3.6)	2.21 (*br dd*, 14.9, 4.3)	2.23 (*br dd*, 14.2, 3.9)	2.19 (*br dd*, 12.8, 2.9)	2.21 (*br d*, 10.0)	2.39 (*m*)	2.24 (*br dd*, 11.6, 4.3)
3	3.46 (*dd*, 11.4, 4.1)	3.60 (*dd*, 11.4, 5.2)	3.40 (*dd*, 11.2, 4.1)	3.40 (*dd*, 11.7, 3.9)	3.39 (*dd*, 11.5, 4.4)	3.47 (*dd*, 11.3, 4.2)	3.40 (*dd*, 11.7, 4.1)
5	0.85[a]	0.93[a]	0.86 (*br d*, 11.9)	0.84 (*d*, 12.2)	0.78 (*br d*, 11.9)	0.80 (*br d*, 12.2)	0.82 (*t*, 12.0)
6	1.30 (*qd*, 12.6, 2.7)	1.46 (*qd*, 13.0, 2.1)	1.25[a]	1.22[a]	1.28 (*qd*, 13.3, 4.3)	1.25 (*br d*, 9.0)	1.34 (*qd*, 13.8, 5.0)
	1.58 (*br d*, 14.2)	1.69 (*br d*, 12.0)	1.57 (*br d*, 14.0)	1.54 (*br d*, 15.3)	1.50[a]	1.53[a]	1.50[a]
7	1.23 (*dt*, 12.8, 3.2)	1.27 (*dt*, 12.4, 3.0)	1.25[a]	1.20[a]	1.22[a]	1.21[a]	1.50[a]
	1.42 (*td*, 13.1, 3.9)	1.51 (*td*, 13.3, 3.0)	1.47 (*dd*, 13.2, 2.9)	1.33[a]	1.39[a]	1.41 (*br d*, 15.8)	1.58 (*td*, 12.9, 3.0)
9	1.55 (*dd*, 10.5, 7.3)	1.61 (*dd*, 10.3, 6.8)	1.59 (*dd*, 10.8, 7.3)	1.57 (*dd*, 10.1, 7.6)	1.50 (*br t*, 8.6)	1.53 (*br d*, 9.8)	2.78 (*br t*, 13.3)
11	1.79 (2H, *m*)	1.84 (2H, *m*)	1.80 (2H, *m*)	1.81 (2H, *m*)	1.74 (2H, *m*)	1.78 (2H, *m*)	1.88 (2H, *m*)
12	5.24 (*t*-like, 3.4)	5.32 (*t*-like, 3.2)	5.31 (*t*-like, 3.5)	5.32[a]	5.11 (*t*-like, 3.9)	5.12 (*t*-like, 3.2)	5.61 (*t*-like, 3.5)
15	0.99 (*dt*, 12.4, 3.2)	0.99 (*br d*, 12.8)	0.98 (*br d*, 13.3)	0.98 (*br d*, 12.4)	0.93 (*br d*, 13.5)	0.98 (*br d*, 12.6)	1.86 (*br d*, 11.9)
	1.67 (*td*, 13.6, 3.9)	1.85 (*td*, 12.6, 4.6)	1.83 (*dt*, 13.7, 4.6)	1.83 (*dt*, 13.1, 4.4)	1.79 (*td*, 14.7, 4.7)	1.80 (*dt*, 14.7, 4.4)	2.31 (*br dd*, 15.2, 3.9)
16	1.19 (*br d*, 12.1)	1.12 (*br d*, 11.9)	1.10 (*br dd*, 15.6, 4.9)	1.09 (*br dd*, 11.4)	1.11 (*br d*, 10.1)	1.10 (*br t*, 10.1)	5.27 (*br s*)
	2.08 (*td*, 13.1, 3.7)	1.99 (*td*, 13.1, 4.4)	1.96 (*td*, 12.1, 4.1)	1.96 (*td*, 12.6, 3.7)	1.96 (*td*, 13.7, 3.6)	1.95 (*qd*, 10.7, 5.0)	
18	2.38 (*dd*, 13.7, 3.9)	2.46 (*dd*, 13.5, 3.2)	2.44 (*dd*, 13.0, 2.9)	2.43 (*dd*, 13.0, 3.0)	2.40 (*br d*, 13.6)	2.43 (*dd*, 12.6, 3.0)	3.56 (*dd*, 14.4, 3.6)
19	1.31 (*m*)	1.33[a]	1.32[a]	1.31 (*dd*, 13.7, 6.1)	1.30 (*m*)	1.33 (*br d*, 10.5)	1.77[a]
	2.17 (*dd*, 13.7, 11.2)	2.08 (*br t*, 13.8)	2.07 (*t*, 13.7)	2.06 (*br t*, 13.8)	2.03 (*br t*, 14.0)	2.07 (*br t*, 13.8)	1.30 (*d*, 8.0)
21	2.13 (*d*, 13.8)	3.90 (*d*, 2.1)	3.89 (*d*, 1.1)	3.90 (*d*, 1.2)	3.98 (*d*, 6.9)	3.90[a]	1.36 (*dd*, 13.8, 5.0)
	2.58 (*d*, 13.9)						2.42 (*td*, 12.6, 4.3)
22		3.76 (*d*, 2.7)	3.75 (*d*, 2.8)	3.75 (*d*, 2.5)	3.97 (*d*, 6.2)	3.76 (*d*, 2.8)	2.20 (*td*, 13.0, 4.0)
							2.33 (*dt*, 14.8, 3.9)
23	1.38 (*s*)	1.56 (*s*)	1.34 (*s*)	1.32 (*s*)	1.41 (*s*)	1.34 (*s*)	1.29 (*s*)
24	3.41 (*d*, 11.2)	3.67 (*d*, 11.9)	3.29 (*d*, 11.5)	3.25 (*d*, 11.3)	3.37 (*d*, 11.5)	3.36 (*d*, 10.8)	0.99 (*s*)
	4.32 (*d*, 11.5)	4.40 (*d*, 12.0)	4.28 (*d*, 11.4)	4.25 (*d*, 11.7)	4.32 (*d*, 11.3)	4.30 (*d*, 10.8)	
25	0.74 (*s*)	0.81 (*s*)	0.69 (*s*)	0.67 (*s*)	0.70 (*s*)	0.68 (*s*)	0.85 (*s*)
26	0.87 (*s*)	0.94 (*s*)	0.90 (*s*)	0.89 (*s*)	0.87 (*s*)	0.89 (*s*)	1.08 (*s*)
27	1.27 (*s*)	1.30 (*s*)	1.30 (*s*)	1.29 (*s*)	1.24 (*s*)	1.25 (*s*)	1.82 (*s*)
28	1.17 (*s*)	1.35 (*s*)	1.43 (*s*)	1.44 (*s*)	1.25 (*s*)	1.42 (*s*)	
29	0.96 (*s*)	1.22 (*s*)	1.21 (*s*)	1.20 (*s*)	1.23 (*s*)	1.21 (*s*)	1.02 (*s*)
30	0.85 (*s*)	1.35 (*s*)	1.34 (*s*)	1.34 (*s*)	1.37 (*s*)	1.34 (*s*)	1.14 (*s*)
$COCH_3$					2.35 (*s*)		

[a] Overlapped signals.

anosyl-(1 → 2)-α-L-arabinopyranosyl moiety by the HMBC correlations between Xyl-H-1 (δ_H 5.17) and Rha-C-2 (δ_C 71.9), Rha-H-1 (δ_H 5.29) and Ara-C-2 (δ_C 75.1), Ara-H-1 (δ_H 6.45) and C-28 (δ_C 175.9). Thus, impatienoside G (**14**) was determined as 3-*O*-β-D-glucocuronopyranosyl-echinocystic acid-28-*O*-β-D-xylopyranosyl-(1 → 4)-α-L-rhamnopyranosyl-(1 → 2)-α-L-arabinopyranoside.

The isolated saponins (**1–19**) were evaluated for their cytotoxic activities against human HL-60 myeloid leukemia, human stomach KATO-III adenocarcinoma, and human lung A549 adenocarcinoma cells. Compound **14** showed moderate cytotoxic activities against HL-60, KATO-III, and A549 cells with IC_{50} values of 21.8 ± 0.4, 36.7 ± 4.4, and 24.8 ± 4.8 μM, respectively, whereas etoposide was used as the positive control showing the IC_{50} values of 0.3 ± 0.04, 0.1 ± 0.01, and 20.6 ± 8.2 μM, respectively. Other compounds were inactive for the three cell lines at 50 μM.

3. Conclusions

As a conclusion, 19 triterpenoid saponins, including seven new compounds, impatienosides A–G, were isolated from the whole plants of *I. siculifer*. Compound **14** showed moderate cytotoxic activities against HL-60, KATO-III, and A549 cells. To our knowledge, in genus *Impatiens*, only five triterpenoid saponins have been reported from the rhizomes of *I. pritzellii* var. *hupehensis* (Zhou et al., 2007). This work described the first phytochemical profile of the saponin contents of *I. siculifer*.

4. Experimental

4.1. General

IR spectra were recorded with a JASO FT/IR-4100 (by a KBr disk method) spectrometer, whereas optical rotations were measured with a JASCO DIP-370 digital polarimeter in a 0.5-dm cell. The ^{1}H and ^{13}C NMR spectra were recorded using a JEOL ECP-500 NMR spectrometer with TMS as the internal reference, and chemical shifts are expressed in δ (ppm). ESIMS and HRESIMS were conducted using a JEOL JMS-T100LP AccuTOF LC-plus mass spectrometer. Diaion HP-20 resin (Mitsubishi Chemical Corporation, Tokyo, Japan), silica gel (silica Gel 60N, Kanto Chemical Co. Inc., Tokyo, Japan), and octadecylsilyl silica gel (ODS) (Chromatorex, 100–200 mesh, Fuji Sylisia Chemical Ltd., Aichi, Japan) were used for column chromatography (CC). For HPLC, a JASCO PU-1580 HPLC system, equipped with a Shodex RI-71 Differential Refractometer detector, was used. TLC was conducted in Kieselgel 60 F_{254} plates (E. Merck). GLC was carried out on a Perkin–Elmer Clarus 500 GC–MS instrument.

4.2. Plant material

The whole plants of *I. siculifer* were collected from Guizhou Province, People's Republic of China in September 1999, and iden-

Table 4
^{1}H NMR spectroscopic data (δ) of sugar moieties of **2**, **7**, **9**, **10**, and **12**–**14** (500 MHz in pyridine-d_5).

Position	2	7	9	10	12	13	14
3-*O*-sugar							
GlcA-1	4.97 (*d*, 7.4)	5.51 (*d*, 7.6)	4.98 (*d*, 7.1)	4.99 (*d*, 7.6)	5.08 (*d*, 7.7)	5.40 (*d*, 7.8)	5.03 (*d*, 7.7)
2	4.34 (*t*, 9.3)	4.03 (*t*, 8.1)	4.49 (*t*, 9.2)	4.58 (*t*, 8.2)	4.26 (*t*, 8.3)	4.23 (*t*, 8.4)	4.14 (*t*, 8.3)
3	4.59 (*t*, 10.3)	4.36 (*t*, 8.0)	4.59 (*t*, 9.1)	4.60[a]	4.72 (*t*, 9.2)	4.68 (*t*, 8.9)	4.34 (*t*, 8.9)
4	4.55 (*t*, 9.4)	4.62 (*t*, 8.6)	4.51[a]	4.46 (*t*, 9.0)	4.55 (*t*, 9.8)	4.38[a]	4.62 (*t*, 9.1)
5	4.38 (*d*, 9.4)	4.74 (*d*, 9.6)	4.52[a]	4.64 (*d*, 9.9)	4.68 (*d*, 9.6)	4.50 (*d*, 10.3)	4.70 (*d*, 9.7)
	Gal		AraI	Gal	Gal	Gal	28-*O*-Ara
1	5.52 (*d*, 7.6)		5.59 (*d*, 7.3)	5.75 (*d*, 7.6)	5.63 (*d*, 7.6)	5.57 (*d*, 7.7)	6.45 (*d*, 3.0)
2	4.51 (*dd*, 9.3, 7.3)		4.52 (*t*, 8.5)	4.54 (*dd*, 9.4, 7.3)	4.55 (*dd*, 9.8, 7.1)	4.56 (*dd*, 9.9, 7.4)	4.55[a]
3	4.11 (*dd*, 9.6, 3.2)		4.02 (*br d*, 9.4)	4.07 (*dd*, 8.0, 2.3)	4.17[a]	4.16[a]	4.53[a]
4	4.43 (*br d*, 2.9)		4.02[a]	4.38 (*t*, 2.7)	4.47[a]	4.45 (*br d*, 2.9)	4.40[a]
5	4.01 (*t*, 5.5)		3.57 (*d*, 11.9)	3.94 (*br t*, 6.2)	3.91 (*t*, 5.4)	3.90 (*t*, 10.4)	3.95 (*dd*, 10.5, 3.6)
			4.14 (*br d*, 11.2)				4.51[a]
6	4.36 (*dd*, 11.7, 1.4)			4.35 (*dd*, 11.5, 1.9)	4.35 (*dd*, 11.9, 4.6)	4.34 (*dd*, 13.5, 5.0)	
	4.48 (*dd*, 11.7, 6.2)			4.40 (*dd*, 11.5, 6.0)	4.42 (*dd*, 11.5, 5.7)	4.44 (*dd*, 11.7, 5.9)	
			Rha	Rha	GlcI	Xyl	Rha
1			6.23 (*br s*)	6.24 (*br s*)	4.99 (*d*, 7.8)	5.11 (*d*, 7.5)	5.29 (*br s*)
2			4.79 *dd*, 3.5, 1.4)	4.81 (*dd*, 3.4, 1.6)	3.85 (*t*, 8.2)	4.02 (*t*, 7.9)	4.56 (*dd*, 3.0, 1.2)
3			4.66 (*dd*, 9.2, 3.5)	4.71 (*dd*, 9.2, 3.4)	4.18[a]	4.16 (*t*, 7.8)	4.59 (*dd*, 9.4, 3.2)
4			4.35 (*t*, 9.2)	4.36 (*t*, 8.9)	4.15[a]	4.19[a]	4.40[a]
5			4.96 (*qd*, 9.4, 3.2)	5.01 (*qd*, 9.2, 5.7)	3.95 (*m*)	3.67 (*br d*, 9.6)	4.41[a]
6			1.78 (*d*, 6.2)	1.80 (*d*, 6.2)	4.35[a]	4.28[a]	1.75 (*d*, 5.5)
					4.52 (*dd*, 11.7, 2.5)		
22-*O*-sugar		Ara	AraII	Ara	Ara	Ara	Xyl
1		4.87 (*d*, 7.8)	4.87 (*d*, 7.1)	4.87 (*d*, 7.8)	5.47 (*d*, 7.5)	4.90 (*d*, 7.8)	5.17 (*d*, 7.5)
2		4.62 (*t*, 8.6)	4.62 (*dd*, 9.1, 8.0)	4.61 (*t*, 8.4)	6.07 (*dd*, 9.4, 8.0)	4.62 (*t*, 8.5)	4.02 (*t*, 7.5)
3		4.13 (*dd*, 8.4, 2.8)	4.12 (*dd*, 9.6, 3.2)	4.14 (*dd*, 9.6, 3.2)	4.19 (*dd*, 9.2, 3.2)	4.09 (*dd*, 9.4, 3.2)	4.07 (*t*, 8.9)
4		4.41 (*br s*)	4.42 (*br d*, 2.8)	4.48 (*br s*)	4.47 (*br d*, 2.2)	4.39 (*br d*, 3.0)	4.15 (*m*)
5		3.62 (*d*, 12.4)	3.65 (*d*, 11.7)	3.67 (*d*, 11.7)	3.60 (*br d*,11.5)	3.72 (*dd*, 12.2, 1.8)	3.48 (*t*, 10.5)
		4.20 (*d*, 12.9)	4.20 (*br d*, 13.3)	4.21 (*br d*, 14.7)	4.15 (*dd*, 13.9, 2.3)	4.28 (*br d*, 13.0)	4.21 (*dd*, 11.3, 5.3)
		Glc	Glc	Glc	GlcII	Glc	
1		5.24 (*d*, 7.8)	5.25 (*d*, 7.8)	5.23 (*d*, 8.1)	5.19 (*d*, 7.8)	5.18 (*d*, 8.0)	
2		4.11 (*t*, 8.9)	4.04 (*t*, 8.8)	4.06 (*dd*, 8.7, 8.1)	4.08 (*t*, 8.9)	4.09 (*t*, 8.4)	
3		4.23 (*t*, 8.2)	4.24 (*t*, 8.7)	4.24 (*t*, 8.9)	4.17[a]	4.19 (*t*, 9.0)	
4		4.22 (*t*, 7.4)	4.20 (*t*, 8.7)	4.18 (*t*, 9.2)	4.12 (*t*, 9.0)	4.15 (*t*, 9.2)	
5		3.98 (*m*)	3.98 (*ddd*, 8.4, 5.2, 2.3)	3.97 (*ddd*, 9.2, 5.5, 2.5)	3.94[a]	3.90[a]	
6		4.34 (*dd*, 11.9, 5.0)	4.36 (*dd*, 11.7, 5.5)	4.32 (*dd*, 11.7, 5.2)	4.27 (*dd*, 11.9, 5.5)	4.30[a]	
		4.53 (*dd*, 11.6, 2.3)	4.53 (*dd*, 11.7, 2.3)	4.53 (*dd*, 11.7, 2.3)	4.60 (*dd*, 11.5, 2.1)	4.58 (*dd*, 11.9, 2.1)	

[a] Overlapped signals.

tified by Prof. Jiang Du (Guiyang Collage of Traditional Chinese Medicine). A specimen of the plant (Toho9908) is kept in the herbarium of the Faculty of Pharmaceutical Sciences, Toho University.

4.3. Extraction and isolation

The whole plants (5.0 kg) of *I. siculifer* were extracted three times with EtOH–H_2O (7:3, v/v) for 1 h each at room temperature. The alcohol extract was concentrated (950 g) then partitioned between EtOAc (2 L × 3) and H_2O (2 L). The H_2O layer was partitioned with *n*-BuOH (2 L × 3). The *n*-BuOH layer was evaporated under reduced pressure below 40 °C to give a residue (67.0 g), which was subjected to passage over a Diaion HP-20 column, and washed with MeOH–H_2O (4:6, v/v) and MeOH. The MeOH fraction (28.6 g) was applied to an ODS column eluted with MeOH–H_2O (4:6, v/v), MeOH–H_2O (8:2, v/v), and MeOH in order to give three fractions. The MeOH–H_2O (8:2) eluates were concentrated (16.5 g) and subjected to a silica gel CC with $CHCl_3$–MeOH–H_2O (60:20:3, v/v/v) as eluate to give three saponin fractions. Further purification of the saponin fractions by repeated preparative HPLC with CH_3CN-H_2O (1:1, v/v) containing 0.06% TFA as eluate, afforded **1** (7 mg), **2** (15 mg), **3** (23 mg), **4** (88 mg), **5** (10 mg), **6** (14 mg), **7** (12 mg), **8** (16 mg), **9** (12 mg), **10** (11 mg), **11** (36 mg), **12** (16 mg), **13** (4 mg), **14** (32 mg), **15** (11 mg), **16** (25 mg), **17** (20 mg), **18** (23 mg), and **19** (18 mg).

4.4. Impatienoside A (**2**)

Amorphous solid, $[\alpha]_D^{22}$ −10.5 (*c* 1.00, MeOH); IR (KBr) λ_{max}: 3434, 2924, 2854, 1702, 1637, 1459, 1379, 1274, 1245 cm^{-1}. For ^{1}H NMR (500 MHz, pyridine-d_5) and ^{13}C NMR (125 MHz, pyridine-d_5) spectroscopic data, see Tables 1–4. ESIMS (positive) *m/z* 817 $[M+Na]^+$. HRESIMS (positive) *m/z* 817.4313 $[M+Na]^+$ (calcd. for $C_{42}H_{66}O_{14}Na$, 817.4350).

4.5. Impatienoside B (**7**)

Amorphous solid, $[\alpha]_D^{22}$ +33.4 (*c* 0.86, MeOH); IR (KBr) λ_{max}: 3408, 2925, 1727, 1628, 1459, 1409, 1384, 1256, 1226, cm^{-1}. For ^{1}H NMR (500 MHz, pyridine-d_5) and ^{13}C NMR (125 MHz, pyridine-d_5) spectroscopic data, see Tables 1–4. ESIMS (positive) *m/z* 967 $[M+Na]^+$. HRESIMS (positive) *m/z* 967.4900 $[M+Na]^+$ (calcd. for $C_{47}H_{76}O_{19}Na$, 967.4879).

4.6. Impatienoside C (**9**)

Amorphous solid, $[\alpha]_D^{22}$ +12.1 (*c* 0.73, MeOH); IR (KBr) λ_{max}: 3406, 2926, 1709, 1628, 1412, 1383, 1257, 1225 cm^{-1}. For ^{1}H NMR (500 MHz, pyridine-d_5) and ^{13}C NMR (125 MHz, pyridine-d_5) spectroscopic data, see Tables 1–4. ESIMS (positive) *m/z* 1245

$[M+Na]^+$. HRESIMS (positive) *m/z* 1245.5923 $[M+Na]^+$ (calcd. for $C_{58}H_{94}O_{27}Na$, 1245.5880).

4.7. Impatienoside D (**10**)

Amorphous solid, $[\alpha]_D^{22}$ +12.0 (*c* 0.83, MeOH); IR (KBr) λ_{max}: 3407, 2926, 1721, 1628, 1412, 1384, 1255, 1224 cm^{-1}. For ^1H NMR (500 MHz, pyridine-d_5) and ^{13}C NMR (125 MHz, pyridine-d_5) spectroscopic data, see Tables 1–4. ESIMS (positive) *m/z* 1275 $[M+Na]^+$. HRESIMS (positive) *m/z* 1275.6033 $[M+Na]^+$ (calcd. for $C_{59}H_{96}O_{28}Na$, 1275.5986).

4.8. Impatienoside E (**12**)

Amorphous solid, $[\alpha]_D^{22}$ +11.8 (*c* 1.00, MeOH); IR (KBr) λ_{max}: 3416, 2926, 1735, 1628, 1408, 1383, 1251 cm^{-1}. For ^1H NMR (500 MHz, pyridine-d_5) and ^{13}C NMR (125 MHz, pyridine-d_5) spectroscopic data, see Tables 1–4. ESIMS (positive) *m/z* 1333 $[M+Na]^+$. HRESIMS (positive) *m/z* 1333.6007 $[M+Na]^+$ (calcd. for $C_{61}H_{98}O_{30}Na$, 1333.6041).

4.9. Impatienoside F (**13**)

Amorphous solid, $[\alpha]_D^{22}$ +11.5 (*c* 0.80, MeOH); IR (KBr) λ_{max}: 3398, 2925, 1720, 1619, 1412, 1384, 1292, 1257 cm^{-1}. For ^1H NMR (500 MHz, pyridine-d_5) and ^{13}C NMR (125 MHz, pyridine-d_5) spectroscopic data, see Tables 1–4. ESIMS (positive) *m/z* 1261 $[M+Na]^+$. HRESIMS (positive) *m/z* 1261.5875 $[M+Na]^+$ (calcd. for $C_{58}H_{94}O_{28}Na$, 1261.5829).

4.10. Impatienoside G (**14**)

Amorphous solid, $[\alpha]_D^{22}$ −31.0 (*c* 1.00, MeOH); IR (KBr) λ_{max}: 3408, 2944, 1735, 1629, 1449, 1388, 1364, 1222 cm^{-1}. For ^1H NMR (500 MHz, pyridine-d_5) and ^{13}C NMR (125 MHz, pyridine-d_5) spectroscopic data, see Tables 1–4. ESIMS (positive) *m/z* 1096 $[M+Na]^+$. HRESIMS (positive) *m/z* 1081.5166 $[M+Na]^+$ (calcd. for $C_{52}H_{82}O_{22}Na$, 1081.5195).

4.11. Acid hydrolysis

Solutions of **1**, **2**, **3** (each 2 mg), **7**, **8**, **9**, **10**, **12**, **13** (each 1 mg) in 1 M HCl (dioxane–H_2O, 1:1, 200 μL), and **14** (5 mg) in 1 M HCl (dioxane–H_2O, 1:1, 1 mL) were separately heated at 100 °C for 2 h. After dioxane was removed, each solution was extracted with EtOAc (1 mL × 3). The EtOAc extracts were analyzed by TLC to identify the aglycone by comparison with authentic samples. TLC condition: $CHCl_3$–MeOH (95:5), R_f, 0.37 for soyasapogenol E, 0.18 for soyasapogenol A, and 0.26 for echinocystic acid. The EtOAc extracts for **1**, **2** and **3**, for **7**, **8**, **9**, **10**, **12** and **13**, and for **14** were combined, respectively, then purified by silica gel CC with $CHCl_3$–MeOH (95:5) as eluate to afford soyasapogenol E (2.8 mg), soyasapogenol A (2.4 mg), and echinocystic acid (2.4 mg), which showed identical spectroscopic data with authentic samples. The aqueous layers obtained above were concentrated under reduced pressure to give sugar fractions. Identification of the monosaccharides present in sugar fractions was carried out by the same procedure as in literature (Li et al., 2007). t_R (min): 11.30 (D-glucuronic acid), 10.67 (D-glucose), 10.57 (D-galactose), 7.55 (L-rhamnose), 6.28 (L-arabinose), and 6.25 (D-xylose).

4.12. Cytotoxic assays

The human HL-60 myeloid leukemia, human stomach KATO-III adenocarcinoma, and human lung A549 adenocarcinoma cell lines assays were performed as the same procedures as in our previous report (Chang et al., 2007).

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