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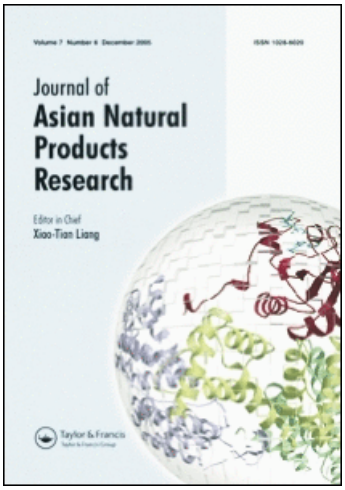

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Triterpene saponins from the leaves of *Ilex kudingcha*

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Three new triterpene saponins, ilekudinosides T–V (**1**–**3**), along with six known saponins were isolated from the 70% ethanolic extract of the leaves of *Ilex kudingcha*. The new saponins were characterized as 3-*O*-β-D-glucopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-3β,19α-dihydroxy-urs-12(13)-en-28,20β-lactone (**1**), 3-*O*-β-D-glucopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-3β,19α-dihydroxy-12-ethoxy-urs-13(18)-ene-28,20β-lactone (**2**), 3-*O*-β-D-glucopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-3β,19α-dihydroxy-11-oxo-urs-13(18)-ene-28,20β-lactone (**3**), respectively. The structures of compounds **1**–**3** were elucidated on the basis of the chemical and spectroscopic evidence, and the structures of known compounds were identified by comparison of their spectroscopic data with those reported in the literature.

Keywords: *Ilex kudingcha*; triterpenoid saponin; ilekudinosides T–V

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1. Introduction

Ilex kudingcha C.J. Tseng (Aquifoliaceae) is an economically very important plant in southern region of China and is also widely cultivated in Guangxi Province. The leaves of this plant are widely used in the form of an aqueous infusion as a beverage or a medicinal plant. The southern Chinese have shared for centuries the custom of drinking this mildly stimulant beverage called 'Ku-Ding-Cha' which has a peculiar flavor and stimulating properties. This infusion is reputed to have a characteristic bitter taste and hypocholesteremic, antioxidant, diuretic, glycogenolytic, and lipolytic properties. Actually, it is used in commercial herbal preparations as a stimulant to the central nervous system, a diuretic, a treatment for sore throats, an aid to weight losing and for the relief of hypertension [1]. Previous studies indicated that saponins are the major constituents of *I. kudingcha* [2–4]. Some authors had also reported inhibition activity of acyl CoA cholesteryl acyl transferase which was related to the compounds isolated from *I. kudingcha* [5,6]. Recent efforts to promote the consumption of Ku-Ding-Cha instead of (or in addition to) other stimulating beverages (coffee, tea, or stimulating carbonated drinks) in China (south region especially) look promising. We initiated a program aimed at the saponins constituents of *I. kudingcha*, with an ultimate goal to the proposal of an efficient methodology based on the identification of some specific triterpenes and the detection of the beverages. In this paper, we report the isolation and structural elucidation of three

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Table 1. ^{13}C NMR spectral data of **1**–**3** (125 MHz in pyridine-d_5).

Triterpene moiety	**1**	**2**	**3**	3-*O*-sugar	**1**	**2**	**3**
1	38.6	39.2	38.0	Ara			
2	26.6	28.5	28.7	1	104.7	104.8	104.5
3	88.0	88.1	87.5	2	74.8	74.7	74.7
4	39.5	39.6	39.2	3	82.0	82.2	82.0
5	56.0	56.1	55.4	4	68.1	68.2	67.9
6	18.5	18.4	18.0	5	64.8	64.9	64.6
7	33.2	35.5	33.7	Glc			
8	40.6	42.4	40.9	1	104.6	104.7	104.4
9	47.5	45.2	49.2	2		74.9	73.6
10	37.1	37.0	36.9	3	78.4	78.6	78.2
11	24.2	26.6	212.5	4	71.3	71.4	69.7
12	125.6	73.3	39.5	5	78.1	78.2	77.9
13	135.9	142.5	144.2	6	62.4	62.5	62.2
14	42.0	44.9	45.7	Rha			
15	26.5	29.4	25.7	1	101.8	101.9	101.6
16	26.2	26.5	26.1	2	72.4	72.5	72.2
17	40.0	44.8	44.6	3	72.3	72.4	72.1
18	49.8	139.5	150.4	4	74.5	73.9	74.3
19	73.8	74.0	72.6	5	69.9	70.0	69.7
20	85.4	85.6	85.1	6	18.5	18.6	18.3
21	31.2	26.7	25.6				
22	25.2	33.1	30.9				
23	27.9	28.0	27.6				
24	16.8	16.8	16.5				
25	15.7	16.9	16.4				
26	16.0	18.1	15.5				
27	23.3	22.2	22.0				
28	178.7	175.1	173.5				
29	26.5	25.5	22.9				
30	20.1	19.5	19.5				
1′		62.7					
2′		15.8					

Assignments were based on DQF-COSY, ^{1}H–^{1}H COSY, DEPT, HSQC, NOESY, and HMBC experiments. Ara, α-L-arabinopyranose; Rha, α-L-rhamnopyranose; Glc, β-D-glucopyranose.

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new triterpenoid saponins from the leaves of *I. kudingcha*, together with six known saponins. The chemical information obtained could be important not only for understanding folk utilization, but also for the future validation of compounds as makers for the assessment of Ku-Ding-Cha infusion.

2. Results and discussion

Compound **1** was obtained as a white powder. It showed a quasi-molecular ion peak at *m/z* 933.4868 $[M + Na]^+$ in its HR-FAB-MS, suggesting a molecular formula of $C_{47}H_{74}O_{17}$. The UV spectrum showed an absorption maximum at 209 nm. The IR spectrum exhibited absorptions at 3429 cm^{-1} (hydroxyl) and 1733 cm^{-1} (γ-lactone). The spectroscopic features and physico-chemical properties suggested that **1** is a triterpene saponin. The ^{13}C NMR spectrum (Table 1) showed 47 carbons, of which 30 were assigned to a triterpene moiety and 17 to the saccharide portion. The ^{1}H NMR spectrum (Table 2) showed seven singlets for tertiary methyls at δ 0.75, 0.82, 1.11, 1.17, 1.19, 1.43, and 1.55, a methine proton at δ 2.57 (brs), a tri-substituted olefinic proton signal at δ 6.16 (m), and a typical signal of axial H-3 at δ 3.26 (dd, $J = 11.5$, 4.5 Hz) in the

Table 2. ^{1}H NMR spectral data (δ) of **1**–**3** (500 MHz in pyridine-d_5).

	1	**2**	**3**
Triterpene moiety			
3	3.26 dd (11.5, 4.5)	3.26 dd (11.5, 4.0)	3.20 dd (11.5, 4.0)
11	5.02 d (7.5)		
12	6.16 m	5.40 brs	
18	2.57 brs		
23	1.19 s	1.21 s	1.13 s
24	1.11 s	1.09 s	1.03 s
25	0.82 s	0.84 s	0.79 s
26	0.75 s	0.88 s	0.76 s
27	1.17 s	1.48 s	0.98 s
29	1.55 s	1.60 s	1.24 s
30	1.43 s	1.47 s	1.35 s
1′-O—CH_2		3.68 m	
		3.60 m	
2′-CH_3		1.08 t	
3-*O*-sugar-Ara			
1	4.83 d (5.5)	4.84 d (5.5)	4.78 d (5.5)
2	4.55 t (7.5)	4.63 t (7.0)	4.20 m
3	4.28 dd (9.0, 4.5)	4.30 m	4.27 dd (8.0, 3.0)
4	4.51 m	4.50 m	4.49 m
5	3.79 d (9.0)	3.75 d (12.0)	3.73 d (10.0)
	4.22 m	4.27 dd (12.0, 4.5)	4.17
Glc			
1	5.06 d (7.5)	5.08 d (8.0)	5.05 d (7.5)
2	3.95 dd (8.0, 8.0)	3.95 m	4.20 m
3	4.15 t (8.5)	4.16 m	4.08 m
4	4.18 m	4.15 m	4.50 m
5	3.91 m	3.93 m	3.87 m
6	4.31 m	4.31 m	4.21 m
	4.47 d (8.0)	4.47 d (9.5)	4.42 d (11.5)
Rha			
1	6.07 brs	6.15 brs	6.07 brs
2	4.57 dd (9.6, 3.0)	4.58 m	4.65 brs
3	4.71 dd (9.5, 2.7)	4.72 m	4.50 m
4	4.27 m	4.26 m	4.56 t (7.0)
5	4.49 dd (9.4, 6.2)	4.57 dd (9.5, 6.5)	4.50 m
6	1.60 d (6.0)	1.62 d (6.5)	1.56 d (6.0)

Assignments were based on DQF-COSY, ^{1}H–^{1}H COSY, DEPT, HSQC, NOESY, and HMBC experiments. Ara, α-L-arabinopyranose; Rha, α-L-rhamnopyranose; Glc, β-D-glucopyranose.

aglycon moiety. The methine proton was assigned to H-18, which correlated to C-17 (δ 40.0) and C-28 (δ 178.7) in the HMBC spectrum and to the carbon at δ 49.8 in the HMQC spectrum. The ^{13}C and DEPT NMR spectra indicated seven carbons at δ 15.7, 16.0, 16.8, 20.1, 23.3, 26.5, and 27.9 and two olefinic carbons at δ 125.6 (C-12) and 135.9 (C-13). These data were used to assign the aglycon moiety of **1** as having a 3β,19α-dihydroxy-ursane-12-en unit. The ^{13}C NMR signals at δ 85.4 (C-20) and 178.7 (C-28) and the carbon resonances of the E ring indicated the presence of a hexacyclic lactone ring. A 19-*O*-substituent on the ursane skeleton was proved by the signal at δ 73.8 (C-19) in the ^{13}C and DEPT NMR spectra. With the additional 2D NMR experiments, the aglycon of **1** was identified as 3β,19α-dihydroxy-ursane-12-en-28,20β-lactone, reported before as the aglycon moiety of ilekudinoside G [6]. The ^{13}C NMR chemical shift of C-3 (δ 88.0) suggested that **1** has

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a glycosyl linkage at C-3. Acid hydrolysis of **1** afforded sugar components identified by TLC and GC analysis as L-arabinose, D-glucose, and L-rhamnose. The presence of three anomeric proton signals at δ 4.83 (d, $J = 5.5$ Hz), 5.06 (d, $J = 7.5$ Hz), and 6.07 (brs) and three corresponding carbon signals at δ 104.7, 104.6, and 101.8 indicated that **1** is a trisaccharide of L-arabinose, D-glucose, and L-rhamnose. The other sugar proton signals were overlapped in the region between δ 3.90 and 4.86. The identities of the monosaccharides and the trisaccharide sequence were determined by a combination of ^{1}H–^{1}H COSY, DQF-COSY, HSQC, HMBC, and NOESY NMR experiments. The ^{1}H–^{1}H COSY and DQF-COSY experiments allowed analyses of the trisaccharide spin systems and assignments of their proton resonances. The assignments of the proton and the carbon resonances revealed the presence of an inner arabinose unit and two terminal units of glucose and rhamnose, respectively. The $^3J_{H1-H2}$ coupling constant (5.5 Hz) indicated the α-anomeric configuration of the arabinose unit. The large $^3J_{H1-H2}$ coupling constant (7.5 Hz) suggested a β-anomeric configuration for the glucose unit. The anomeric proton of the rhamnose unit was observed as a broad singlet. The ^{1}H NMR splitting pattern and the three-bond strong HMBC correlations from the anomeric proton to C-3 and C-5 indicated the approximate 180° dihedral angles between H-1 and C-3 and H-1 and C-5, which suggested an equatorial anomeric proton and its α-configuration in the $^1C_4$ form [4]. The positions and sequence of the trisaccharide moiety were defined unambiguously from the HMBC correlations between signals of H-1 (δ 4.83) of the α-arabinose and C-3 (δ 88.0) of the aglycon, H-1 (δ 5.06) of the terminal β-glucose and C-3 (δ 82.0) of the inner arabinose, and H-1 (δ 6.07) of the terminal α-rhamnose and C-2 (δ 74.8) of the inner arabinose. The same sugar sequence was supported by the NOESY experiment. Therefore, the structure of **1** was elucidated as 3-*O*-β-D-glucopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1→ 2)]-α-L-arabinopyranosyl-3β,19α-dihydroxy-urs-12(13)-en-28,20β-lactone, and named ilekudinoside T.

Compound **2** was obtained as a white powder. It exhibited a quasi-molecular ion peak at *m/z* 977.5107 $[M + Na]^+$ in its positive HR-FAB-MS, consistent with a molecular formula of $C_{49}H_{78}O_{18}$. The UV spectrum showed an absorption maximum at 210 nm. The IR spectrum exhibited absorptions at 3425 cm^{-1} (hydroxyl) and 1731 cm^{-1} (γ-lactone). The NMR spectra suggested that **2** is a triterpene saponin. The ^{13}C NMR spectrum (Table 1) showed 49 carbon signals, 32 from the triterpene moiety and 17 from the saccharide portion. The ^{1}H NMR spectrum (Table 2) displayed signals characteristic for a triterpene of the ursan-13(18)-ene type: seven singlets for tertiary methyls at δ 0.84, 0.88, 1.09, 1.21, 1.47, 1.48, and 1.60, a multiple methyl signal at δ 1.08 (*t*), a typical signal at δ 3.26 (dd, $J = 11.5$, 4.0 Hz) ascribable to an axial H-3, two multiple signals at δ 3.60 (m) and 3.68 (m) ascribable to ethoxy protons, and a low-field H-12 signal at δ 5.40 (brs), which suggested a 12-*O*-substituted ursane triterpenoid proton. Further features in the ^{13}C NMR spectrum were eight sp^3 carbon signals at δ 15.8, 16.8, 16.9, 18.1, 19.5, 22.2, 25.5, and 28.0, a low-field methylene carbon signal at δ 62.7, two olefinic carbons at δ 139.5 (C) and 142.5 (C) assignable to C-13 and C-18, respectively. The signals at δ 74.0 (C-19), 85.6 (C-20), and 175.1 (C-28) and the carbon resonances of the E ring indicated the presence of a 19-*O*-substituent and lactone ring. The ethoxy at the C-12 position was revealed from the HMBC correlations between the signal of H-12 (δ 5.40) and the methylene carbon signal (δ 62.7). The configuration of 12β-OCH_2CH_3 was deduced from the NOESY experiment. The NOE correlation of H-12/H_3-27 showed that H-12 possessed

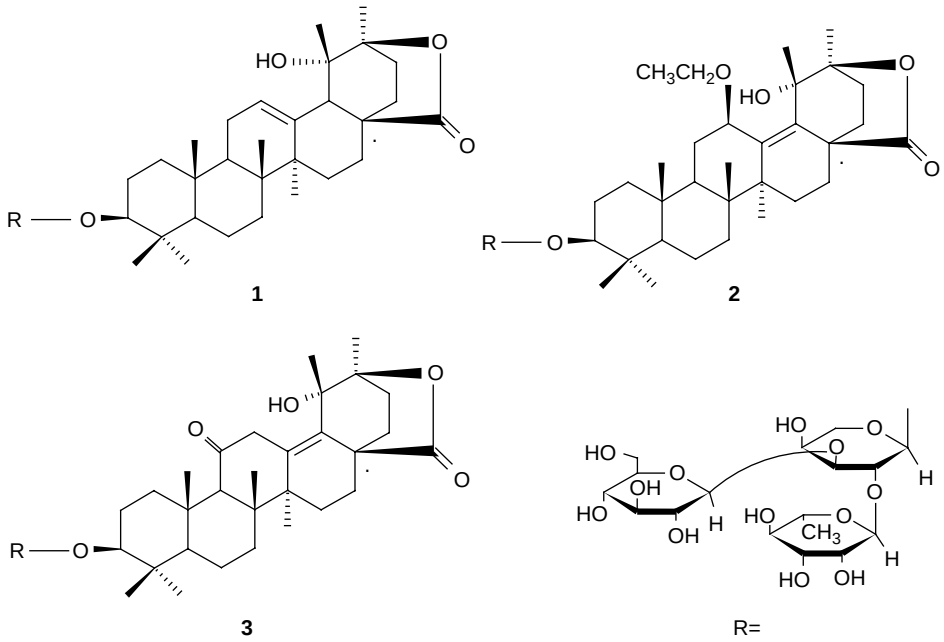

Figure 1. Structures of compounds **1**–**3**.

α-orientation. Therefore, the aglycon of **2** was identified as 3,19-dihydroxy-12-ethoxy-urs-13(18)-ene-28,20-lactone. The ^{13}C NMR chemical shift of C-3 (δ 88.1) revealed that the sugar moiety was attached to C-3 of the aglycon. Acid hydrolysis of **2** yielded L-arabinose, D-glucose, and L-rhamnose, identified by TLC and GC analysis. The overall structural assignments were determined following the same procedure for elucidation as used in compound **1**. The results of 2D NMR experiment indicated the presence of an inner arabinose unit and two terminal units of glucose and rhamnose, respectively. The trisaccharide nature of **2** was deduced from the presence of three sugar units characterized as arabinose (δ 4.84, 104.8), glucose (δ 5.08, 104.7), and rhamnose (δ 6.15, 101.9) units by NMR studies as described above. The attachment of the terminal units of glucose and rhamnose at C-2 and C-3 positions of the inner arabinose unit, respectively, was established from the inter-residue NOEs and HMBC cross-peaks arising from the anomeric protons to the signals involved in the glycosidic linkage. Thus, the structure of **2** was deduced to be 3-*O*-β-D-glucopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-3β,19α-dihydroxy-12-ethoxy-urs-13(18)-ene-28,20β-lactone, and named ilekudinoside U (Figure 1).

Compound **3** was obtained as a white powder. It was assigned a molecular formula of $C_{47}H_{72}O_{18}$ determined on the basis of its positive HR-FAB-MS $[M + Na]^+$ ion peak at *m/z* 947.4658 and its ^{13}C and DEPT NMR spectra. The UV spectrum showed an absorption maximum at 265 nm. The IR spectrum exhibited absorptions at 3426 cm^{-1} (hydoxyl) and 1732 cm^{-1} (γ-lactone). The ^{1}H, ^{13}C, and DEPT NMR spectrum (Tables 1 and 2) indicated that **3** has an aglycon similar to **2** except that the hydroxyl substitution at C-12 was changed to an oxo at C-11. The existence of 11-oxo was evident from the basis of ^{13}C chemical shift value of the signals at δ 36.9, 212.5, and 39.5, ascribed to C-10, C-11, and C-12, respectively.

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Multiple 2D NMR experiments, together with comparison of chemical shifts for the aglycon region of kudinoside F reported in the literature [3], were used to elucidate the aglycon of **3** as 3β,19α-dihydroxy-11-oxo-urs-13(18)-ene-28,20β-lactone. The chemical shift of C-3 (δ 87.5) indicated that the sugar chain was attached to C-3 of the aglycon. On acid hydrolysis, **3** yielded the sugar components as L-arabinose, D-glucose, and L-rhamnose, identified by TLC and GC analysis. Detailed NMR analysis indicated three anomeric signals at δ 4.78 (d, $J = 5.5$ Hz) and 104.5, δ 5.05 (d, $J = 7.5$ Hz) and 104.4, δ 6.07 (brs) and 101.6. The comparison of ^{1}H and ^{13}C NMR spectral data of the sugar portion of **3** with those of **1** and **2** suggested that **3** possesses an identical trisaccharide chain at the C-3 position of the aglycon as **1** and **2**. Confirmation was obtained from the HMBC correlation signals between H-1 (δ 4.78) of the inner arabinose and C-3 (δ 87.5) of the aglycon, H-1 (δ 5.05) of the terminal glucose and C-3 (δ 82.0) of the inner arabinose, and H-1 (δ 6.07) of the terminal rhamnose and C-2 (δ 74.7) of the inner arabinose. The anomeric configuration of the sugar units was similar to the corresponding ones in **1** and **2**. Therefore, the structure of **3** was elucidated as 3-*O*-β-D-glucopyranosyl-(1 → 3)-[α-L-rhamnopyranosyl-(1 → 2)]-α-L-arabinopyranosyl-3β,19α-dihydroxy-11-oxo-urs-13(18)-ene-28,20β-lactone, and named ilekudinoside V.

Additionally, the previously reported compounds latifolosides A, C, G, H [7,8], kudinosides G and H [2] were also isolated, and they were identified by spectroscopic data comparison with literature values.

3. Experimental

3.1 General experimental procedures

Optical rotations were carried out on a Perkin-Elmer 243B digital polarimeter. UV spectra were measured with a Shimadzu spectrometer. IR spectra were measured in a Nicolet Avatar 360 FT-IR spectrometer as KBr pellets. FAB-MS were measured on a Jmx-sx mass spectrometer. HR-FAB-MS were measured on an Auto Spec Ultima-TOF mass spectrometer. NMR spectra were taken on an Inova500 apparatus at 500 MHz (^{1}H) or 125 MHz (^{13}C); respective saponins were analyzed in pyridine-d_5 with TMS as an internal standard. Semi-preparative HPLC was performed on a Waters 2487 instrument (Waters ODS column, 7.8 mm i.d. × 300 mm, detected at UV 260 and 210 nm). GC analysis was carried out on an Agilent 6890N gas chromatograph using a HP-5 capillary column (28 m × 0.32 mm i.d.), with FID detector (detector temperature: 260°C; column temperature: 180°C; carrier gas: N_2; flow rate: 40 ml/min). Column chromatography was performed on AB-8 porous polymer resin (Tianjin Chemical Industry, Tianjin, China), silica gel (200–300 mesh; Qingdao Marine Chemical Industry, Qingdao, China), and Rp-18 gel (ODS, Fuji Sylisia Chemical Ltd, Aichi, Japan).

3.2 Plant material

The leaves of *I. kudingcha* were purchased from Daxing County, Guangxi Province, South China, in June 2003 and were identified by Prof. P.-F. Tu. A voucher specimen (KDC 20030601) is deposited at the Herbarium of Peking University, Modern Research Center for Traditional Chinese Medicine.

3.3 Extraction and isolation

The dried and powdered leaves (10 kg) were extracted twice with 70% ethanol under reflux, each for 2 h and filtered. The filtrate was concentrated under reduced pressure and extracted with petroleum ether, $CHCl_3$, and *n*-BuOH successively. Evaporation of the solvent under reduced pressure from the petroleum ether, $CHCl_3$,

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and *n*-BuOH-soluble fractions yielded 126, 89, and 630 g of each residue, respectively. The *n*-BuOH-soluble fraction (240 g) was decolorized on porous polymer resin (AB-8) column with a gradient aqueous MeOH to give the crude saponins. The crude saponin mixture (100 g) was first chromatographed over a silica gel column and eluted in a step gradient manner of $CHCl_3$–MeOH (15:1 to 2:1, v/v) to yield fractions I–V. Fractions II (1 g) and V (6 g) were subjected to chromatography over RP-18 with MeOH–H_2O (30–100%) to give subfractions designated 2-2, 2-3, 5-2, and 5-3, respectively. Each subfraction was repeatedly subjected to semi-preparative HPLC {ODS column 2.5 cm × 30 cm [H_2O–MeOH (35:65) for fractions 2-2 and 2-3, (40:60) for fractions 5-2 and 5-3], flow rate 2.5 ml/min, UV 210 and 254 nm} to yield **1** (14 mg), **2** (25 mg), **3** (9 mg), ilekudinoside I (15 mg), latifoloside A (11 mg), kudinoside G (42 mg), kudinoside H (6 mg), latifoloside C (7 mg), latifoloside G (248 mg), and latifoloside H (25 mg), respectively.

3.3.1 *Ilekudinoside T (**1**)*

An amorphous powder; $[\alpha]_D^{20}$ − 19.8 ($c = 0.10$, MeOH); UV (MeOH) λ_{max} 209 (log ε 3.34) nm; IR (KBr) ν_{max} 3429, 2943, 1733, 1075 cm^{-1}. ^1H NMR spectral data (500 MHz, pyridine-d_5), see Table 1; ^{13}C NMR spectral data (125 MHz, pyridine-d_5), see Table 2; FAB-MS (positive ion mode) *m/z*: 933 $[M + Na]^+$; HR-FAB-MS *m/z*: 933.4868 $[M + Na]^+$ (calcd for $C_{47}H_{74}O_{17}Na$, 933.4824).

3.3.2 *Ilekudinoside U (**2**)*

An amorphous powder; $[\alpha]_D^{20}$ −22.7 ($c = 0.10$, MeOH); UV (MeOH) λ_{max} 210 (log ε 3.02) nm; IR (KBr) ν_{max} 3425, 2940, 1731, 1070 cm^{-1}. ^1H NMR spectral data (500 MHz, pyridine-d_5), see Table 1; ^{13}C NMR spectral data (125 MHz, pyridine-d_5), see Table 2; FAB-MS (positive ion mode) *m/z*: 977 $[M + Na]^+$; HR-FAB-MS *m/z*: 977.5107 $[M + Na]^+$ (calcd for $C_{49}H_{78}O_{18}Na$, 977.5086).

3.3.3 *Ilekudinoside V (**3**)*

An amorphous powder; $[\alpha]_D^{20}$ −20.9 ($c = 0.10$, MeOH); UV (MeOH) λ_{max} 265 (log ε 3.65) nm; IR (KBr) ν_{max} 3426, 2939, 1733, 1072 cm^{-1}. ^1H NMR spectral data (500 MHz, pyridine-d_5), see Table 1; ^{13}C NMR spectral data (125 MHz, pyridine-d_5), see Table 2; FAB-MS (positive ion mode) *m/z*: 947 $[M + Na]^+$; HR-FAB-MS *m/z*: 947.4658 $[M + Na]^+$ (calcd for $C_{47}H_{72}O_{18}Na$, 947.4616).

3.4 *Acid hydrolysis of saponins*

Each saponin (3 mg) was heated in 3 ml of 10% HCl–dioxane (1:1) at 80°C for 4 h. After the dioxane was removed, the solution was extracted with EtOAc (3 ml × 3) to yield the aglycon and the sugar, respectively.

3.5 *Sugar analyses by TLC and GC*

The sugar components in the aqueous layer left after acid hydrolysis of **1**–**3** were analyzed by silica gel TLC by comparison with standard sugars. The solvent system was $CHCl_3$–MeOH–H_2O (8:5:1) and spots were visualized by spraying with 95% EtOH–H_2SO_4–anisaldehyde (9:0.5:0.5, v/v), then heated at 120°C for 10 min. For sugars of **1**–**3**, the R_f of glucose, arabinose, and rhamnose by TLC were 0.30, 0.55, and 0.50, respectively. The results were confirmed by GC analysis of the methyl sugar peracetates. The aqueous layer was evaporated and dissolved in anhydrous pyridine (100 μl), 0.1 M L-cysteinemethyl ester hydrochloride (200 μl) was added, and the mixture was warmed at 60°C for 1 h. The trimethylsilylation reagent HMDS–TMCS (hexamethyldisilazane–trimethylchlorosilane–pyridine, 2:1:10; Acros Organics, Geel, Belgium) was added and

warmed at 60°C for 30 min. The thiazolidine derivatives were analyzed by GC for sugar identification. The retention times of L-arabinose (t_R, 5.31 min), D-glucose (t_R, 12.45 min), and L-rhamnose (t_R, 5.39 min) were confirmed by comparison with those of authentic standards [9,10].

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