Chemical constituents from *Hedyotis cryptantha*

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Abstract: Eleven known compounds were isolated from the whole plant of *Hedyotis cryptantha*. On the basis of spectroscopic data, their structures were identified as asperuloside (**1**), asperulosidic acid (**2**), asperulosidic acid methyl ester (**3**), asperulosidic acid ethyl ester (**4**), 3,4-dihydro-3-methoxy asperuloside (**5**), kaempferol (**6**), kaempferol 3,7-di-*O*-β-D-glucoside (**7**), kaempferol 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-galactopyranoside (**8**), ursonic acid (**9**), stigmasterol (**10**), and β-sitosterol (**11**). All of these compounds were isolated from this plant for the first time, while compounds **7** and **8** were found from the genus *Hedyotis* L. for the first time.

Key words: *Hedyotis cryptantha*; iridoid glycosides; flavnoids; triterpenoids; sterols

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闭花耳草的化学成分研究

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摘 要： 从闭花耳草(*Hedyotis cryptantha*)全草中分离得到 11 个化合物，经波谱数据分析鉴定为车叶草苷 (**1**)，车叶草苷酸(**2**)，车叶草苷酸甲酯(**3**)，车叶草酸乙酯(**4**)，3,4-二氢-3-甲氧基车叶草苷 (**5**)，山奈酚 (**6**)，kaempferol 3,7-di-*O*-β-D-glucoside (**7**)，kaempferol

3-*O*-*β*-D-galactopyranosyl-(1→3)-*β*-D-galactopyranoside (**8**)， 乌索酸 (**9**), 豆甾醇 (**10**), *β*-谷甾醇 (**11**)。所有化合物均为首次从闭花耳草植物中分离得到，其中化合物 **7** 和 **8** 首次从耳草属(*Hedyotis*)植物中分离得到。

关键词：闭花耳草；环烯醚萜；黄酮；三萜；甾醇

The *Hedyotis* L. (Rubiaceae), a large genus (ca. 699 specie) of herbs or somewhat shrubby plants distributed throughout the tropics and subtropics and with 62 species and 7 varieties found in China (38 endemic), has been widely used as a traditional medicine in a number of Asian countries (Wang, *et al*., 2001; Ahmad, *et al*., 2005). Various species of *Hedyotis* were known to have a range of important pharmacological effects such as anticancer, antimicrobial, anti-inflammatory, antiulcer and immunomodulating activities, and used to treat various diseases including snakebites, sphagitis, bronchitis, hepatitis, pneumonia in children, appendicitis, pelvitis, tonsillitis, nephritis, rheumatic arthritis, infectious fever, dysentery, and some tumors in liver, lung and stomach (Peng, *et al*., 1998; Zhang, *et al*., 2010; Zhang, *et al*., 2011). Previous chemical investigations of this genus have led to the isolation of a series of iridoid glycosides (Peng, *et al*., 1999; Jiangsu Provincial Institute of Botany, 1991), triterpenoids (Si, *et al*., 2010; Masatak, *et al*., 1998; Hui, *et al*., 1977), flavonoids (Kim, *et al*., 2001; Ju, 2007), anthraquinones (Shi, *et al*., 2008; Yu, *et al*., 2009; Rohaya, *et al*., 2005), phenolic glycosides (Wang, *et al*., 2013) and alkaloids (Peng, *et al*., 1997; Phuong, *et al*., 1999), and some of these compounds have significant bioactivities.

Hedyotis cryptantha Dunn is endemic to Hainan Island, China, which has been used as a traditional medicine by Li ethnic group for the treatment of injury. It was firstly reported in ethnobotanical investigation in Hainan Province (Zheng, *et al*., 2009a; Zheng, *et al*., 2009b). To the best of our knowledge, there is little phytochemical study on this plant. The present work is the first detailed phytochemical study on the species *H. cryptantha*. In total 11 compounds were isolated from the whole plant: asperuloside (**1**), asperulosidic acid (**2**), asperulosidic acid methyl ester (**3**), asperulosidic acid ethyl ester(**4**), 3,4-dihydro-3-methoxy asperuloside (**5**), kaempferol (**6**), kaempferol 3,7-di-*O*-*β*-D-glucoside (**7**), kaempferol3-*O*-*β*-D-galactopyranosyl-(1→3)-*β*-D-galactopyranoside (**8**), ursonic acid (**9**), stigmasterol (**10**), and *β*-sitosterol (**11**).

1 Metierial and Methods

1.1 Experimental Instruments

^{1}H and ^{13}C NMR spectra were recorded on Bruker AM-600 and Bruker DRX-500 spectrometers using TMS as an internal standard. HR-ESI-MS analyses were carried out on an LCT premier XE TOF mass spectrometer (Waters, Manifold, MA) instrument. Silica gel (80−100 and 300−400 mesh, Yantai Chemical Industry Institute Co., Ltd., China), MCI gel CHP 20P

(75–150 μm, Mitsubishi Chemical Corporation, Tokyo, Japan), HSCCC (TBE-20A and TBE-300B, Shanghai Tauto Biotechnique, Shanghai, China), Rp-C_{18} (20–45 μm, BUCHI Labortechnic AG, Switzerland) and SephadexLH-20 (GE Healthcare Bio-Xciences AB) were used for column chromatography, and silica gel GF254 (Yantai) was used for preparative TLC in the form of precoated plates. TLC spots were visualized under UV light and by dipping into 5% H_2SO_4 in EtOH followed by heating.

1.2 Plant Material

The whole plants of *H. cryptantha* were collected from Lingshui County, Hainan, China in August 2010 and identified by one of our authors (Prof. Chun-Lin Long). The voucher specimen (No. 20100814) was deposited at the Herbarium of Minzu University of China.

2 Extraction and Isolation

Dried and powdered samples (10.0 kg) of *H. cryptantha* were extracted with 90% EtOH for three times under reflux to give ethanol extract. After evaporation under reduced pressure, the extract residue was suspended in water and partitioned successively with petroleum ether, ethyl acetate, and *n*-butanol. The *n*-butanol extract (56.0 g) was subjected to macroporous resin TM XAD16 and successfully eluted with H_2O and different concentrations of EtOH to obtain 5 fractions (A–E). Fraction C (50%) was further separated by silica gel (200–300 mesh) column chromatography eluting with $CHCl_3$–MeOH gradient (35:1-4:1) to yield nine fractions (Fr.C1–Fr.C9). Fr.C2 (3.2 g) was chromatographed on HSCCC with a $CHCl_3$-MeOH-H_2O-BuOH solvent system (4-3-2-0.8) to yield Compounds **1** (96 mg), **3** (21.9 mg), and **4** (17.1 mg). Fr.C5 (161.1 mg), Fr.C6 (31.0 mg) and Fr.C8 (22.0 mg) were subjected to the Sephadex LH-20 eluting with MeOH to yield Compounds **6** (3.1 mg), **7** (9.7 mg), and **8** (3.4 mg) respectively. Fr.B (30%) was further separated by silica gel (200–300 mesh) column chromatography eluting with $CHCl_3$–MeOH gradient (30:1→2:1) to yield seven fractions (Fr.B1–Fr.B6). Fr.B1(8.93 g) was separated by MPLC gradient eluted with a MeOH-H_2O solvent system (20:80, 40:60, 60:40, 80:20, and 100:0) and further purified by gel permeation chromatography on Sephadex LH-20 in MeOH to yield compound **2** (4.4 mg), Compounds **3** (20.8 mg) and **5** (7.8 mg). Ethyl acetate extract (30 g) was also separated on macroporous adsorption resin column (MeOH/H_2O gradient 0:1-0:1) to afford five fractions (F1–F5). Fraction F5 was subjected to repeated column chromatography (silica gel, Sephadex LH-20 (MeOH)) to yield Compound **9** (6 mg). Fraction F1 was applied to column chromatography (silica gel, $CHCl_3$/MeOH 50:1; petroleum ether (PE)/acetone 9:1) to yield compound **10** (8.0 mg). Fraction F2 was subjected to repeated column chromatography (silica gel, $CHCl_3$/MeOH 50:1; EtOH/MeOH/H_2O 30:1:1) to yield compound **1** (20 mg). The petroleum ether extract (50.0 g) was separated with silica gel column (PE/acetone gradient 100:0-0:100) and purified by Sephadex LH-20 (MeOH) and recrystallizing to yield compound **11** (30 mg).

3 Results and Isolation

Fig. 1 The chemical structures of compounds **1**–**11**

Asperuloside (1) Colorless needles (methyl alcohol), ESI-MS *m/z* 415 [M + H]$^+$, $C_{18}H_{22}O_{11}$. ^{1}H NMR (600 MHz, MeOD) δ: 7.31 (1H, s, H-3), 5.96 (1H, s, H-1α), 5.73 (1H, s, H-7), 5.57 (1H, d, *J* = 6.5 Hz, H-6β), 4.78 (1H, s, H-1′), 4.67 (2H, t, *J* = 10.7 Hz, H-10), 3.93 (1H, m, H-6′a), 3.67 (2H, m, H-6′b, H-5β), 3.37 (2H, m, H-, H-5′), 3.27 (2H, m, H-, H-4′), 3.19 (1H, t, *J* = 8.6 Hz, H-2′), 2.08 (3H, s, H-COC$\underline{H}_3$); ^{13}C NMR (150 MHz, MeOD) δ: 93.899.4 (C-1), 150.8 (C-3), 106.7 (C-4), 38.0(C-5), 86.8 (C-6), 129.4 (C-7), 144.8 (C-8), 45.8 (C-9), 62.4 (C-10), 172.8 (C-11), 173.1 (C-$\underline{C}$OCH$_3$), 21.1 (C-COC$\underline{H}_3$), 100.5 (C-1′), 75.2 (C-2′), 78.4 (C-3′), 72.1 (C-4′), 78.9 (C-5′), 63.3 (C-6′). This compound was identified by comparison of its spectral data with those reported (Bergera, *et al.*, 2011).

Asperulosidic acid (2) Colorless needles (methyl alcohol), ESI-MS m/z 433[M + H]$^+$, $C_{18}H_{24}O_{12}$, ^{1}H NMR (600 MHz, MeOD) δ: 7.63 (1H, s, H-3), 5.99 (1H, s, H-7), 5.03 (1H, d, J = 9.0 Hz, H-1α), 4.91 (1H, d, J =14.9 Hz, H-6β), 4.84-4.78 (2H, m, H-10), 4.70 (1H, d, J = 7.8 Hz, H-1′), 3.82 (1H, dd, J = 1.5, 12.0 Hz, H-6′a), 3.58 (1H, dd, J = 12.0, 5.9 Hz, H-6′b), 3.36 (1H, t, J = 8.5 Hz,H-5′), 3.24 (3H, m, H-2′, H-3′, H-4′), 2.98 (1H, m, H-5β), 2.60 (1H, m, H-9β), 2.06 (3H, s, H-COC$\underline{H}_3$); ^{13}C NMR (150MHz, MeOD) δ: 101.4 (C-1), 155.6 (C-3), 108.4 (C-4), 42.5 (C-5), 75.5 (C-6), 132.0 (C-7), 146.0 (C-8), 46.3 (C-9), 63.9 (C-10), 170.8 (C-11), 172.7 (C-$\underline{C}$OCH$_3$), 20.9 (C-CO$\underline{C}$H$_3$), 100.7 (C-1′), 75.0 (C-2′), 77.9 (C-3′), 71.7 (C-4′), 78.6 (C-5′), 63.1 (C-6′).The spectral data was consistent with those reported (Bergera, *et al*., 2011).

Asperulosidic acid methyl ester (3) Colorless needles (methyl alcohol), ESI-MS m/z 447 [M + H]$^+$, $C_{19}H_{26}O_{12}$. ^{1}H NMR (600 MHz, MeOD) δ: 7.62 (1H, s, H-3), 5.99 (1H, s, H-7), 5.02 (1H, d, J = 9.0 Hz, H-1α), 4.90 (1H, d, J = 15.6 Hz, H-6β), 4.77 (2H, m, H-10), 4.69 (1H, d, J = 7.8 Hz, H-1′), 3.82 (1H, dd, J =12.0, 1.8 Hz, H-6′a), 3.71 (3H, s, H-COOC$\underline{H}_3$), 3.58 (1H, dd, J =12.0, 6.0 Hz, H-6′b), 3.35 (1H, m, H-5′), 3.26 (2H, m, H-2′, H-3′), 3.21 (1H, m, H-4′), 3.00 (1H, m, H-5β), 2.60 (1H, m, H-9β), 2.06 (3H, s, H-COC$\underline{H}_3$); ^{13}C NMR (150MHz, MeOD) δ: 99.9 (C-1), 154.0 (C-3), 108.2 (C-4), 40.9 (C-5), 74.1 (C-6), 130.1 (C-7), 146.0 (C-8), 44.8 (C-9), 62.2 (C-10), 169.4 (C-11), 50.6 (C- COO$\underline{C}$H$_3$), 172.6 (C-$\underline{C}$OCH$_3$), 20.9 (C-CO$\underline{C}$H$_3$), 99.2 (C-1′), 73.5 (C-2′), 77.2 (C-3′), 70.1 (C-4′), 76.5 (C-5′), 61.6 (C-6′).The spectral data was in accordance with those reported (Bergera, *et al*., 2011; Guvenalp, *et al*., 2006).

Asperulosidic acid ethyl ester (4) Colorless needles (methyl alcohol), ESI-MS m/z 461 [M + H]$^+$, $C_{20}H_{28}O_{12}$. ^{1}H NMR (600 MHz, DMSO-d$_6$) δ: 7.58 (1H, s, H-3), 5.97 (1H, s, H-7), 5.14 (1H, d, J = 4.2 Hz, H-1α), 4.96 (1H, d, J = 8.9 Hz, H-6β), 4.78 (1H, d, J =14.8 Hz, H-10a), 4.70 (1H, d, J =14.8 Hz, H-10b), 4.12 (2H, q, J = 7.1 Hz, H-C$\underline{H}_2$CH$_3$), 3.66 (1H, dd, J = 11.3, 2.9 Hz, H-6′a), 3.43 (1H, m, H-6′b), , 3.16 (2H, dt, J = 15.3, 7.3 Hz, H-2′, H-3′), 3.07 (1H, t, J = 9.1 Hz, H-4′), 3.00 (1H, d, J = 8.2 Hz, H-5′), 2.90 (1H, t, J = 6.7 Hz, H-5β), 2.53 (1H, d, J = 8.7 Hz, H-9β), 2.07 (3H, s, H-COC$\underline{H}_3$), 1.23 (3H, s, H-COOCH$_2$C$\underline{H}_3$); ^{13}C NMR (150MHz, DMSO-d$_6$) δ: 99.9 (C-1), 153.4 (C-3), 107.9 (C-4), 41.1 (C-5), 74.1 (C-6), 132.3 (C-7), 143.1 (C-8), 45.1 (C-9), 62.5 (C-10), 166.8 (C- $\underline{C}$OOCH$_2$CH$_3$), 59.8 (C- COO$\underline{C}$H$_2$CH$_3$), 14.7 (C- COOCH$_2\underline{C}$H$_3$), 172.6 (C-$\underline{C}$OCH$_3$), 21.1 (C-CO$\underline{C}$H$_3$), 99.4 (C-1′), 73.6 (C-2′), 77.6 (C-3′), 70.1 (C-4′), 77.0 (C-5′), 61.5 (C-6′). The spectral data resembled those reported (Peng, *et al*., 1999; Bergera, *et al*., 2011).

3,4-dihydro-3-methoxy asperuloside (5) Colorless amorphous substance (methyl alcohol), ESI-MS m/z 447 [M + H]$^+$, $C_{19}H_{26}O_{12}$. ^{1}H NMR (600 MHz, MeOD) δ: 6.01 (1H, s, H-7), 5.39 (1H, d, J = 6.7 Hz, H-6β), 5.02 (1H, d, J = 6.1 Hz, H-1α), 5.13 (1H, d, J = 3.6 Hz, H-3α), 4.98 (1H, s, H-10), 4.76 (1H, s, H-10), 4.71 (1H, s, H-1′), 3.86 (1H, m, H-6′a),3.67 (1H, m, H-6′b) , 3.54 (3H, s, H-OCH$_3$), 3.40 (H, dd, J = 8.5, 17.7 Hz, H-5β), 3.35 (H, dd, J = 8.5, 9.1 Hz, H-3′), 3.28 (2H, m, H-4′, H-5′), 3.26 (1H, dd, J = 3.6, 8.0 Hz, H-4), 3.22 (1H, dd, J = 9.2, 8.0 Hz, H-2′), 3.05 (1H, dd, J = 8.5, 6.5 Hz, H-9β), 2.13 (3H, s, H-COC$\underline{H}_3$); ^{13}C NMR (150MHz, MeOD) δ: 98.7 (C-1), 96.9 (C-3), 44.7 (C-4), 37.8 (C-5), 87.9 (C-6), 126.4 (C-7), 152.2 (C-8), 45.3 (C-9), 62.9 (C-10), 177.3

(C-11), 173.7.1 (C-$\underline{C}$OCH$_3$), 20.8 (C-CO$\underline{C}$H$_3$), 56.6 (C-O$\underline{C}$H$_3$), 99.7 (C-1′), 75.1 (C-2′), 78.2 (C-3′), 71.7 (C-4′), 78.4 (C-5′), 62.9 (C-6′). Compound 5 was characterized as 3, 4-dihydro-3-methoxy asperuloside by comparison of the physical and spectral data with those reported (Quang, *et al.*, 2002; Bergera, *et al.*, 2011).

Kaempferol (6) Yellow powder (methyl alcohol), ESI-MS *m/z* 316 [M + H]$^+$, C$_{15}$H$_{10}$O$_6$, ^{1}H NMR (600 MHz, MeOD) δ: 8.11 (2H, d, *J* = 8.9 Hz, H-2′, 6′); 6.93 (2H, d, *J* = 8.8 Hz, H-3′, 5′), 6.42 (1H, d, *J* = 1.9 Hz, H-8), 6.21 (1H, d, *J* = 2.0 Hz, H-6); ^{13}C NMR (150 MHz, MeOD) δ: 148.2 (C-2), 137.3 (C-3), 177.5 (C-4), 165.8 (C-5), 99.4 (C-6), 162.7 (C-7), 94.6 (C-8), 160.7 (C-9), 104.7 (C-10), 123.9 (C-1′), 130.8 (C-2′, 6′), 116.5 (C-3′, 5′), 158.4 (C-4′). Compound 6 was characterized as kaempferol by comparison of the physical and chemical properties and spectral data with those reported (Ju, 2007).

Kaempferol 3,7-di-*O*-β-D-glucoside (7) Yellow powder (methyl alcohol), ESI-MS *m/z* 316 [M + H]$^+$, C$_{27}$H$_{30}$O$_{16}$. ^{1}H NMR (600 MHz, DMSO-d$_6$) δ: 12.7 (1H, s, H-5O$\underline{H}$), 8.08 (2H, d, *J* = 8.7 Hz, H-2′, 6′); 6.89 (2H, d, *J* = 8.6 Hz, H-3′, 5′), 6.44 (1H, d, *J* = 1.9 Hz, H-8), 6.19 (1H, d, *J* = 2.0 Hz, H-6), 5.32 (1H, s, H-1″), 3.60 (1H, m, H-2″), 3.48 (1H, m, H-3″), 3.41 (1H, m, H-4″), 3.26 (1H, m, H-5″), 3.74 (1H, m, H-6″a), 3.60 (1H, m, H-6″b), 4.93 (1H, s, H-1‴), 3.48 (1H, m, H-2‴), 3.71 (1H, m, H-3‴), 3.23 (1H, m, H-4‴), 3.13 (1H, m, H-5‴), 3.67 (1H, m, H-6‴a), 3.60 (1H, m, H-6‴b); ^{13}C NMR (150 MHz, DMSO-d$_6$) δ: 157.4 (C-2), 133.5 (C-3), 177.9 (C-4), 160.4 (C-5), 98.4 (C-6), 164.8 (C-7), 93.3 (C-8), 157.1 (C-9), 103.4 (C-10), 121.3 (C-1′), 131.0 (C-2′, 5′), 114.9 (C-3′, 6′), 160.2 (C-4′),100.1 (C-1″), 78.9 (C-2″), 76.8 (C-3″), 68.0 (C-4″), 76.3 (C-5″), 61.2 (C-6″), 104.3 (C-1‴), 73.8 (C-2‴), 80.9 (C-3″), 69.9 (C-4‴), 74.1 (C-5‴), 60.6 (C-6″). This compound was characterized as kaempferol 3,7-*O*-β-D-diglucoside by comparison of the physical and spectral data with those reported (Kamiya, *et al.*, 1997).

Kaempferol 3-*O*-β-D-galactopyranosyl-(1→3)-β-D-galactopyranoside (8) Yellow powder (methyl alcohol), ESI-MS *m/z* 649[M + Na]$^+$, C$_{27}$H$_{30}$O$_{16}$. ^{1}H NMR (600 MHz, MeOD) δ: 8.09 (2H, d, *J* = 8.7 Hz, H-2′, 6′); 6.90 (2H, d, *J* = 8.6 Hz, H-3′, 5′), 6.40 (1H, d, *J* = 1.9 Hz, H-8), 6.20 (1H, d, *J* = 2.0 Hz, H-6), 5.36 (1H, s, H-1″), 3.84 (1H, m, H-2″), 3.70 (1H, m, H-3″), 4.06 (1H, m, H-4″), 3.45 (1H, m, H-5″), 3.52 (1H, m, H-6″a), 3.37 (1H, m, H-6″b), 4.75 (1H, s, H-1‴), 3.38 (1H, m, H-2‴), 3.35 (1H, m, H-3‴), 3.79 (1H, m, H-4‴), 3.35 (1H, m, H-5‴), 3.60 (1H, m, H-6‴a), 3.52 (1H, m, H-6‴b); ^{13}C NMR (150 MHz, MeOD) δ: 158.7 (C-2), 133.3 (C-3), 180.1 (C-4), 161.7 (C-5), 100.0 (C-6), 166.1 (C-7), 94.9 (C-8), 159.0 (C-9), 106.2 (C-10), 123.0 (C-1′), 132.8 (C-2′, 5′), 116.4 (C-3′, 6′), 163.3 (C-4′), 101.6 (C-1″),76.0 (C-2″), 80.8 (C-3″), 70.6 (C-4″), 78.7 (C-5″), 62.5 (C-6″), 105.0 (C-1‴), 75.4 (C-2‴), 77.5 (C-3‴), 71.8 (C-4‴), 78.4 (C-5‴), 63.1 (C-6‴). The spectral data was consistent with those reported (Hirayama, *et al.*, 2013).

Ursolic acid (9) Colorless needles (methyl alcohol), ESI-MS *m/z* 457[M+H]$^+$, C$_{30}$H$_{50}$O$_3$, ^{1}H NMR (600 MHz, Pyr-d$_5$) δ: 14.76 (1H, br s, -COOH), 5.48 (1H, s, H-12), 3.45 (1H, dd, *J* = 10.3,

5.5 Hz, H-3α), 2.63 (1H, d, J = 11.3 Hz, H-18), 1.23 (6H, d, J = 9.8 Hz, H-23 Me, 27 Me), 1.04 (3H, s, H-26 Me), 1.01 (3H, s H-24 Me), 0.99 (3H, d, J = 6.4 Hz, H-29 Me), 0.94 (3H, d, J = 6.2 Hz, H-30 Me), 0.88 (3H, s, H-25 Me); ^{13}C NMR (150 MHz, Pyr-d_5) δ: 37.6 (C-1), 28.3 (C-2), 78.3 (C-3), 56.0 (C-5), 18.9 (C-6), 33.7 (C-7), 39.6 (C-8), 48.2 (C-9), 23.8 (C-11), 125.8 (C-12), 28.3 (C-15), 25.1 (C-16), 16.7 (C-25), 17.6 (C-26), 24.1 (C-27), 17.7 (C-29), 21.6 (C-30). The spectral data was in consistent with those reported (Tundis, *et al*., 2002).

Stigmasterol (10) Colorless needles (methyl alcohol), ESI-MS *m/z* 412[M]$^+$, $C_{29}H_{48}O$. ^{1}H NMR (600 MHz, CDCl$_3$) δ: 5.38 (1H, d, J = 3.8 Hz, H-6), 5.17 (1H, dd, J = 15.2, 8.6 Hz, H-22), 5.04 (1H, dd, J = 15.0, 8.5 Hz, H-23), 3.55 (1H, m, H-3). Six methyls: 1.03 (3H, s, H-19), 0.95 (3H, d, J = 6.5 Hz, H-21), 0.87 (3H, m, H-29), 0.85–0.78 (6H, m, H-26, 27), 0.71 (3H, d, J = 11.2 Hz, H-18). ^{13}C NMR (150 MHz, CDCl$_3$) δ: 37.2 (C-1), 31.6 (C-2), 71.8 (C-3), 42.3 (C-4), 140.1 (C-5), 121.8 (C-6), 31.9 (C-7), 31.9 (C-8), 50 (C-9), 37.2 (C-10), 21.1 (C-11), 40.6 (C-12), 42.2 (C-13), 56.9 (C-14), 24.3 (C-15), 29.8 (C-16), 55.9 (C-17), 12.1 (C-18), 19.4 (C-19), 42.1 (C-20), 21.1 (C-21), 138.6 (C-22), 129.2 (C-23), 51.2 (C-24), 31.8 (C-25), 22.2 (C-26), 18.9 (C-27), 25.4 (C-28), 12.2 (C-29). The spectral data was in agreement with those reported (Itoh, *et al*., 1978).

β-Sitosterol (11) White powder (methyl alcohol), $C_{29}H_{50}O$. It was characterized by comparing it with an authentic sample on TLC.

4 Discussions and Conclusion

In this study, 11 compounds were isolated from the whole plant of *H. cryptantha*, in which iridoid glycosides (IGs), especially asperuloside (1), are the major compounds. IGs are one of the most important types of natural products with bioactivities of anti-oxidation, anti-inflammatory and immunomodulatory. Kaempferol and its glycosides are also known to be good antioxidants. Although largely untested for the bioactivities, it could be assumed that the IGs and flavonids found in the present study contribute to the treatment of injure and thus explain the current use of the species in ethnomedicine. In addition, all of these five iridoid glycosides have also been isolated from *H. diffusa* or other species in this genus. *H. diffusa*, as an important traditional Chinese medicine, has been widely used to treat snake bite, cancer, appendicitis, hepatitis, furunculosis, enteritis, and bleeding in China and also found to possess notable effect on many types of cancers in clinical application (Jiangsu New Medical College, 1977). The ethanol extract of *H. diffusa* could inhibit colorectal cancer growth *in vivo* via inhibition of SHH-mediated tumor angiogenesis (Lin, *et al.*, 2013), induce apoptosis via activation of the mitochondrion-dependent pathway in human colon carcinoma cells (Lin, *et al.*, 2010), exhibited antileukemia activity in WEHI-3 cell-induced leukemia *in vivo* and promoted T- and B-Cell proliferation (Lin, *et al.*, 2011)

and the water extract possessed high cytotoxicity towards human breast cancer MCF7 cells (Ling, *et al.*, 2013). Not only the extract of *H. diffusa* exhibited strong anti-cancer effect but also the compounds isolated from genus of *Hedyotis* presented potent bioactivities. For example, 2-hydroxy-3-methylanthraquinone from *H. diffusa* induced THP-1 cell apoptosis in a time- and dose-dependent manner (Wang, *et al.*, 2010), 6-*O*-*β*-D-apiofuranosyl-*β*-D- glucopyranoside and grevilloside G isolated from *H. scandens* showed antiviral activity against respiratory syncytial virus with IC_{50} values of 20 and 25 μ g/mL, respectively (Wang, *et al*., 2013). Asperuloside isolated from *Eucommia ulmoides* also showed important anti-obesity effects (Hirata, *et al*., 2011). We can deduce that compounds from *H. cryptantha* also have some bioactivities which are similar to those from *H. diffusa*.

Furthermore, as an important Li medicinal plant species, *H. cryptantha* has only been reported in the ethnopharmacological investigations in recent years. There are too many medicinal plants which are only known by the local Li ethnic group but have potential medicinal values, such as *H. ovata* (Böjthe-Horváth, *et al*., 1980). Ethnobotanical investigation can play an important role in discovering new resources. The traditional knowledge of these plants are disappearing inevitably as Li people are without their own writing characters. We hope more people would engage in the study on ethnobotany of Li ethnic group to rescue this cultural treasure. And more people would be attracted by these valuable plants and their associated traditional knowledge.

Acknowledgements

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