The Antitumor Constituents from Hedyotis Diffusa Willd

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Abstract: As a TCM, Hedyotis diffusa Willd. has been using to treat malignant tumors, and many studies also showed that the extracts from Hedyotis diffusa Willd. possessed evident antitumor activities. Therefore, we carried out chemical study on Hedyotis diffusa Willd. and investigated the cytotoxicity of the obtained compounds on a panel of eight tumor cell lines. As a result, four new compounds were isolated from Hedyotis diffusa Willd., including three iridoid glycosides of Shecaoiridoidside A–C (1–3) and a cerebroside of shecaocerenoside A (4). Also, six known iridoid compounds (5–10) were also obtained. The cytotoxicity of all compounds against human tumor cell lines of HL-60, HeLa, HCT15, A459, HepG2, PC-3, CNE-2, and BCG-823 were also evaluated in vitro. New compound 3 exhibited evident cytotoxicity to all tumor cell lines except the Hela, and the IC₅₀ values are from 9.6 µM to 62.2 µM, while new compound 4 showed moderate cytotoxicity to all the cell lines, and the IC₅₀ values are from 33.6 µM to 89.3 µM. By contrast, new compound 1 and known compound 9 showed moderate cytotoxicity to HCT15, A459, and HepG2 selectively. Known compound 7 also exhibited moderate cytotoxicity to HCT15 and A459 selectively.

Keywords: Hedyotis diffusa Willd.; antitumor; iridoid glycosides; cerebrosides

1. Introduction

As an annual herb, Genus of Hedyotis has been widely distributed in subtropical area of the world [1]. 62 species of Hedyotis are distributed in China, among which more than 20 species are used as medicines, including Hedyotis diffusa Willd. (H. diffusa Willd.). H. diffusa Willd. has been recorded in Chinese pharmacopoeia (2015 edt) and possesses the efficacies of diuresis to reduce edema, clearing away the heat evil and detoxifying, and promoting blood circulation to arrest pain [2]. Clinically, H. diffusa Willd. has been using to treat urinary tract infection, tonsillitis, appendicitis, pharyngitis, hepatitis, dysentery, diarrhea, and snake bites [1–3]. But more importantly, H. diffusa Willd. also showed significant effective on malignant tumors of breast, gastric, colon, rectal, and ovarian [2,4,5]. The components of iridoids, triterpenes, flavonoids, lignans, anthraquinones, alkaloids, cerebrosides, coumarins, and sterols were discovered during the chemical studies of H. diffusa Willd. [2,6–8]. There are some differences of the chemical constituents if the H. diffusa Willd. grown in different parts of China. The contents of anthraquinones and iridoids in H. diffusa Willd. from Guangdong province were higher than the H. diffusa Willd. from the provinces of zhejiang, Jiangxi, Hubei, and Fujian. These include 2,7-dihydroxy-3-methyl anthraquinone, 2-hydroxy-3-methyl-1-methoxy anthraquinone, 2-hydroxy-3-methoxy-7-methoxy
anthraquinone, 2-methyl-3-hydroxy anthraquinone, 2-methyl-3-hydroxy-4-methoxy anthraquinone, deacetyl asperulosidic acid, scadsoside, E-6-O-p-coumaroyl scadsoside methyl ester [9]. Various hepatoprotective, immunoregulation, anti-tumor, anti-inflammatory, antibacterial, analgesia, sedative, and anti-oxidant activities can be found in pharmacological studies of *H. diffusa* Willd [3,6,10–13], but more studies found that the extracts from *H. diffusa* Willd possessed evident anticancer activities [1,2,14–20]. *H. diffusa* Willd has also been used to treat cancers adjuvantly for a long time in China. With increasing incidence and mortality in China, cancer has become the leading cause of death and caused serious public health problems. According to the latest report, in 2015, about 4,292,000 new cancer cases and 2,814,000 cancer deaths occurred in China, with lung cancer being the most common incident cancer and the leading cause of cancer death. Stomach, esophageal, and liver cancers were also commonly diagnosed and identified as leading causes of cancer death [21]. Therefore, screened active components from *H. diffusa* Willd. might be helpful. In this study, we carried out chemical study on *H. diffusa* Willd., and four new (1–4) along with six known (5–10) compounds were obtained. The structures of known compounds were determined by detailed NMR and ESI-MS spectra analyses, as well as comparing the data with the literature. In this paper, we describe the isolation of compounds 1–4 and elucidate their structures. The cytotoxic activity of all compounds against tumor cell lines of HL-60 (human leukemia cells), HeLa (human cervical cancer cells), HCT15 (human colon cancer cells), HL-60 (human leukemia cells), HeLa (human cervical cancer cells), HCT15 (human colon cancer cells), HL-60 (human leukemia cells), HeLa (human cervical cancer cells), HCT15 (human colon cancer cells), HL-60 (human leukemia cells), HeLa (human cervical cancer cells), HCT15 (human colon cancer cells), HL-60 (human leukemia cells), HeLa (human cervical cancer cells), HCT15 (human colon cancer cells), HL-60 (human leukemia cells), HeLa (human cervical cancer cells), HCT15 (human colon cancer cells), HL-60 (human leukemia cells), HeLa (human cervical cancer cells), HCT15 (human colon cancer cells), HL-60 (human leukemia cells), HeLa (human cervical cancer cells), HCT15 (human colon cancer cells), HL-60 (human leukemia cells), HeLa (human cervical cancer cells), HCT15 (human colon cancer cells), HL-60 (human leukemia cells), 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HeLa (human cervical cancer cells), HCT15 (human colon cancer cell...
Table 1. Cont.

| 1<sup>a</sup> | 2<sup>a</sup> | 3<sup>a</sup> | 4<sup>b</sup> |
|---|---|---|---|
| H | δ<sub>H</sub> (J, Hz) | δ<sub>H</sub> (J, Hz) | δ<sub>H</sub> (J, Hz) | H | δ<sub>H</sub> (J, Hz) |
| 6 | 4.04, d (2.5) | 2.34, dd (8.4, 13.4); 2.19, m | 2.74, m; 2.26, brd (16.4) | 3 | 4.77, m |
| 7 | 3.36, d (2.5) | 3.87, m | 5.78, brs | 4 | 5.86, m |
| 9 | 2.05, m | 3.08, d (10.7) | 5 | 5.98, m |
| 10 | 3.69, d (2.8) | 1.59, s | 4.11, brd (10.0) | 6 | 2.06, m |
| 11 | 4.21, d (11.6); 4.35, d (11.6) | 5.08, 5.11, s | 4.92, 4.91, d (2.0) | 7-22 | 1.16-1.42, brs |
| 1′ | 4.41, d (7.8) | 4.72, d (7.8) | 23 | 0.88, d (6.4) |
| 2′ | 5.62, s | 3.16, t (8.2) | 3.27, m | 24 | 0.86, t (6.4) |
| 3′ | 3.30, m | 3.43, m | 2′ | 4.61, m |
| 4′ | 2.16, m | 3.29, m | 3′ | 1.85, m |
| 5′ | 0.89, t (7.4) | 3.34, m | 3.62, m | 4′ | 1.73, m; 1.16-1.42, brs |
| 6′ | 2.15, s | 3.59, 3.95, m | 4.60, brd, (11.6); 4.42, dd, (11.8, 4.8) | 5′-17′ | 1.16-1.42, brs |
| 1′′ | 4.72, d (8.1) | 5.01, d (1.7) | 18′ | 0.88, t (6.4) |
| 2′′ | 3.35, m | 3.87, m | 7.88, d (8.8) | 1′ | 4.90, d (7.6) |
| 3′′ | 4.05, m | 6.81, d (8.8) | 2′ | 4.02, m |
| 4′′ | 3.49, m | 3.95, 3.75, m | 3′ | 4.22, m |
| 5′′ | 3.59 (1H, m) | 3.58, s | 6.81, d (8.8) | 4′ | 4.22, m |
| 6′′ | 3.67, m; 3.86, dd (1.5, 11.5) | 7.88, d (8.8) | 5′ | 3.88, m |
| 6″ | 4.36, 4.50, m |

<sup>a</sup> Measured in CD<sub>3</sub>OD at 30 °C; <sup>b</sup> Measured in C<sub>5</sub>D<sub>4</sub>N-d<sub>5</sub> at 30 °C.

Table 2. <sup>13</sup>C-NMR data of compounds 1–4 (100 MHz, δ in ppm).

| 1<sup>a</sup> | 2<sup>a</sup> | 3<sup>a</sup> | 4<sup>b</sup> |
|---|---|---|---|
| C | δ<sub>C</sub> | C | δ<sub>C</sub> | C | δ<sub>C</sub> | C | δ<sub>C</sub> |
| 1 | 90.8, CH | 1 | 175.2, C | 1 | 72.8, CH<sub>2</sub> | 1 | 70.2, CH<sub>2</sub> |
| 3 | 142.4, CH | 3 | 71.5, CH<sub>2</sub> | 3 | 72.8, CH<sub>2</sub> | 2 | 54.5, CH |
| 4 | 109.8, C | 4 | 144.5, C | 4 | 156.2, C<sup>b</sup> | 3 | 72.3, CH |
| 5 | 35.4, CH | 5 | 41.2, CH | 5 | 49.8, CH | 4 | 131.6, CH |
| 6 | 59.9, CH | 6 | 40.0, CH<sub>2</sub> | 6 | 39.2, CH<sub>2</sub> | 5 | 132.7, CH |
| 7 | 60.3, CH | 7 | 90.1, CH | 7 | 131.4, CH | 6 | 34.2, CH<sub>2</sub> |
| 8 | 80.2, C | 8 | 86.1, C | 8 | 144.4, C | 7-20 | 29.5–30.5, CH<sub>2</sub> |
| 9 | 43.6, CH | 9 | 54.2, CH | 9 | 99.8, C | 21 | 35.7, CH |
| 10 | 67.2, CH<sub>2</sub> | 10 | 22.5, CH<sub>3</sub> | 10 | 59.3, CH<sub>2</sub> | 22 | 30.5, CH<sub>2</sub> |
| 11 | 69.8, CH<sub>2</sub> | 11 | 113.8, CH<sub>2</sub> | 11 | 105.4, CH<sub>2</sub> | 23 | 19.6, CH<sub>2</sub> |
| 1′ | 165.8, C | 1′ | 99.9, CH | 1′ | 103.9, CH | 24 | 11.8, CH<sub>2</sub> |
| 2′ | 114.6, CH | 2′ | 75.5, CH | 2′ | 74.8, CH | 1′ | 175.6, C |
| 3′ | 162.1, C | 3′ | 78.7, CH | 3′ | 77.7, CH | 2′ | 72.5, CH |
| 4′ | 33.8, CH<sub>3</sub> | 4′ | 72.3, CH | 4′ | 72.3, CH | 3′ | 35.8, CH<sub>2</sub> |
| 5′ | 11.7, CH<sub>3</sub> | 5′ | 78.3, CH | 5′ | 76.0, CH | 4′ | 26.2, CH<sub>2</sub> |
| 6′ | 19.0, CH<sub>3</sub> | 6′ | 68.3, CH<sub>2</sub> | 6′ | 65.2, CH<sub>2</sub> | 5′-15′ | 29.5–30.5, CH<sub>2</sub> |
| 1″ | 100.4, CH | 1″ | 111.5, CH | 1″ | 122.4, C<sup>b</sup> | 16′ | 32.2, CH<sub>2</sub> |
| 2″ | 72.6, CH | 2″ | 76.2, CH | 2″ | 132.9, CH<sub>2</sub> | 17′ | 22.8, CH<sub>2</sub> |
| 3″ | 73.2, CH | 3″ | 80.8, C | 3″ | 116.6, CH | 18′ | 14.2, CH<sub>2</sub> |
| 4″ | 69.2, CH | 4″ | 75.4, CH<sub>2</sub> | 4″ | 164.2, C | 1″ | 105.6, CH |
| 5″ | 75.6, CH | 5″ | 65.8, CH<sub>2</sub> | 5″ | 116.6, CH | 2″ | 75.2, CH |
| 6″ | 63.4, CH<sub>2</sub> | 6″ | 132.9, CH | 3″ | 78.6, CH | 5″ | 78.7, CH |
| 7″ | 167.8, C | 7″ | 71.5, CH | 4″ | 71.5, CH |
| 8″ | 62.6, CH<sub>2</sub> |

<sup>a</sup> Measured in CD<sub>3</sub>OD at 30 °C; <sup>b</sup> Measured in C<sub>5</sub>D<sub>4</sub>N-d<sub>5</sub> at 30 °C.
Compound 2 was isolated as a white amorphous powder. HRESIMS ([M + Na]⁺ m/z 515.1737, calc. 515.1741) determined the molecular formula of 2 as C_{21}H_{22}O_{13}. Hydrolysis experiment of 2 liberated D-glucose and D-apiose which determined by GC-MS analysis. In the 1H-NMR spectrum of 2 (Table 1), signals of three methenyl groups at δ 3.34 (1H, m, H-5), 3.87 (1H, m, H-7), 3.08 (1H, d, J = 10.7 Hz, H-9), three methylene groups at δ 5.10 (1H, d, J = 11.0 Hz, H-3a), 4.44 (1H, d, J = 11.6 Hz, H-3b), 2.34 (1H, dd, J = 8.4, 13.4 Hz, H-6a), 2.19 (1H, m, H-6b), 5.08 (s, H-11a), 5.011 (s, H-11b), and a methyl group at δ 1.59 (3H, s, H-10) could be observed. The β-configuration of glucopyranosyl moiety was confirmed by the coupling constant of H-1’ (J = 7.8 Hz). The 13C-NMR and DEPT spectra of 2 (Table 2) showed 21 carbon signals, except for the 6 carbon signals at δ_{C} 99.9, 75.5, 78.7, 72.3, 78.3, 68.3 belong to a C-6’ substituted β-D-glucopyranosyl moiety and 5 carbon signals at δ_{C} 111.5, 76.2, 80.8, 75.4, 65.8 belong to a terminal β-D-apiofuranosyl moiety [22]. The left 10 carbon signals were similar to those of jatamanin A [23]. The main difference lies in the chemical shift value of C-7 in 2 was shifted downfield by 8.3 compared to that of jatamanin A, which confirmed that the β-D-glucopyranosyl moiety was located at C-7. The HSQC and 1H−1H COSY spectra of 2 showed the coupling sequence of (C(9)−C(5)−C(6)−C(7) (Figure 1). The cyclopenta[c]pyran-type iridoid structure for 2 was established by the HMBC correlations from H-7 to C-8, H-7 to C-9, H-5 to C-1, and H-3 to C-5. The HMBC correlations from H-1” to C-6’ and H-1’ to C-8 suggested that the apiofuranosyl moiety was located at C-6’ and glucopyranosyl moieties was located at C-7 (Figure 1).

The stereo-configuration of 2 was determined by NOESY spectrum. The NOE correlations (Figure 2) of H-9/CH3-10 and H-5/CH3-10, but absence of the correlations of H-9/H-7 and H-5/H-7, suggested that H-5, H-9 and CH3-10 were β-oriented, while 8-OH and H-7 was α-oriented. Therefore, the structure of 2 was founded to be (5S,7S,8S,9S)-8-hydroxy-8-methyl-4-methylenehexahydrocyclopenta[c]pyran-1(3H)-one 7-O-(6-O-β-D-apiofuranosyl)-β-D-glucopyranosyl and named Shecaoiridoidside B (Figure 2).

Compound 3 was obtained as a white amorphous powder. HRESIMS ([M + Na]⁺ m/z 487.1576, calc. 487.1580) established the molecular formula of 3 as C_{23}H_{25}O_{10}. Hydrolysis experiment of 3 liberated D-glucose which determined by GC-MS analysis. In the 1H-NMR of 3 (Table 1), signals of two oxygenated methylenes at δ 3.96 (1H, d, J = 10.4 Hz, H-1a), 3.76(1H, d, J = 10.4 Hz, H-1b), 4.37 (1H, d, J = 12.6 Hz, H-3a) and 4.18 (1H, d, J = 12.6 Hz, H-3b), a nonoxygenated methylene at δ 2.74 (1H, m, H-6a) and 2.26 (1H, brd, J = 16.4 Hz, H-6b), a noxygenatedmethine at δ 3.25 (1H, m, H-5), and three olefinic protons at δ 5.78 (1H, brs, H-7), 4.91 (1H, d, J = 2.0 Hz, H-11a) and 4.92 (1H, d, J = 2.0 Hz, H-11b), and a p-substituted benzene protons at 7.88 (2H, d, J = 8.8 Hz) and 6.81 (2H, d, J = 8.8 Hz) were observed. The β-configuration of glucopyranosyl moiety was confirmed by coupling constant of H-1’ (J = 7.8 Hz). The 13C-NMR and DEPT spectra of 3 (Table 2) showed 23 carbon signals, except for the 6 carbon signals at δ_{C} 103.9, 74.8, 77.7, 72.3, 76.0, 65.2 belong to a C-6’ substituted β-D-glucopyranosyl moiety and 7 carbon signals at δ_{C} 122.4, 132.9 × 2, 116.6 × 2, 164.2, 167.8 belong to a p-hydroxybenzoyl moiety, the left 10 carbon signals were similar with those of patriridoside G [24]. The main difference lies in the signal at δ_{C} 121.1 (CH$_3$-10) in patriridoside G was substituted by the signal at δ_{C} 59.3 (CH$_2$-10) in 3, which indicated that CH$_3$-10 of patriridoside G was substituted by a hydroxyl group.

The HSQC and 1H−1H COSY spectra of 3 showed the coupling sequences of C-3/C-4/C-11, C-11/C-4/C-5/C-6/C-7/C-8/C-10, C-2”/C-3”, and C-5”/C-6” (Figure 2). HMBC correlations of from H-10 to C-7, C-8, and C-9, H-1 to C-5 and C-9, H-11 to C-3, C-4, and C-5, H-3 to C-9, H-1’ to C-1, H-6’ to C-7” and H-2”/H-6” to C-7” established the structure of 3 (Figure 1). The NOE correlation of H-5β/H-1 in NOESY spectra suggested a β-orientation for C-1 (Figure 1). As a result, the structure of 3 was identified as (5R,9S)-6-O-(6-O-hydroxybenzoyl-β-D-glucopyranosyl)-8-hydroxymethyl-4-methylene-4,5,6,9-tetrahydro-3H-cyclopenta[b]furan-9-yl-methanol and named Shecaoiridoidside C (Figure 2).

Compound 4 was obtained as white amorphous powder. HRESIMS m/z 828.6924 [M + H]⁺ (calc. for 828.6929) determined the molecular formula of 4 as C$_{48}$H$_{63}$NO$_8$. Methanolysis experiment of 4 liberated D-glucose which determined by GC-MS analysis. In 1H- and 13C-NMR spectra of 4, signals of anomic proton δ$_{H}$ (4.90, 1H, d, J = 7.6 Hz) and δ$_{C}$ (105.6, 75.2, 78.6, 71.5, 78.7, and 62.6)
indicated the presence of a β-D-glucopyranosyl moiety. The characteristics of a cerebrosidé with a 2-hydroxy fatty acid fraction in 4 could be confirmed by analyzing its $^{1}H$- and $^{13}C$-NMR data (Tables 1 and 2). A fatty acid methyl ester (FAME) and a long-chain base (LCB) were obtained respectively by methanolysis of 4. GC-MS analysis determined the structure of FAM as 2-hydroxyoctadecanoic acid methyl ester. The absolute configuration of C-25 was determined by the specific rotation $[a]_{D}^{22} = -4.8^\circ$ ($c$ 0.05, CHCl$_{3}$) of the FAM [25]. The NMR data of C-2 and C-3 were compared with those of in literatures [26,27] and determined their stereo-configurations as 25 and 3R, respectively. The correlations of $\delta_{H}$ 4.77 (1H, m, H-4), 0.88 (3H, d, $J$ = 6.4 Hz, CH$_{3}$-23), and 0.86 (3H, t, $J$ = 6.4 Hz, CH$_{3}$-24) could be observed. Therefore, the branched methyl group was located in the LCB. The $^{1}H$- and $^{13}C$-NMR data (Tables 1 and 2) were further assigned by the spectra of DEPT, HSQC, $^{1}H$-H COSY, and HMBC. Thus, 4 was established as 1-O-β-D-glucopyranosyl-(2S,3R,4E)2-[(2'R)-2-hydroxyoctadecanamideamino]-21-methyl-4-tetracosene-1,3-diol which was named as shecaocerenoside A (Figure 1).

The known compounds were identified as jatamanin E (5) [28], 11-methoxyviburtinal (6) [29], 15-Demethylisoplumieride (7) [28], suspensolide F (8) [30], kanokoside A (9) [31], and patrinoside (10) [32] by comparing their physico-chemical constants and NMR spectroscopic data with those of in literatures (Figure 2).

The cytotoxicity of compounds 1–10 against tumor human cell lines of HL-60, HeLa, HCT15, A459, HepG2, PC-3, CNE-2, and BCG-823 were investigated in vitro. The MTT method was used to determine the IC$_{50}$ values. New compound 3 exhibited evident cytotoxicity to all tumor cell lines except the Hela, and the IC$_{50}$ values are from 9.6 µM to 62.2 µM, while new compound 4 showed moderate cytotoxicity to all the cell lines and the IC$_{50}$ values are from 33.6 µM to 89.3 µM. By contrast, new compound 1 and known compound 9 showed moderate cytotoxicity to HCT15, A459, and HepG2 selectively. Known compound 7 also exhibited moderate cytotoxicity to HCT15 and A459 selectively (Table 3). Compounds 1 and 9 with the structural stem-nucleus 8-hydroxy-8-hydroxymethyl-6,7-epoxyxyclopept-[c]pyran-1-O-4-methylscieneioxyl-11-hydroxymethyl-3-en 11-O-β-D-glycoside were tend to show cytotoxicity to HepG2, which was consist with the reference reported [33]. While HCT15 was tend to sensitive to compound 3. The cytotoxicity of sfingolipids has been reported in many references, and depend on its LCB, FAM, double bonds and glycosyl group to show moderate or weak activity to most of tumor cell lines [34–37], as well as compound 4.

| Compounds | HL-60 | Hela | HCT15 | A459 | HepG2 | PC-3 | CNE-2 | BCG-823 |
|-----------|-------|------|-------|------|-------|------|-------|--------|
| 1         | >100.0 | >100.0 | 87.6 ± 1.2 | 77.7 ± 1.6 | 37.6 ± 1.4 | >100.0 | >100.0 | >100.0 |
| 2         | >100.0 | >100.0 | >100.0 | >100.0 | >100.0 | >100.0 | >100.0 | >100.0 |
| 3         | 17.1 ± 0.7 | 62.2 ± 0.5 | 9.6 ± 0.8 | 14.8 ± 0.9 | 114 ± 1.6 | 26.2 ± 1.3 | 215 ± 0.6 | 134 ± 1.1 |
| 4         | 74.8 ± 1.3 | 89.3 ± 1.8 | 37.3 ± 1.5 | 33.6 ± 1.1 | 49.5 ± 1.4 | 64.0 ± 0.9 | 55.2 ± 1.1 | 44.1 ± 1.7 |
| 5         | >100.0 | >100.0 | >100.0 | >100.0 | >100.0 | >100.0 | >100.0 | >100.0 |
| 6         | >100.0 | >100.0 | >100.0 | >100.0 | >100.0 | >100.0 | >100.0 | >100.0 |
| 7         | >100.0 | >100.0 | 71.3 ± 1.2 | 50.4 ± 1.1 | >100.0 | 342 ± 1.3 | >100.0 | >100.0 |
| 8         | >100.0 | >100.0 | 89.8 ± 1.2 | 91.3 ± 0.7 | >100.0 | >100.0 | >100.0 | >100.0 |
| 9         | >100.0 | >100.0 | 96.1 ± 1.6 | 78.3 ± 0.8 | 97.9 ± 1.4 | >100.0 | >100.0 | >100.0 |
| 10        | >100.0 | >100.0 | >100.0 | >100.0 | >100.0 | >100.0 | >100.0 | >100.0 |

5-Fluorouracil: 7.5 ± 0.6, 10.4 ± 0.4, 4.7 ± 0.4, 14.7 ± 1.1, 22.8 ± 1.4, 33.2 ± 0.7, 11.6 ± 0.8, 17.8 ± 0.7

Key: All results are expressed as IC$_{50}$ values in µM. Compounds with IC$_{50}$ > 100 µM were inactive for the tumor cell lines.

Table 3. In vitro antitumor activity of compounds 1–10 in a panel of 8 tumor cell lines.
3. Materials and Methods

3.1. General

Column chromatographies such as Macroporous resin (AB-8 Crosslinked Polystyrene, Shanxi Lanshen Resin, Xi’an, China), silica gel (200–300 mesh, Hejie Technology Co. Ltd., Shanghai, China), and ODS-A (120A, 50 mm; DAIISO, Kyoto, Japan) were used for isolations. Compounds were prepared on a preparative HPLC (Waters, Milford, MA, USA). Bruker AVANCE 400 MHz NMR
instrument (Bruker SpectroSpin, Karlsruhe, Germany) was used to measure all the NMR spectra, including 1D-NMR and 2D-NMR spectra. Measured and analyzed the HRESIMS data was conducted on a Xero Q Tof MS spectrometer (Waters, Milford, MA, USA). IR Spectra data was recorded on FTIR-8400S (Shimadzu, Kyoto, Japan). The GC-MS (Agilent, Palo Alto, CA, USA) instrument was used to analyse the volatile derivatives from compounds. The growth of the tumor cell lines was monitored with a microplate reader (BMG FLUOStar OPTIMA, Ortenberg, Germany).

3.2. Plant Materials

The aerial part of *H. diffusa* Willd. was collected from Guangdong province of China and identified by Shuyuan Li of Guangdong Pharmaceutical University. The voucher specimen (No. 20160987) is deposited at the Herbarium of Guangdong Pharmaceutical University, Guangzhou, China.

3.3. Extraction and Isolation

The dried *H. diffusa* Willd. (10.0 Kg) were extracted two times (each for 2 h) with 75% EtOH (100 L) under reflux. The extract (1611 g) was suspended in water (15 L), and then extracted with petroleum ether (60–90 °C), EtOAc and n-butanol, respectively. Solvents were removed under vacuum to give extracts of petroleum ether (74.3 g), EtOAc (135.3 g), n-butanol (196.5 g), and remained water (1152.4 g). The EtOAc fraction (150.0 g) was subject to silica gel column column and eluted with a gradient of CH₂Cl₂/MeOH (30:1 to 0:1) to yield fractions of F₁–F₅. F₂ (28.4 g) was further chromatographed on silica gel column and eluted with petroleum ether/EtOAc (15:1 to 1:1) to yield subfractions of A₁–A₄. The sub-fraction A₂ (6.2 g) was repeated chromatographed on silica gel column and eluted with petroleum ether/EtOAc (5:1) to yield compound 4 (58 mg). F₃ (30.6 g) was chromatographed on silica gel column and eluted with a gradient of CH₂Cl₂/MeOH (20:1 to 5:1) to yield sub-fractions B₁–B₅. B₂ (10.4 g) was repeated chromatographed on silica gel column and eluted with CH₂Cl₂/MeOH (15:1) to yield compound 4 (46 mg). B₄ (5.6 g) was repeated chromatographed on silica gel column and eluted with CH₂Cl₂/MeOH (8:1) to yield compound 5 (41 mg). F₄ (62.4 g) was chromatographed on silica gel column and eluted with a gradient of CH₂Cl₂/MeOH (15:1–1:1) to yield sub-fractions C₁–C₆. C₃ (11.2 g) was chromatographed on silica gel column and eluted with a gradient of CH₂Cl₂/MeOH (10:1 to 3:1), and then purified on a preparative HPLC with Hypersil-ODS II column (10 µm, 20 × 300 mm) eluted with MeOH/H₂O (18%, flow rate 8 mL/min) to yield compounds 8 (48 mg, tR = 15 min), 10 (57 mg, tR = 27 min), 1 (62 mg, tR = 31 min), and 9 (53 mg, tR = 35 min). C₅ (14.4 g) was chromatographed on silica gel column and eluted with CH₂Cl₂/MeOH (5:1), and then purified on a preparative HPLC with Hypersil-ODS II column (10 µm, 20 × 300 mm) eluted with MeOH/H₂O (8%, flow rate 8 mL/min) to yield compounds 2 (48 mg, tR = 11 min), 7 (43 mg, tR = 18 min), and 3 (55 mg, tR = 23 min).

***Shecaoiridoidside A* (1). white amorphous powder; [α]D 22° = −25.4 (c = 0.20, CH₃OH); IR (KBr) νmax 3433, 3384, 2921, 2871, 1723, 1648, 1455, 1353, 1252, 1082, 880 cm⁻¹; ESIMS m/z 511 (100) [M + Na]⁺; HRESIMS [M + Na]⁺ m/z 511.1788 calc. 511.1791 for C₂₂H₃₂O₁₂Na; ¹H- and ¹³C-NMR data, see Tables 1 and 2.

***Shecaoiridoidside B* (2). white amorphous powder, [α]D 22° + 109.4° (c 0.10, MeOH); IR (KBr) νmax 3462, 3430, 2974, 2858, 1712, 1648, 1428, 1373, 1235, 1104 cm⁻¹; ESIMS m/z 515 (100) [M + Na]⁺; HRESIMS [M + Na]⁺ m/z 515.1737, calcld. 515.1741 for C₂₁H₃₂O₁₂Na; ¹H- and ¹³C-NMR data, see Tables 1 and 2.

***Shecaoiridoidside C* (3). white amorphous powder, [α]D 22° + 44.6° (c 0.12, MeOH); IR (KBr) νmax 3518, 3421, 2875, 1674, 1447, 1384, 1325, 1169, 1080, 891, 595 cm⁻¹; ESIMS m/z 487 (100) [M + Na]⁺; HRESIMS [M + Na]⁺ m/z 487.1576, calcld. 487.1580 for C₂₁H₂₈O₁₀Na; ¹H- and ¹³C-NMR data, see Tables 1 and 2.

***Shecaocerenoside A* (4). white amorphous powder; [α]D 22° = +6.4 (c = 0.15, C₅H₅N); IR (KBr) νmax 3411, 2941, 2838, 1635, 1532, 1455, 1162, 724 cm⁻¹; ESIMS m/z 828 (100) [M + H]⁺; HRESIMS [M + H]⁺ m/z 828.6924 calc. 828.6929 for C₄₈H₉₄NO₁₆H; ¹H- and ¹³C-NMR data, see Tables 1 and 2.
3.4. Acid Hydrolysis of 1–3

Acid hydrolysis experiment was carried out as the method in reference [24]. Briefly, the sugar residues were obtained by hydrolyzing of compounds 1–3 (2.0 mg) with 2 mol/L H2SO4 (2.0 mL), and then treated with trimethylchlorosilane, respectively. The sugar derivatives were further analyzed by GC-MS. As a result, the sugar derivatives from compounds 1 and 3 were determined to be D-galactose ($t_R = 19.46$ min) and D-glucose ($t_R = 11.33$ min), respectively. The sugar derivatives from compound 2 was determined to be D-glucose ($t_R = 11.33$ min) and D-apiose ($t_R = 14.53$ min).

3.5. Methanolsysis of 4

Methanolsysis of 4 was carried out according to the previous study [38]. In short, compound 4 (5.0 mg) was dissolved in in 82 % aqueous MeOH (20 mL) with 5% HCl and refluxed for 18 h. The FAME of 4 was obtained by extracting the reaction mixture with n-hexane. The FAME of 4 was a white amorphous powder, $[\alpha]_D^{20} = -4.8^\circ$ (c 0.02, CHCl3). Analyzed the FAME by GC-MS and the characteristic fragment ions ($m/z$ 314 [$M^+$], 256 [$M – COOMe$]) were obtained. As a result, the FAME of 4 was identified as 2-hydroxyoctadecanoic acid methyl ester. The remained solution was analyzed by GC-MS and the monosaccharide of 4 was identified as D-glucose ($t_R = 11.33$ min). After that the remained solution was evaporated MeOH and the aqueous ammonia was added to adjust pH 9.0, and then extracted the solution with Et2O to obtain the LCB. The fragment ions of $m/z$ 384 [$M + H]^+$ and 366 [$M – H_2O + H]^+$ from ESI-MS analysis led the LCB of 4 was identified as 2-aminotetracos-7-ene-1,3-diol (Figure 2).

3.6. Cytotoxicity Assay of Compounds 1–10

The cytotoxicity of all compounds against human tumor cell lines of HL-60, HeLa, HCT15, A459, HepG2, PC-3, CNE-2 and BCG-823 was assayed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method in vitro. The assay protocol was conducted by previous published paper [24,39]. The tested compounds 1–10 were dissolved in DMSO and adjusted to the final concentrations from 1.0 μM to 300 μM by diluting with the growth medium. 5-Fluorouracil was used as the positive drug.

4. Conclusions

We investigated the chemical constituents of H. diffusa Willd. based on its clinical application of treating malignant tumors and 10 compounds were obtained, including three new iridoid glycosides and a new cerebroside. The structures of new compounds were identified as (15,5S,9S,6S,7R,8S)-8-hydroxy-8-hydroxymethyl-6,7-epoxycyclopenta[c]pyran-1-O-4-methylseneicoyl-oxyl-11-hydroxymethyl-3-en-11-O-β-D-glucopyranoside (1), (6S,7S,8S,9S)-8-hydroxy-8-methyl-4-methylenehexahydrocyclopenta[c]pyran-1(3H)-one 7-O-(6-O-β-D-apiofuranosyl)-β-D-glucopyranoside (2), (5R,9S)-6-O-(6-O-4-hydroxybenzoyl-β-D-glucopyranosyl)-8-hydroxymethyl-4-methylene-4,5,6,9-tetrahydro-3H-cyclopenta[b]furan-9-yl-methanol (3), and 1-O-β-D-glucopyranosyl-(2S,3R,4E)-2-[(2′R)-2-hydroxyoctadecanamideino]-21-methyl-4-tetracosene-1,3-diol (4), respectively. Antitumor assays in vitro discovered cytotoxic compounds 1, 4, 7, and 9, especially found that new compound 3 exhibited evident cytotoxicity to all tumor cell lines except the Hela.

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References

1. Cai, Q.Y.; Lin, J.M.; Wei, L.H.; Zhang, L.; Wang, L.L.; Zhan, Y.Z.; Zeng, J.W.; Xu, W.; Shen, A.L.; Hong, Z.F.; et al. Hedyotis diffusa Wild Inhibits Colorectal Cancer Growth in Vivo via Inhibition of STAT3 Signaling Pathway. *Int. J. Mol. Sci.* **2012**, *13*, 6117–6128. [CrossRef] [PubMed]

2. Niu, Y.; Meng, Q.X. Chemical and preclinical studies on Hedyotis diffusa with anticancer potential. *J. Asian Nat. Prod. Res.* **2013**, *15*, 550–565. [CrossRef] [PubMed]

3. Zheng, W. Preparation of Graphene-Multi-Walled Carbon Nanotube Composite for Quantitive Determination of 2-hydroxy-3-Methylanthraquinone in Hedyotis diffusa. *Int. J. Electrochem. Sci.* **2017**, *12*, 629–638.

4. Chao, T.H.; Fu, P.K.; Chang, C.H.; Chang, S.N.; Chiahung, M.F.; Lin, C.H. Prescription patterns of Chinese herbal products for post-surgery colon cancer patients in Taiwan. *J. Ethnopharmacol.* **2014**, *155*, 702–708. [CrossRef] [PubMed]

5. Yeh, Y.C.; Chen, H.Y.; Yang, S.H.; Lin, Y.H.; Chiu, J.H.; Lin, Y.H.; Chen, J.L. *Hedyotis diffusa* Combined with *Scutellaria barbata* Are the Core Treatment of Chinese Herbal Medicine Used for Breast Cancer Patients: A Population-Based Study. *Evid. Based Complement. Altern. Med.* **2014**, *2014*, 202378. [CrossRef] [PubMed]

6. Chen, R.; He, J.; Tong, X.; Tang, L.; Liu, M. The *Hedyotis diffusa* willd. (rubiaceae): A review on phytochemistry, pharmacology, quality control and pharmacokinetics. *Molecules* **2016**, *21*, 710. [CrossRef] [PubMed]

7. Ye, J.H.; Liu, M.H.; Zhang, X.L.; He, J.Y. Chemical profiles and protective effect of *Hedyotis diffusa* willd in lipopolysaccharide-induced renal inflammation mice. *Int. J. Mol. Sci.* **2015**, *16*, 27252–27269. [CrossRef] [PubMed]

8. Li, D.; Schmitz, O.J. Comprehensive two-dimensional liquid chromatography tandem diode array detector (DAD) and accurate mass QTof-MS for the analysis of flavonoids and iridoid glycosides in *Hedyotis diffusa*. *Anal. Bioanal. Chem.* **2015**, *407*, 231–240. [CrossRef] [PubMed]

9. Cheng, Q.Q.; Cheng, C.S.; Liu, Z.Z.; Ouyang, Y.; Lin, J.R.; Zhang, Z.F.; Liu, Z.Q.; Zhou, H. Comparison of identification and medicinal progress of *Hedyotis diffusa* and *H. corymbosa*. *Chin. Tradit. Herb. Drugs* **2017**, *48*, 4328–4338.

10. Chen, Y.; Lin, Y.; Yachan, L.I.; Candong, L.I. Total flavonoids of *Hedyotis diffusa* willd inhibit inflammatory responses in lps-activated macrophages via suppression of the nf-kb and mapk signaling pathways. *Exp. Ther. Mol. Biol.* **2016**, *11*, 1116–1122. [CrossRef] [PubMed]

11. Shen, H.; Bai, Y.; Huo, Z. The protective effect of *Hedyotis diffusa* on collagen induced arthritis rats. *Int. J. Clin. Exp. Med.* **2016**, *9*, 12880–12887.

12. Gao, X.; Li, C.; Tang, Y.L.; Zhang, H. Chan, S.W. Effect of *Hedyotis diffusa* water extract on protecting human hepatocyte cells (LO) from HO-induced cytotoxicity. *Pharm. Biol.* **2015**, *54*, 1–8. [CrossRef] [PubMed]

13. Kuo, Y.J.; Lin, J.P.; Hsiao, Y.T.; Chou, G.L.; Tsai, Y.H.; Chiang, S.N.; Lin, J.G.; Chung, J.G. Ethanol Extract of *Hedyotis diffusa* Willd Affects Immune Responses in Normal Balb/c Mice In Vivo. *Molecules* **2016**, *21*, 3875–3881. [CrossRef] [PubMed]

14. Hu, E.; Wang, D.; Chen, J.; Tao, X. Novel cyclotides from *Hedyotis diffusa* induce apoptosis and inhibit proliferation and migration of prostate cancer cells. *Int. J. Clin. Exp. Med.* **2015**, *8*, 4059–4065. [PubMed]

15. Zhang, Y.; Xie, R.F.; Xiao, Q.G.; Li, R.; Shen, X.L.; Zhu, X.G. *Hedyotis diffusa* Willd extract inhibits the growth of human glioblastoma cells by inducing mitochondrial apoptosis via AKT/ERK pathways. *J. Ethnopharmacol.* **2014**, *158*, 404–411. [CrossRef] [PubMed]

16. Kuo, Y.J.; Liu, Y.J.; Way, T.D. Synergistic inhibition of leukemia wehi-3 cell growth by arsenic trioxide and *Hedyotis diffusa* willd extract in vitro and in vivo. *Exp. Ther. Med.* **2017**, *13*, 3388–3396. [CrossRef] [PubMed]

17. Jiang, J.; Wang, B.; Li, J.; Ye, B.; Lin, S.; Qian, W.; Shan, L.; Effertth, T. Total coumarins of *Hedyotis diffusa*, induces apoptosis of myelodysplastic syndrome skm-1 cells by activation of caspases and inhibition of pi3k/akt pathway proteins. *J. Ethnopharmacol.* **2017**, *196*, 253–260. [CrossRef] [PubMed]

18. Zhang, L.; Zhang, J.; Qi, B.; Jiang, G.; Liu, J.; Zhang, P.; Ma, Y.; Li, W. The anti-tumor effect and bioactive phytochemicals of *Hedyotis diffusa* willd on ovarian cancer cells. *J. Ethnopharmacol.* **2016**, *192*, 132–139. [CrossRef] [PubMed]

19. Xin, G.; Chang, L.; Tang, Y.L.; Zhang, H.; Chan, S.W. Effect of *Hedyotis diffusa* water extract on protecting human hepatocyte cells (lo2) from H2O2-induced cytotoxicity. *Pharm. Biol.* **2016**, *54*, 1148–1155.

20. Sun, G.; Wei, L.; Feng, J.; Lin, J.; Peng, J. Inhibitory effects of *Hedyotis diffusa* willd on colorectal cancer stem cells. *Oncol. Lett.* **2016**, *11*, 3875–3881. [CrossRef] [PubMed]
21. Chen, W.; Zheng, R.; Baade, P.D.; Zhang, S.; Zeng, H.; Bray, F.; Ahmedin, J.; Xue, Q.Y.; Jie, H. Cancer statistics in China, 2015. *CA Cancer J. Clin.* 2016, 66, 115–132. [CrossRef] [PubMed]

22. Jiang, Z.Y.; Li, S.Y.; Li, W.J.; Guo, J.M.; Tian, K.; Hu, Q.F.; Huang, X.Z. Phenolic glycosides from *Ficus tikosa* and their cytotoxic activities. *Carbohydr. Res.* 2013, 382C, 19–24. [CrossRef] [PubMed]

23. Lin, S.; Chen, T.; Liu, X.H.; Shen, Y.H.; Li, H.L.; Shan, L.; Liu, R.H.; Xu, X.K.; Zhang, W.D.; Wang, H. Iridoids and lignans from *Valeriana jatamansi*. *J. Nat. Prod.* 2010, 73, 632–638. [CrossRef] [PubMed]

24. Li, N.; Di, L.; Gao, W.C.; Wang, K.J.; Zu, L.B. Cytotoxic iridoids from *Patrinia scabra*. *J. Nat. Prod.* 2012, 75, 1723–1728. [CrossRef] [PubMed]

25. Shibuya, H.; Kawashima, K.; Sakagami, M.; Shimomura, M.; Ohashi, K.; Kitagawa, I. Sphingolipids and glycerolipids. I. Chemical structures and ionophoretic activities of soyacerebrosides I and II form soybean. *Chem. Pharm. Bull.* 1990, 38, 2933–2938. [CrossRef] [PubMed]

26. Satoshi, K.; Kazufumi, N.; Masanori, I.; Ryuichi, H. Isolation and Structure Determination of Six Glucocerebrosides from the Starfish *Luidia maculate*. *Chem. Pharm. Bull.* 2002, 50, 1091–1096.

27. Koji, Y.; Noriko, W.; Hiroyuki, O.; Rei, M.; Ryuichi, I.; Masanori, I.; Ryuichi, H. Constituents of Holothuroidea, Isolation of Ante-iso Type Regio isomer on Long Chain Base Moiety of Glucocerebroside from the Sea Cucumber *Holothuria leucospilota*. *Chem. Pharm. Bull.* 2005, 53, 788–791.

28. Dinda, B.; Debnath, S.; Harigaya, Y. Naturally occurring secoiridoids and bioactivity of naturally occurring iridoids and secoiridoids. A review, part 2. *Chem. Pharm. Bull.* 2007, 58, 206–212. [CrossRef] [PubMed]

29. Murshid, S.S.A.; Badr, J.M.; Youssef, D.T.A. Penicillosides A and B: New cerebrosides from the marine-derived fungus *Penicillium* species. *Rev. Bras. Farmacogn.* 2016, 26, 29–33. [CrossRef] [PubMed]

30. Tian, X.R.; Tang, H.F.; Feng, J.T.; Li, Y.S.; Lin, H.W.; Fan, X.P.; Zhang, X. Neritinaceramides A–E, New Ceramides from the Marine Bryozoan *Bugula neritina* inhabiting South China Sea and their cytotoxicity. *Mar. Drugs* 2014, 12, 1987–2003. [CrossRef] [PubMed]

31. Catoni, F.; Zilic, J.; Zachigna, M.; Procida, G. Cerebrosides with antiproliferative activity from *Euphorbia peplus* L. *Fitoterapia* 2010, 81, 97–103. [CrossRef] [PubMed]

32. Lei, X.; Li, J.; Li, B.; Zhang, N.; Liu, H. Separation and identification of four new compounds with antibacterial activity from *Portulaca oleracea*. *Molecules* 2015, 20, 16375–16387. [CrossRef] [PubMed]

33. Nguyen, T.H.; Pham, H.V.; Pham, N.K.; Quach, N.D.; Padhom, K.; Hansen, P.E.; Phung Nguyen, K.P. Chemical constituents from *Sonneratia ovata* backer and their in vitro cytotoxicity and acetylcholinesterase inhibitory activities. *Bioorg. Med. Chem. Lett.* 2015, 25, 2366–2371. [CrossRef] [PubMed]

Sample Availability: Samples of the Shecaoiridoidside A–C and shecaocerenoside A are available from the authors.