

## 梭果黄芪的化学成分和生物活性研究

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**摘要:** 从梭果黄芪甲醇提取物中分离鉴定了 14 个化合物, 经波谱数据分别鉴定为羽扇烯酮(1)、 $\beta$ -D-Glucopyranoside-3,4-dihydro-3-(2-hydroxy-3,4-dimethoxyphenyl)-2H-1-benzopyran-7-yl(2)、甘草素(3)、(3R)-8,2'-dihydroxy-7,4'-dimethoxyisoflavane(4)、异甘草素(5)、蔗糖(6)、7 $\alpha$ -羟基谷甾醇(7)、3 $\beta$ -羟基-5 $\alpha$ ,8 $\alpha$ -过氧化麦角甾-6,22-二烯(8)、三亚油酸甘油酯(9)、正三十三烷(10)、正十八烷(11)、二十八醇(12)、正二十七烷(13)、 $\beta$ -谷甾醇(14)。其中化合物 1-13 为首次从该植物中分离得到。活性研究结果显示, 化合物 2 对胃癌细胞 MGC-803、肝癌细胞 HepG2、人卵巢癌细胞 SKOV3 有一定抑制作用。

**关键词:** 梭果黄芪; 化学成分; 细胞毒

中图分类号: R284.2

文献标识码: A

Chemical and Biological Studies of *Astragalus ernestii* H. F. Comber

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**Abstract:** Fourteen compounds were separated from ethyl acetate fraction of the root extract of *Astragalus ernestii* H. F. Comber, including lupiniketene (1),  $\beta$ -D-glucopyranoside, 3,4-dihydro-3-(2-hydroxy-3,4-dimethoxyphenyl)-2H-1-benzopyran-7-yl (2), liquiritigenin (3), (3R)-8,2'-dihydroxy-7,4'-dimethoxy isoflavane (4), isoliquiritigenin (5), sucrose (6), 7 $\alpha$ -hydroxysterol (7), 5 $\alpha$ ,8 $\alpha$ -epidioxy-(22E,24R)-ergosta-6,22-dien-3 $\beta$ -ol (8), trilinolein (9), *n*-tritriacontane (10), *n*-octadecane (11),  $\rho$ tacosanol (12), *n*-heptacosane (13), and  $\beta$ -sitosterol (14). According to bioassays, compound 2 showed moderate cytotoxicities against the human gastric cancer cell line (MGC-803), the human hepatoma cell line (HepG2), and the human ovarian cancer cell line (SKOV3).

**Key words:** *Astragalus ernestii*; chemical constituents; cytotoxicity

## Introduction

Genus *Astragalus* is the largest one in the Fabaceae family<sup>[1]</sup>. As a member of the genus, *Astragalus ernestii* is mainly distributed in southwest China, including the northwest Sichuan, northwest Yunnan, and east Tibet, with an altitude between 3900-4500 m. This plant is often used as substitute of Chinese medicine "Huang Qi"<sup>[2-3]</sup> by local folks, and therefore to be thought to have similar medicinal function with Huangqi, such as accelerate the metabolism, antifatigue effects, adjust the

body's immunological function, anti-hypoxic, radiation resistance, liver protection and so on<sup>[4-8]</sup>. So far, only several chemical constituents have been reported from *A. ernestii*<sup>[9]</sup>. As a part of the project to better understand chemical and bioactive properties of *Astragalus* plants, we recently investigated *A. ernestii* collected from northwest Yunnan. As a result, fourteen known compounds were isolated and identified. And one of the compounds showed cytotoxicities against the human gastric cancer cell line (MGC-803), the human hepatoma (HepG2), and the human ovarian cancer cell line (SKOV3).

## Materials and Methods

## Apparatus and reagents

NMR spectra were recorded on Bruker AM-400 with TMS as reference. Silica gel (200-300 mesh, 300-400

Received: April 11 2013 Accepted: June 13 2013

Foundation Item: This project was supported by National Natural Science Foundation of China (No. 31170313) and the grant (2009CD090) from Yunnan Provincial Science and Technology Department.

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mesh) used for column chromatography and silica gel GF<sub>254</sub> TLC were purchased from Qingdao Marine Chemical Factory (Qingdao, China). Sephadex LH-20 and MCI-gel (CHP-20P) were purchased from Amersham Biosciences (Amersham, Sweden). Spots of TLC were colored by spraying with 10% H<sub>2</sub>SO<sub>4</sub> followed by heating. Reagents used in the studies were all of analytical purity.

### Plant material

The plant sample was collected from Zhongdian (Yunnan province, China) and authenticated as *Astragalus ernestii* H. F. Comber by Dr. Zhang De-quan who was a botanist working at Dali University. A voucher specimen was deposited at Prof. Jiang Bei's laboratory, College of Pharmacy and Chemistry, Dali University.

### Extract preparation and compound isolation

The finely powdered roots of *A. ernestii* (1.26 kg) were extracted six times with methanol at room temperature. The filtered solvent was evaporated to yield crude extract (168 g), which was suspended in H<sub>2</sub>O and partitioned with ethyl acetate. The EtOAc fraction (35 g) was subjected to silica gel column chromatography (200–300 mesh) and eluted with CHCl<sub>3</sub>–CH<sub>3</sub>COCH<sub>3</sub> (100:0–0:100) to afford fractions 1–10. Fr. 2 (2.0 g) was separated by silica gel column chromatography (CC) and eluted with petroleum–EtOAc system (150:1) to give compound **1** (30 mg), **9** (20 mg), and **8** (10 mg). Fr. 3 (1.5 g) was separated by silica gel CC and developed with petroleum–EtOAc system (20:1), and the appropriate subfractions were further purified by sephadex LH-20 (eluted with MeOH), silica gel CC and recrystallization, and silica gel CC (eluted with CHCl<sub>3</sub>–MeOH 70:1), to yield compounds **12** (6 mg), **14** (1.0 g), and **11** (6 mg), respectively. Fr. 8 was chromatographed over an MCI column eluted with MeOH–H<sub>2</sub>O gradient system (20%–100%) to give compounds **2** (800 mg), **3** (8 mg), **4** (10 mg), **7** (8 mg), **5** (10 mg), **10** (10 mg), and **13** (6 mg). Fr. 10 was chromatographed over an MCI column eluted with MeOH–H<sub>2</sub>O gradient system (10%–100%) to give compound **6** (20 mg).

## Structural identification results

**Lupiniketene (1)**, colorless needle crystal

(CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>, 400 MHz) δ: 4.69 (1H, br s, H-29b), 4.57 (1H, br s, H-29a), 1.68 (3H, s, H-30), 1.08 (6H, s, H-23, 26), 1.04 (3H, s, H-24), 0.96 (3H, s, H-27), 0.94 (3H, s, H-25), 0.80 (3H, s, H-28); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 100 MHz) δ: 217.9 (s, C-3), 150.8 (s, C-20), 109.4 (t, C-29), 54.9 (s, C-5), 49.8 (d, C-9), 48.3 (d, C-18), 47.9 (d, C-19), 47.3 (s, C-4), 43.0 (s, C-17), 42.8 (s, C-14), 40.8 (s, C-8), 40.0 (t, C-22), 39.6 (t, C-1), 37.4 (d, C-13), 36.9 (s, C-10), 35.6 (t, C-16), 34.1 (t, C-2), 33.6 (t, C-7), 29.8 (t, C-21), 27.4 (t, C-15), 26.6 (q, C-23), 25.1 (t, C-12), 21.5 (t, C-11), 21.0 (q, C-24), 19.7 (t, C-6), 19.3 (q, C-30), 18.0 (q, C-28), 15.9 (q, C-25), 15.8 (q, C-26), 14.5 (q, C-27). These data are consistent with the literature values<sup>[10]</sup>. Thus **1** was determined to be lupiniketene.

### **β-D-Glucopyranoside 3-O-(2-hydroxy-3,4-dimethoxyphenyl)-2H-1-benzopyran-7-yl (2)**, colorless needle crystal (MeOH).

<sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>OD) δ: 6.98 (1H, d, J = 8.5 Hz, H-5), 6.77 (1H, d, J = 9.0 Hz, H-6'), 6.62 (1H, dd, J = 8.5, 2.5 Hz, H-6), 6.54 (1H, d, J = 2.5 Hz, H-8), 6.46 (1H, d, J = 9.0 Hz, H-5''), 4.85 (1H, d, J = 8.0 Hz, H-1'), 4.20 (1H, ddd, J = 10.5, 3.5, 1.5 Hz, H-2), 3.96 (1H, t, J = 10.5 Hz, H-2), 3.75 (3H, s, 4'-OCH<sub>3</sub>), 3.69 (1H, dd, J = 12.0, 2.0 Hz, H-7''), 3.69 (3H, s, 3'-OCH<sub>3</sub>), 3.46 (1H, dd, J = 12.0, 6.0 Hz, H-6''), 3.36 (1H, dddd, J = 10.5, 3.5, 11.0, 5.0 Hz, H-3), 3.29 (1H, ddd, J = 9.0, 6.0, 2.0 Hz, H-5''), 3.26 (1H, d, J = 9.0 Hz, H-3''), 3.20 (1H, dd, J = 9.0, 8.0 Hz, H-2''), 3.15 (1H, t, J = 9.0 Hz, H-4''), 2.81 (1H, ddd, J = 16.5, 5.0, 1.5 Hz, H-4); <sup>13</sup>C NMR (100 MHz, CD<sub>3</sub>OD) δ: 158.4 (s, C-7), 156.3 (s, C-9), 153.2 (s, C-4'), 149.5 (s, C-3'), 137.6 (s, C-2'), 131.1 (d, C-5), 122.8 (d, C-6'), 122.3 (s, C-1'), 117.0 (s, C-10), 110.1 (d, C-6), 105.6 (d, C-8), 104.4 (d, C-5'), 102.5 (d, C-1'), 78.2 (q, C-5''), 78.0 (s, C-3''), 74.9 (d, C-2''), 71.9 (q, C-4'), 71.0 (t, C-2), 62.5 (t, C-6''), 61.0 (q, -OCH<sub>3</sub>), 56.2 (q, -OCH<sub>3</sub>), 33.5 (d, C-3), 31.1 (t, C-4). These data are consistent with the reported values<sup>[11]</sup>. Thus **2** was determined to be the title compound.

**Liquiritigenin (3)** was obtained as yellow powder

(MeOH).  $^1\text{H NMR}$  (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$ : 8.16 (1H, d,  $J = 8.8$  Hz, H-5), 7.53 (2H, br. d,  $J = 8.7$  Hz, H-2', 6'), 7.21 (2H, br. d,  $J = 8.16$  Hz, H-3', 5'), 6.88 (1H, dd,  $J = 2.0, 8.8$  Hz, H-6), 6.80 (1H, d,  $J = 2.0$  Hz, H-8), 5.55 (1H, dd,  $J = 2.8, 13.0$  Hz, H-2 $\beta$ ), 3.25 (1H, dd,  $J = 13.6, 16.5$  Hz, H-3 $\alpha$ ), 2.75 (1H, dd,  $J = 2.8, 16.9$  Hz, H-3 $\beta$ );  $^{13}\text{C NMR}$  (100 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$ : 190.4 (s, C-4), 166.5 (s, C-7), 164.5 (s, C-9), 159.3 (s, C-4'), 130.2 (s, C-1'), 129.5 (d, C-5), 128.7 (d, C-2', 6'), 116.5 (d, C-3', 5'), 114.9 (s, C-10), 111.5 (d, C-6), 103.7 (d, C-8), 80.3 (d, C-2), 44.4 (t, C-3). These data for **3** are consistent with the literature values for liquiritigenin<sup>[12]</sup>.

(**3R**)-**8**, **2'**-Dihydroxy-**7**, **4'**-dimethoxy isoflavane (**4**) was obtained as white crystal ( $\text{CH}_3\text{COCH}_3$ ).  $^1\text{H NMR}$  (acetone- $d_6$ , 400 MHz)  $\delta$ : 6.88 (1H, d,  $J = 8.4$  Hz, H-6'), 6.82 (1H, d,  $J = 9.0$  Hz, H-5), 6.49 (1H, d,  $J = 9.0$  Hz, H-6), 6.35 (1H, dd,  $J = 8.5, 2.6$  Hz, H-5'), 6.26 (1H, d,  $J = 2.4$  Hz, H-3'), 4.24 (1H, brd,  $J = 10.2$  Hz, H-2 $\beta$ ), 3.97 (1H, t,  $J = 10.2$  Hz, H-2 $\alpha$ ), 3.81 (1H, s, 4'-OMe), 3.78 (1H, s, 7-OMe), 3.45 (1H, m, H-3), 2.96 (1H, dd,  $J = 16.2, 10.8$  Hz, H-4 $\beta$ ), 2.82 (1H, ddd,  $J = 16.2, 5.2, 1.9$  Hz, H-4 $\alpha$ );  $^{13}\text{C NMR}$  (100 MHz, acetone- $d_6$ )  $\delta$ : 157.5 (s, C-7), 156.0 (s, C-4'), 152.6 (s, C-9), 148.9 (s, C-2'), 136.8 (s, C-8), 130.9 (d, C-6'), 122.4 (d, C-5), 121.5 (s, C-1'), 114.1 (s, C-10), 108.7 (d, C-5'), 104.2 (d, C-6), 103.6 (d, C-3'), 70.3 (t, C-2), 60.7 (4'-OCH<sub>3</sub>), 50.0 (7-OCH<sub>3</sub>), 32.9 (d, C-3), 30.8 (t, C-4). These data for **4** are highly consistent with those reported values for (**3R**)-**8**, **2'**-dihydroxy-**7**, **4'**-dimethoxy isoflavane<sup>[13]</sup>.

**Isoliquiritigenin** (**5**) was obtained as yellow powder (MeOH).  $^1\text{H NMR}$  ( $\text{CD}_3\text{OD}$ , 400 MHz)  $\delta$ : 8.01 (1H, d,  $J = 8.9$  Hz, H-6'), 7.82 (1H, d,  $J = 14.5$  Hz, H- $\alpha$ ), 7.65 (3H, overlap, H-2, 6,  $\beta$ ), 6.87 (2H, d,  $J = 8.5$  Hz, H-3, 5), 6.44 (1H, dd,  $J = 9.1, 2.7$  Hz, H-5'), 6.30 (1H, d,  $J = 2.3$  Hz, H-3');  $^{13}\text{C NMR}$  ( $\text{CD}_3\text{OD}$ , 100 MHz)  $\delta$ : 192.1 (CO), 166.1 (s, C-4'), 164.9 (s, C-2'), 160.2 (s, C-4), 144.3 (d, C- $\beta$ ), 131.9 (s, C-6'), 130.4 (d, C-2, 6), 126.4 (s, C-1), 116.9 (d, C- $\alpha$ ), 115.5 (d, C-2, 5), 113.3 (d, C-1'), 107.7 (d, C-5'), 102.4 (d, C-3'). These data are con-

sistent with those reported values<sup>[14]</sup>. Therefore **5** was determined to be isoliquiritigenin.

**Sucrose** (**6**) was obtained as colorless crystal (DMSO).  $^1\text{H NMR}$  (DMSO- $d_6$ , 400 MHz)  $\delta$ : 5.42 (1H, d,  $J = 3.3$  Hz, H-1), 4.23 (1H, dd,  $J = 8.7, 2.5$  Hz, H-3'), 4.06 (1H, dd,  $J = 8.4, 2.4$  Hz, H-4'), 3.57 (1H, dd,  $J = 9.8, 3.5$  Hz, H-2);  $^{13}\text{C NMR}$  (DMSO- $d_6$ , 100 MHz)  $\delta$ : 103.6 (d, C-2'), 92.1 (s, C-1), 81.3 (d, C-5'), 76.3 (d, C-3'), 73.9 (d, C-4'), 72.3 (t, C-5), 72.5 (d, C-3), 71.0 (d, C-2), 69.1 (d, C-4), 62.3 (t, C-6'), 61.2 (d, C-1'), 60.0 (t, C-6). These data for **6** are consistent with the literature values for sucrose<sup>[15]</sup>.

**7 $\alpha$ -Hydroxysitosterol** (**7**), colorless needle crystal ( $\text{CHCl}_3$ ), EIMS  $m/z$  (rel. int. %): 412 [M-H<sub>2</sub>O].  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 5.60 (1H, d,  $J = 5.1$  Hz, H-6), 3.85 (1H, brs, H-7), 3.59 (1H, m, H-3), 0.99 (3H, s, Me-19), 0.92 (3H, d,  $J = 6.5$  Hz, Me-21), 0.86 (3H, overlap, Me-26), 0.84 (3H, overlap, Me-29), 0.80 (3H, overlap, Me-27), 0.70 (3H, s, Me-18);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 100 MHz)  $\delta$ : 146.2 (s, C-5), 123.8 (d, C-6), 71.3 (d, C-3), 65.3 (d, C-7), 55.7 (d, C-17), 49.4 (d, C-14), 45.8 (d, C-24), 42.3 (s, C-13), 42.2 (d, C-9), 42.0 (t, C-4), 39.2 (t, C-12), 37.5 (d, C-8), 37.4 (s, C-10), 37.0 (t, C-1), 36.1 (d, C-20), 33.9 (t, C-22), 31.4 (t, C-2), 29.7 (t, C-16), 29.0 (d, C-25), 28.3 (t, C-23), 24.3 (t, C-15), 23.1 (t, C-28), 20.7 (t, C-11), 19.9 (q, C-27), 19.0 (q, C-19), 18.8 (q, C-26), 18.2 (q, C-21), 11.9 (q, C-29), 11.6 (q, C-18). These data for **7** are consistent with the literature values for 7 $\alpha$ -hydroxysitosterol<sup>[16]</sup>.

**5 $\alpha$  8 $\alpha$ -Epidioxy-(22E, 24R)-ergosta-6, 22-dien-3 $\beta$ -ol** (**8**), white powder ( $\text{CHCl}_3$ );  $^1\text{H NMR}$  ( $\text{CDCl}_3$ , 400 MHz)  $\delta$ : 6.53 (1H, d,  $J = 8.4$  Hz, H-7), 6.22 (1H, d,  $J = 8.7$  Hz, H-6), 5.24 (1H, dd,  $J = 7.9, 15.3$  Hz, H-22), 5.24 (1H, dd,  $J = 7.3, 15.1$  Hz, H-23), 3.99 (1H, m, H-3), 1.09 (3H, s, H-19), 1.00 (3H, d,  $J = 6.6$  Hz, H-21), 0.90 (3H, s, H-18), 0.89 (3H, m, H-28), 0.84 (3H, d,  $J = 6.5$  Hz, H-26), 0.82 (3H, d,  $J = 4.1$  Hz, H-27);  $^{13}\text{C NMR}$  ( $\text{CDCl}_3$ , 100 MHz)  $\delta$ : 135.4 (d, C-6), 135.1 (d, C-22), 132.3 (d, C-23), 130.6 (d, C-7), 82.2 (s, C-5), 79.4 (d, C-8), 66.5 (d, C-3), 56.2 (d, C-17), 51.6 (d, C-14), 51.0 (d, C-

9) 44.5 (s, C-13) 42.7 (d, C-24) 39.5 (d, C-20) , 39.3 (t, C-12) 37.0 (t, C-4) 36.9 (s, C-10) 34.6 (t, C-1) 33.0 (d, C-25) 30.0 (t, C-2) 28.6 (t, C-16) 23.3 (t, C-11) 20.6 (t, C-15) 20.6 (21-CH<sub>3</sub>) , 19.8 (26-CH<sub>3</sub>) 19.6 (27-CH<sub>3</sub>) 18.1 (19-CH<sub>3</sub>) 17.5 (28-CH<sub>3</sub>) 12.9 (18-CH<sub>3</sub>) . These data are consistent with the literature values<sup>[17]</sup>. Thus **8** was determined to be 5 $\alpha$  8 $\alpha$ -epidioxy-(22*E* 24*R*)-ergosta-6 22-dien-3 $\beta$ -ol.

**Trilinolein (9)** , colorless oil (CHCl<sub>3</sub>) ; <sup>1</sup>H NMR (CDCl<sub>3</sub> , 400 MHz)  $\delta$ : 5.31-5.41 (12H, m) , 5.26 (1H, m) , 4.28 (2H, dd, *J* = 4.2, 11.9 Hz) , 4.13 (2H, dd, *J* = 6.0, 12.0 Hz) , 2.75 (4H, t, *J* = 6.5 Hz) 2.29 (2H, overlap) 2.28 (4H, overlap) 2.00-2.07 (12H, overlap) 1.60 (6H, m) 1.22-1.38 (overlap) 0.87 (9H, t, *J* = 6.7) ; <sup>13</sup>C NMR (CDCl<sub>3</sub> , 100 MHz)  $\delta$ : 173.1 (s, C-1', 1'') , 172.7 (s, C-1') , 130.1 (d, C-10', 10'', 10''') , 129.9 (d, C-12', 12'', 12''') , 128.0 (s, C-13', 13'', 13''') , 127.8 (s, C-9', 9'', 9''') 68.8 (d, C-13) 62.0 (t, C-2) 34.1 (t, C-2', 2'') , 33.9 (t, C-2'') , 31.4 (t, C-3', 3'', 3''') , 29.0-29.6 (t, C-4'-7', 4''-7'' , 4'''-7''') , 15', 15'' 15''' 27.1 (t, C-8', 8'', 8''') , 25.5 (t, C-14', 14'', 14''') 24.8 (t, C-11', 11'', 11''') , 24.7 (t, C-16', 16'', 16''') 22.5 (t, C-17', 17'', 17''') , 14.0 (q, C-18', 18'', 18''') . These data for **9** are agreed with the literature values for trilinolein<sup>[18]</sup>.

***n*-Tritriacontane (10)** was obtained as white powder (CHCl<sub>3</sub>) . EI-MS: 464 [M<sup>+</sup> , 6.2] , 449 (8.8) , 435 (10.0) 421 (12.5) , 407 (21.9) , 393 (28.1) , 379 (43.8) , 113 (25.0) , 99 (28.8) , 85 (68.8) , 71 (90.6) 57 (100) . These data are consistent with the literature values<sup>[19]</sup>. Thus **10** was determined to be *n*-tritriacontane.

***n*-Octadecane (11)** was obtained as white powder (CHCl<sub>3</sub>) . EI-MS: 254 [M<sup>+</sup> , 8.1] , 239 (8.1) , 225 (8.1) , 211 (8.1) , 197 (8.1) , 149 (29.4) , 111 (32.5) 97 (38.8) , 71 (62.5) 57 (100) . These data are consistent with the literature values<sup>[20]</sup>. Thus **11** was determined to be *n*-octadecane.

**Octacosanol (12)** , white powder (CHCl<sub>3</sub>) ; EI-MS: 410 [M<sup>+</sup> , 12.5] , 392 (6.3) , 364 (19) , 336 (12.5) , 308 (6.3) , 280 (6.3) , 195 (6.3) , 181 (9.4) , 167

(12.6) , 153 (17) , 139 (25) , 125 (34.4) , 111 (46.9) 97 (81.3) 83 (87.5) , 71 (62.5) 57 (100) . These data are consistent with the literature values<sup>[21]</sup>.

Therefore **12** was determined to be octacosanol.

***n*-Heptacosane (13)** , white powder (CHCl<sub>3</sub>) ; EI-MS: 380 [M<sup>+</sup> , 16.5] , 365 (6.5) , 351 (6.5) , 337 (6.55) , 323 (6.5) , 309 (6.5) , 295 (6.5) , 71 (81) 57 (100) . These data are agreed with the literature values<sup>[22]</sup>. Thus **13** was determined to be *n*-heptacosane.

**$\beta$ -Sitosterol (14)** , white needle crystal (CHCl<sub>3</sub>) ; <sup>1</sup>H NMR (CDCl<sub>3</sub> , 400 MHz)  $\delta$ : 5.36 (1H, dd, *J* = 4.5, 2.8 Hz, H-6) 3.53 (1H, m, H-3a) 1.01 (3H, s, H-19) , 0.93 (3H, d, *J* = 6.6 Hz, H-21) 0.86 (3H, t, *J* = 6.0 Hz, H-26) 0.83 (3H, d, *J* = 6.8 Hz, H-29) 0.81 (3H, d, *J* = 6.6 Hz, H-28) 0.68 (3H, s, H-18) ; <sup>13</sup>C NMR (CDCl<sub>3</sub> , 100 MHz)  $\delta$ : 140.7 (s, C-5) 121.6 (d, C-6) 71.8 (d, C-3) 56.8 (d, C-14) 56.0 (d, C-17) , 51.0 (d, C-9) 45.9 (d, C-24) 42.4 (s, C-13) 42.0 (t, C-4) 39.8 (t, C-12) 37.3 (t, C-1) 36.5 (s, C-10) 36.1 (d, C-20) 33.9 (t, C-22) 31.9 (d, C-8) , 31.6 (t, C-2) 32.0 (t, C-7) 29.1 (q, C-27) 28.3 (t, C-16) 26.1 (t, C-23) 24.1 (t, C-15) 23.0 (d, C-25) 21.1 (t, C-11) 19.7 (q, C-29) 19.4 (q, C-19) , 19.2 (t, C-28) 18.6 (q, C-21) 11.8 (q, C-18) 11.0 (q, C-26) . These data for **14** are consistent with the literature values for  $\beta$ -sitosterol<sup>[23]</sup>.

## Cytotoxic assay

### Cell lines

The human gastric cancer cell line (MGC-803) , the human hepatoma cell line (HepG2) , and the human ovarian cancer cell line (SKOV3) was obtained from the Key Laboratory of Medical Insects and Spiders Resources for Development and Utilization, Yunnan Province.

### Cell culture

Cell line (MGC-803) was maintained in RPMI-1640 (GIBCO) and HepG2, SKOV3 were maintained in DMEM (GIBCO) , supplement with 10% fetal bovine serum FBS (GIBCO) , 100 IU/mL penicillin and 100  $\mu$ g/mL streptomycin (Life Technologies) . Cells were grown in 25 cm<sup>2</sup> tissue culture flasks in a humidified atmosphere containing 5% CO<sub>2</sub> at 37  $^{\circ}$ C. Once the

cells reach 80% confluence, 1 mL of trypsin-EDTA solution was added to the flask for 5 min to detach the monolayer cells. The cells were occasionally observed under the inverted microscope until the cell layer was dispersed. Then 2 mL of complete growth medium was added to the flask followed by repeated gentle pipetting to split apart the cell clumps. Approximately  $0.5-1 \times 10^6$  cells were sub cultured into a new 25 cm<sup>2</sup> flask containing 8 mL of fresh medium.

### MTT colorimetric assay

The MTT assay is commonly used in the screening of anti-cancer compounds, and this method was first developed in 1983. The tetrazolium salt (MTT) is used as a developing dye. The tetrazolium ring of MTT can be cleaved by dehydrogenases in the mitochondria of living cells to produce a purple formazan. The MTT soluble formazan reaction was only partially soluble in the medium, and so the [10% SDS-5% isobutanol-0.012 mol/L HCl (w/v/v)] was used to dissolve the formazan and the optical densities at 570 nm are read by a scanning multi-well spectrophotometer<sup>[26]</sup>.

Briefly, exponentially growing cells were seeded into 96-well plate at a density of approximately  $1 \times 10^5$  cells/90  $\mu$ L/well and allowed to adhere overnight. Treatments in the final concentration range between 3.0 and 300  $\mu$ g/mL were introduced. Meanwhile, the control wells were treated with 0.3% of DMSO equivalent to the amount of DMSO used as a vehicle in the sample treated wells. After 48 h of incubation, 15  $\mu$ L of MTT solution (5.0 mg/mL) was added and incubation for an additional 4 h. Medium and excessive MTT were aspirated and formazan formed was solubilized by the addition of 100  $\mu$ L [10% SDS-5% isobutanol-0.012 mol/L HCl (w/v/v)]. The optical densities at 570 nm are read by a scanning multi-well spectrophotometer. The results were listed in the table 1.

**Table 1 Cytotoxicities of the samples from *A. ernestii***

Sample	IC <sub>50</sub> ( $\mu$ g/mL)		
	HepG2	MGC-803	SKOV3
DDP	7.3	2.2	11.1
The raw extract	—	—	—
The EtOAc fraction	—	—	—

The <i>n</i> -BuOH fraction	—	—	—
The H <sub>2</sub> O fraction	—	—	—
Compound 2	145.5	164.6	253.7

## Conclusion

Among the fourteen compounds obtained from *A. ernestii*, compound 2 and  $\beta$ -sitosterol were the major constituents of the EtOAc fraction. According to the cytotoxic experiments on the samples including raw extract, ethyl acetate fraction, butanol fraction, water fraction, and compound 2 showed moderate cytotoxicities against human gastric cancer cell line (MGC-803), human hepatoma cancer cell line (HepG2), and human ovarian cancer cell line (SKOV3). However, the other samples didn't have activities on these cell lines.

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## Conclusion

The results presented in this study showed that fruit-EFr, leaf-EFr, root-EFr and root-BFr had high  $\alpha$ -glucosidase and  $\alpha$ -amylase inhibitory activity, and are effective on promoting glucose consumption. This study gave basic scientific support for the use of *G. xanthochymus* as a treatment of diabetes, and therefore can help to develop medicinal preparations or nutraceutical and functional foods for diabetics.

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