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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种分离鉴定了 14 个化合物,经波谱数据分别鉴定为羽扇烯酮(**1**)、β-D-Glucopyranoside-3,4-dihydro-3-(2-hydroxy-3,4-dimethoxyphenyl)-2*H*-1-benzopyran-7-yl(**2**)、甘草素(**3**)、(3*R*)-8,2′-dihydroxy-7,4′-dimethoxyisoflavane(**4**)、异甘草素(**5**)、蔗糖(**6**)、7α-羟基谷甾醇(**7**)、3β-羟基-5α,8α-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**)、β-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

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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 lupineketene (**1**), β-*D*-glucopyranoside, 3, 4-dihydro-3-(2-hydroxy-3, 4-dimethoxyphenyl) -2*H*-1-benzopyran-7-yl(**2**), liquiritigenin(**3**), (3*R*)-8,2′-dihydroxy-7,4′-dimethoxy isoflavane(**4**), isoliquiritigenin(**5**), sucrose(**6**), 7α-hydroxysitosterol(**7**), 5α,8α-epidioxy-(22*E*,24*R*)-ergosta-6,22-dien-3β-ol(**8**), trilinolein(**9**), *n*-tritriacontane(**10**), *n*-octadecane(**11**), octacosanol(**12**), *n*-heptacosane(**13**), and β-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 *Astraglus* is the largest one in the Fabaceae family[1]. 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"[2-3] 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[4-8]. So far, only several chemical constituents have been reported from *A. ernestii*[9]. 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).

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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

mesh) used for column chromatography, and silica gel GF_{254} 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_2SO_4 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_2O and partitioned with ethyl acetate. The EtOAc fraction (35 g) was subjected to silica gel column chromatography (200-300 mesh) and eluted with $CHCl_3$-CH_3COCH_3 (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 $CHCl_3$-MeOH, 70:1), to yield compounds **12** (6 mg), **14** (1.0 g), and **11** (6 mg), respectively. Fr. 8 was chromatography over an MCI column eluted with MeOH-H_2O 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_2O gradient system (10%-100%) to give compound **6** (20 mg).

Structural identification results

Lupineketene (**1**), colorless needle crystal ($CHCl_3$); ^{1}H NMR ($CDCl_3$, 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); ^{13}C NMR ($CDCl_3$, 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[10]. Thus, **1** was determined to be lupineketene.

***β*-*D*-Glucopyranoside 3,4-dihydro-3-(2-hydroxy-3,4-dimethoxyphenyl)-2*H*-1-benzopyran-7-yl** (**2**), colorless needle crystal (MeOH). ^{1}H NMR (400 MHz, CD_3OD) 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_3), 3.69 (1H, dd, J = 12.0, 2.0 Hz, H-7″), 3.69 (3H, s, 3′-OCH_3), 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 (H, t, J = 9.0 Hz, H-4″), 2.81 (1H, ddd, J = 16.5, 5.0, 1.5 Hz, H-4); ^{13}C NMR (100 MHz, CD_3OD) 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_3), 56.2 (q, -OCH_3), 33.5 (d, C-3), 31.1 (t, C-4). These data are consistent with the reported values[11]. Thus, **2** was determined to be the title compound.

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

(MeOH). ^{1}H NMR (400 MHz, CD_3OD) δ: 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-2β), 3.25 (1H, dd, J = 13.6, 16.5 Hz, H-3α), 2.75 (1H, dd, J = 2.8, 16.9 Hz, H-3β); ^{13}C NMR (100 MHz, CD_3OD) δ: 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[12].

(3*R*)-8, 2′-Dihydroxy-7, 4′-dimethoxy isoflavane (4) was obtained as white crystal (CH_3COCH_3). ^{1}H NMR (acetone-d_6, 400 MHz) δ: 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-2β), 3.97 (1H, t, J = 10.2 Hz, H-2α), 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-4β), 2.82 (1H, ddd, J = 16.2, 5.2, 1.9 Hz, H-4α); ^{13}C NMR (100 MHz, acetone-d_6) δ: 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_3), 50.0 (7-OCH_3), 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[13].

Isoliquiritigenin (5) was obtained as yellow powder (MeOH). ^{1}H NMR (CD_3OD, 400 MHz) δ: 8.01 (1H, d, J = 8.9 Hz, H-6′), 7.82 (1H, d, J = 14.5 Hz, H-α), 7.65 (3H, overlap, H-2, 6, β), 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}C NMR (CD_3OD, 100 MHz) δ: 192.1 (CO), 166.1 (s, C-4′), 164.9 (s, C-2′), 160.2 (s, C-4), 144.3 (d, C-β), 131.9 (s, C-6′), 130.4 (d, C-2, 6), 126.4 (s, C-1), 116.9 (d, C-α), 115.5 (d, C-2, 5), 113.3 (d, C-1′), 107.7 (d, C-5′), 102.4 (d, C-3′). These data are consistent with those reported values[14]. Therefore, **5** was determined to be isoliquiritigenin.

Sucrose (6) was obtained as colorless crystal (DMSO). ^{1}H NMR (DMSO-d_6, 400 MHz,) δ: 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, td, J = 8.4, 2.4 Hz, H-4′), 3.57 (1H, dd, J = 9.8, 3.5 Hz, H-2); ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 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[15].

7α-Hydroxysitosterol (7), colorless needle crystal ($CHCl_3$), EIMS m/z (rel. int. %): 412 [M-H_2O]. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 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}C NMR ($CDCl_3$, 100 MHz) δ: 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 7α-hydroxysitosterol[16].

5α, 8α-Epidioxy-(22*E*, 24*R*)-ergosta-6, 22-dien-3β-ol (8), white powder ($CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 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-l8), 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}C NMR ($CDCl_3$, 100 MHz) δ: 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-l4), 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_3), 19.8 (26-CH_3), 19.6 (27-CH_3), 18.1 (19-CH_3), 17.5 (28-CH_3), 12.9 (18-CH_3). These data are consistent with the literature values[17]. Thus, **8** was determined to be 5α,8α-epidioxy-(22*E*,24*R*)-ergosta-6,22-dien-3β-ol.

Trilinolein (**9**), colorless oil ($CHCl_3$), ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 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); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 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-1, 3), 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[18].

***n*-Tritriacontane** (**10**) was obtained as white powder ($CHCl_3$). EI-MS: 464 [M^+, 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[19]. Thus, **10** was determined to be *n*-tritriacontane.

***n*-Octadecane** (**11**) was obtained as white powder ($CHCl_3$). EI-MS: 254 [M^+, 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[20]. Thus, **11** was determined to be *n*-octadecane.

Octacosanol (**12**), white powder ($CHCl_3$); EI-MS: 410 [M^+, 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[21]. Therefore, **12** was determined to be octacosanol.

***n*-Heptacosane** (**13**), white powder ($CHCl_3$); EI-MS: 380 [M^+, 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[22]. Thus, **13** was determined to be *n*-heptacosane.

β-Sitosterol (**14**), white needle crystal ($CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 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); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 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 β-sitosterol[23].

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 μg/mL streptomycin (Life Technologies). Cells were grown in 25 cm^2 tissue culture flasks in a humidified atmosphere containing 5% CO_2 at 37 ℃. 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 × 10^6 cells were sub cultured into a new 25 cm^2 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[26].

Briefly, exponentially growing cells were seeded into 96-well plate at a density of approximately 1 × 10^5 cells/90 μL/well and allowed to adhere overnight, Treatments in the final concentration range between 3.0 and 300 μg/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 μL of MTT solution(5.0 mg/mL) was added and incubation for an addition 4 h. Medium and excessive MTT were aspirated and formazan formed was solubilized by the addition of 100 μL [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_{50} (μg/mL)		
	HepG2	MGC-803	SKOV3
DDP	7.3	2.2	11.1
The raw extract	-	-	-
The EtOAc fraction	-	-	-
The *n*-BuOH fraction	-	-	-
The H_2O fraction	-	-	-
Compound **2**	145.5	164.6	253.7

Conclusion

Among the fourteen compounds obtained from *A. ernestii*, compound **2** and β-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**, **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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