

Chemical constituents of *Swertia delavayi* and their anti-hepatitis B virus activity

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[Abstract] Fifteen known compounds were isolated from *Swertia delavayi* by silica gel, Sephadex LH-20 and Rp-18 column chromatographies. Based on extensive spectroscopic analysis (MS, ^1H , ^{13}C -NMR), their structures were identified as erythrocentaurin (**1**), erythrocentaurindimethylacetal (**2**), sweroside (**3**), swertiamarin (**4**), gentiopicroside (**5**), swertiakoside A (**6**), 2'-*O*-acetylswertiamarin (**7**), 4'-*O*-[(Z)-coumaroyl]swertiamarin (**8**), 1,5,8-trihydroxy-3-methoxyxanthone (**9**), 8-*O*- β -D-glucopyranosyl-4-hydroxy-2,3,5-trimethoxyxanthone (**10**), 8-*O*-[β -D-xyl-opyranosyl-(1→6)- β -D-glucopyranosyl]-7,8-dihydroxy-3-methoxyxanthone (**11**), isovitexin (**12**), β -sitosterol (**13**), daucosterol (**14**), and oleanolic acid (**15**). Among them, ten ones (**1~4, 7~11, 13**) were obtained from *S. delavayi* for the first time. The isolates were evaluated for their anti-HBV activities in HepG 2.2.15 cell line in vitro. The results showed that compound **1, 2, 6, 7, 9** and **12** exhibited significant inhibitory activity on HBV DNA replication with IC₅₀ values from 0.05 to 1.46 mmol·L⁻¹.

[Key words] *Swertia delavayi*; Gentianaceae; chemical constituents; anti-hepatitis B virus activity

丽江獐牙菜化学成分及抗乙肝病毒活性研究

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[摘要] 利用各种色谱方法对丽江獐牙菜 *Swertia delavayi* 全草的化学成分进行提取、分离和纯化, 得到 15 个单体化合物, 通过波谱数据分析 (MS, ^1H , ^{13}C -NMR) 对其结构进行鉴定, 分别为红白金花内酯 (*erythrocentaurin*, **1**)、红白金花内酯缩二甲醇 (*erythrocentaurin dimethylacetal*, **2**)、獐牙菜苷 (*sweroside*, **3**)、獐牙菜苦苷 (*swertiamarin*, **4**)、龙胆苦苷 (*gentiopicroside*, **5**)、贵州獐牙菜苷 A (*swertiakoside A*, **6**)、2'-*O*-乙酰基獐牙菜 (**2'-O-acetylswertiamarin**, **7**)、4'-*O*-[(Z)-对香豆酰基]獐牙菜苦苷 (**4'-O**-[(Z)-coumaroyl]-*swertiamarin*, **8**)、1,5,8-三羟基-3-甲氧基山酮 (**1,5,8-trihydroxy-3-methoxyxanthone**, **9**)、8-*O*- β -D-吡喃葡萄糖-1-羟基-2,3,5-三甲基山酮 (**8-O- β -D-glucopyranosyl-4-hydroxy-2,3,5-trimethoxyxanthone**, **10**)、8-*O*-[β -D-吡喃木糖-(1→6)- β -D-吡喃葡萄糖]-1-羟基-2,3,5-三甲氧基山酮 (**8-O-[β -D-xylopyranosyl-(1→6)- β -D-glucopyranosyl]-4-hydroxy-2,3,5-methoxyxanthone**, **11**)、异牡荆素 (*isovitexin*, **12**)、齐墩果酸 (*oleanolic acid*, **13**)、 β -谷甾醇 (β -sitosterol, **14**) 和胡萝卜苷 (*daucosterol*, **15**), 其中化合物 **1~4, 7~11, 13** 是首次从丽江獐牙菜中分离得到。在体外利用 HepG 2.2.15 细胞系对得到的单体化合物进行了抗乙肝病毒活性测试, 其中化合物 **1, 2, 6, 7, 9** 和 **12** 具有明显的抑制 HBV DNV 的复制活性, 其 IC₅₀ 值为 0.05~1.46 mmol·L⁻¹。

[关键词] 龙胆科; 丽江獐牙菜; 化学成分; 抗乙肝病毒活性

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Swertia delavayi Franch known as "hepatic herb" (Gan-Yan-Cao) or "Zou-Dan-Cao", growing in the

northwestern Yunnan and southern Sichuan of China, is widely used as a folk medicine to treat jaundice,

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hepatitis, and cholecystitis in Tibetan and Naxi medicine^[1]. Previous phytochemical investigation revealed that xanthones, flavonoids, iridoid glycosides and triterpenoids were main compounds^[1-4]. According to our preliminary bioassay *in vitro*, the ethanol extracts of *S. delavayi* exhibited significant inhibition on the secretion of hepatitis B antigen(HBsAg) and hepatitis B e antigen(HBeAg) with the IC₅₀ value of 1.64 g · L⁻¹(SI = 2.27) and 1.13 g · L⁻¹(SI = 3.58), respectively, and HBV DNA replication with IC₅₀ value of 0.34 g · L⁻¹(SI > 7.26). However, the active substances responsible for anti-hepatitis B virus have not been unclear. As a continuous search for anti-HBV active compounds from natural sources, further investigation on the ethanol extract of *S. delavayi* yielded fifteen known compounds, erythrocentaurin (1), erythrocentaurin dimethylacetal(2), sweroside(3), swertiamarin(4), gentiopicroside(5), swertiakoside A(6), 2'-O-acetyl-swertiamarin(7), 4'-O-[(Z)-coumaroyl] swertiamarin(8), 1,5,8-trihydroxy-3-methoxyxanthone(9), 8-O-β-D-glucopyranosyl-4-hydroxy-2,3,5-trimethoxyxanthone(10), 8-O-[β-D-xyl-opyranosyl-(1→6)]-β-D-glucopyranosyl]-7, 8-dihydroxy-3-methoxyxanthone(11), isovitexin(12), β-sitosterol(13), daucosterol(14), and oleanolic acid(15). In this study, we reported the isolation and structure elucidation of all isolates, and reports their anti-HBV activity.

1 General experimental procedures and plant material

1D NMR(¹H, ¹³C, DEPT) experiments were recorded on Bruker AM-400, DRX-500 spectrometers (Bruker, Bremerhaven, Germany). The chemical shifts were given in δ scale and referenced to deuterated solvent signal. Mass spectra were acquired on VG Auto Spec-3000(VG, Manchester, UK) and LC-MS-IT-TOF(Shimadzu, Kyoto, Japan) spectrometer. Column chromatography was performed on silica gel(200-300 mesh; Qingdao Makall Chemical Company, Qingdao, China). Sephadex LH-20(20-450 μm, Pharma-cia, Uppsala, Sweden) and Rp-18(40-63 μm, Fuji, Jappan). Fractions were monitored by TLC, and spots were visualized by heating silica gel plates sprayed with 10% H₂SO₄ in EtOH.

The whole plants of *S. Delavayi* Franch were collected in Lijiang, Yunnan province, China, in September 2008 and identified by Prof. Dr. Li-Gong Lei, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen(No. 2008-10-21) was deposited at the Laboratory of Antivirus and Natural Medicinal Chemistry, Kunming Institute of Botany, Chinese Academy of Sciences.

2 Extraction and isolation

The air-dried whole plants of *S. delavayi* (5.0 kg) were powdered and refluxed with 70% EtOH (50 × 3, 2 h). The EtOH extract was concentrated with a rotary evaporator under reduced pressure to give dark-brown residue, which was suspended in H₂O(6 L) and partitioned with petroleum ether(PE) (5 L × 3), EtOAc(5 L × 4) and n-butanol(4 L × 3), successively. The EtOAc part(140 g) of residue was subjected to silica gel column chromatography(CC) eluted with CHCl₃-MeOH(100: 0-70: 30), to yield 5 fractions (Frs. A1-A5). Fr. A1 was re-chromatographed on silica gel CC eluting with PE-EtOAc(95: 5-85: 15) to give compounds 1(721 mg) and 13(364 mg). compound 15(58 g) was obtained from Fr. A2 by silica gel CC using CHCl₃-MeOH(98: 2) as the eluent. Repeated chromatography of Fr. A4 with silica gel column (PE-EtOAc, 80 : 20-50 : 50) and Sephadex LH (CHCl₃-MeOH, 50: 50) gave compounds 9(18 mg) and 2(12 mg). The n-butanol part(B, 217 g) of residue was chromatographed on silica gel column successively eluted with CHCl₃-MeOH-H₂O(95: 5: 0, 90: 10: 1, 80: 20: 2, 60: 40: 4), to yield four sub-fractions (Fr. B1-B4). Fr. B1 was subjected to silica gel CC eluting with PE-Me₂CO(93: 7) and Sephadex LH (CHCl₃-MeOH, 50: 50), to generate compounds 6(18 mg) and 7(37 mg). Fr. B2 was re-chromatographed on silica gel CC(2 cm × 35 cm, 36 g) using CHCl₃-MeOH-H₂O(85: 15: 1.5) as the eluent and further subjected to Rp-18 CC eluted with MeOH-H₂O (20: 80-70: 30) to give compounds 3(27 mg), 4(125 g) and 5(1.34 g). Fr. B3 and B4 was subjected to Rp-18 CC eluted with MeOH-H₂O(10: 90-80: 20) to afford compounds 8(23 mg), 10(23 mg), 11(28

mg) , **12**(13 mg) , and **14**(1.23g) .

3 Results and discussion

Compound 1 Pink needle crystal(MeOH) . ^1H -NMR(CDCl_3 , 500 MHz) δ : 10.22(1H ,s , H-1) 8.35(1H ,dd , J = 7.5 ,1.0 Hz , H-6) ,8.15(1H ,dd , J = 7.5 ,1.0 Hz , H-8) 7.52(1H ,t , J = 7.5 Hz , H-7) ,4.45(2H ,t , J = 6.0 Hz , H-3) ,3.57(2H ,t , J = 6.0 Hz , H-4) . ^{13}C -NMR(125 MHz , CDCl_3) δ : 164.0(s , C-1) 66.5(t , C-3) ,24.6(t , C-4) ,132.6(s , C-5) ,138.2(d , C-6) ,127.8(d , C-7) ,135.3(d , C-8) ,127.5(s , C-9) ,141.8(s , C-10) ,191.7(s , C-11) . The above spectral data were coincident with those reported for erythrocentaurin^[5] .

Compound 2 Colorless needle crystal(MeOH) . ^1H -NMR(CDCl_3 , 500 MHz) δ : 7.94(1H ,d , J = 7.7 Hz , H-8) 7.64(1H ,d , J = 7.7 Hz , H-8) 7.26(1H ,t , J = 7.7 Hz , H-7) 5.33(1H ,s , H-11) 4.37(2H ,t , J = 6.0 Hz , H-3) ,3.03(2H ,t , J = 6.0 Hz , H-4) ,3.20(6H ,s ,OMe) . ^{13}C -NMR(125 MHz , CDCl_3) δ : 165.0(s , C-1) ,66.7(t , C-3) ,24.4(t , C-4) ,125.7(s , C-5) ,132.1(d , C-6) ,126.7(d , C-7) ,130.4(d , C-8) ,134.9(s , C-9) ,138.3(s , C-10) ,101.3(d , C-11) ,53.0(q ,OMe \times 2) . These data were identical with those of erythrocentaurin dimethylacetal^[5] .

Compound 3 White powder ,negative HR-ESI-MS m/z 403.1239 [M + HCOO]⁻ . ^1H -NMR(CD_3OD ,400 MHz) δ : 7.60(1H ,d , J = 2.0 Hz , H-3) ,5.55(1H ,m , H-4) 5.30(2H ,m , H-10) 4.69(1H ,d , J = 7.9 Hz , $\text{H-4}'$) 4.41(2H ,m , H-7) ,3.89(1H ,dd , J = 11.9 ,1.7 Hz , $\text{H-6}'\text{a}$) ,3.66(1H ,dd , J = 11.9 ,5.8 Hz , $\text{H-6}'\text{b}$) ,2.71(1H ,dd , J = 8.1 ,5.5 Hz , H-9) ,1.77(1H ,m , H-6a) ,1.68(1H ,m , H-6b) . ^{13}C -NMR(CD_3OD ,100 MHz) δ : 98.0(d , C-1) ,154.0(d , C-3) ,106.0(s , C-4) ,28.4(d , C-5) ,25.9(t , C-6) ,69.8(t , C-7) ,133.3(d , C-8) ,43.8(d , C-9) ,120.9(t , C-10) ,168.6(s , C-11) ,99.7(d , $\text{C-1}'$) ,74.7(d , $\text{C-2}'$) ,77.8(d , $\text{C-3}'$) ,71.5(d , $\text{C-4}'$) ,78.3(d , $\text{C-5}'$) ,62.6(t , $\text{C-6}'$) . These data were coincident with those reported for sweroside^[6] .

Compound 4 Faint yellow powder. ^1H -NMR(CD_3OD ,400 MHz) δ : 7.60(1H ,s , H-3) 5.74(1H ,br s , H-4) 5.40(3H ,m , H-8 ,10) 4.71(1H ,dd , J =

11.2 ,5.0 Hz , H-7a) ,4.63(1H ,d , J = 7.9 Hz , $\text{H-1}'$) 4.35(1H ,dd , J = 11.2 ,5.0 Hz , H-7b) ,3.86(1H ,br d , J = 11.9 Hz , $\text{H-6}'\text{a}$) ,3.66(1H ,dd , J = 11.9 ,5.6 Hz , $\text{H-6}'\text{b}$) ,3.38-3.23(4H ,m , $\text{H-2}'$,3' ,4' ,5') ,2.91(1H ,d , J = 8.9 Hz , H-9) ,1.93(1H ,ddd , J = 13.2 ,11.2 ,5.0 Hz , H-6a) ,1.73(1H ,d , J = 13.2 Hz , H-6b) . ^{13}C -NMR(CD_3OD ,100 MHz) δ : 99.4(d , C-1) ,154.7(d , C-3) ,109.0(s , C-4) ,64.2(s , C-5) ,33.7(t , C-6) ,66.1(t , C-7) ,133.8(d , C-8) ,52.1(d , C-9) ,121.1(t , C-10) ,168.0(s , C-11) ,100.1(d , $\text{C-1}'$) ,74.3(d , $\text{C-2}'$) ,77.5(d , $\text{C-3}'$) ,71.2(d , $\text{C-4}'$) ,78.4(d , $\text{C-5}'$) ,62.4(t , $\text{C-6}'$) . Compound 4 was identified as swertiamarin by comparison of those data with the reported data^[7] .

Compound 5 White powder ,negative HR-ESI-MS m/z 401.1074 [M + HCOO]⁻ . ^1H -NMR(CD_3OD ,400 MHz) δ : 7.46(1H ,s , H-3) 5.76(1H ,ddd , J = 17.2 ,10.3 ,6.9 Hz , H-6) 5.67(1H ,d , J = 2.9 Hz , H-4) 5.62(1H ,br s , H-10a) 5.22(1H ,m , H-10b) ,5.03(2H ,m , H-7) 4.66(1H ,d , J = 7.9 Hz , $\text{H-4}'$) ,3.90(1H ,dd , J = 11.8 ,1.7 Hz , $\text{H-6}'\text{a}$) ,3.65(1H ,dd , J = 11.8 ,6.1 Hz , $\text{H-6}'\text{b}$) ,3.44-3.22(4H ,m , $\text{H-2}'$,3' ,4' ,5') ,2.91(1H ,d , J = 8.9 Hz , H-9) ,1.93(1H ,ddd , J = 13.2 ,11.2 ,5.0 Hz , H-6a) ,1.73(1H ,d , J = 13.2 Hz , H-6b) . ^{13}C -NMR(CD_3OD ,100 MHz) δ : 98.5(d , C-1) ,150.7(d , C-3) ,104.9(s , C-4) ,126.9(s , C-5) ,117.3(d , C-6) ,71.0(t , C-7) ,135.0(d , C-8) ,46.6(d , C-9) ,118.7(t , C-10) ,166.3(s , C-11) ,100.2(d , $\text{C-1}'$) ,74.5(d , $\text{C-2}'$) ,77.9(d , $\text{C-3}'$) ,71.5(d , $\text{C-4}'$) ,78.4(d , $\text{C-5}'$) ,62.8(t , $\text{C-6}'$) . Combining literature identified that the compound 5 was gentiopicroside^[7] .

Compound 6 Amorphous solids ,negative HR-ESI-MS m/z 355.1034 [M - H]⁻ . ^1H -NMR(CD_3OD ,500 MHz) δ : 5.76(1H ,d , J = 2.2 Hz , H-4) ,5.67(1H ,m , H-8) 5.33(1H ,s , H-3) 5.32(2H ,m , H-10) 4.96(1H ,d , J = 7.1 Hz , $\text{H-1}'$) 4.4(2H ,m , H-7) ,3.86(1H ,dd , J = 12.0 ,1.9 Hz , $\text{H-6}'\text{a}$) ,3.64(1H ,dd , J = 12.0 ,5.4 Hz , $\text{H-6}'\text{b}$) ,3.54(1H ,t , J = 8.8 Hz , $\text{H-3}'$) ,3.11(1H ,d , J = 8.4 Hz , H-9) ,2.74(1H ,m , H-6a) ,2.39(1H ,m , H-6b) . ^{13}C -NMR(CD_3OD ,125 MHz) δ : 91.3(d , C-4) ,95.4(d , C-3) ,

121.5(s ,C-4) ,158.6(s ,C-5) ,28.7(t ,C-6) ,66.7(t ,C-7) ,132.9(d ,C-8) ,50.8(d ,C-9) ,121.2(t ,C-10) ,163.9(s ,C-11) ,99.0(d ,C-1') ,81.6(d ,C-2') ,76.2(d ,C-3') ,70.7(d ,C-4') ,79.1(d ,C-5') ,62.6(t ,C-6') . Combining literature identified that the compound 6 was Swertiakoside A [8].

Compound 7 White amorphous powder, ESI-MS *m/z* 415 [M - H]⁻. ¹H-NMR(CD₃OD, 400 MHz) δ: 7.63(1H ,s ,H-3) ,5.56(1H ,s ,H-4) ,5.43(1H ,m ,H-8) ,5.37(1H ,m ,H-10a) ,5.29(1H ,dd ,J = 9.4 ,2.4 Hz ,H-10b) ,4.63(1H ,d ,J = 7.8 Hz ,H-4') ,4.35(1H ,m ,H-7a) ,4.24(1H ,dd ,J = 12.0 ,5.6 Hz ,H-7b) ,3.61-3.31(4H ,m ,H-3' ,5' ,6') ,3.19(1H ,t ,J = 8.4 Hz ,H-2') ,2.91(1H ,d ,J = 8.6 Hz ,H-9) ,1.91(1H ,td ,J = 14.0 ,5.2 Hz ,H-6a) ,1.75(1H ,d ,J = 14.2 Hz ,H-6b). ¹³C-NMR(CD₃OD, 100 MHz) δ: 99.3(d ,C-4) ,154.7(d ,C-3) ,108.9(s ,C-4) ,64.2(s ,C-5) ,33.7(t ,H-6) ,66.0(t ,C-7) ,133.9(d ,C-8) ,52.0(d ,C-9) ,121.2(t ,C-10) ,167.9(s ,C-11) ,100.3(d ,C-1') ,75.7(d ,C-2') ,74.3(d ,C-3') ,71.3(d ,C-4') ,77.5(d ,C-5') ,64.5(t ,C-6') ,172.7(s ,C = O) ,20.7(q ,Me) . Combining literature identified that the compound 7 was 2'-O-acetylswertiamarin [7].

Compound 8 White amorphous powder, ESI-MS *m/z* 519 [M - H]⁻. ¹H-NMR(CD₃OD, 500 MHz) δ: 7.67(1H ,d ,J = 15.9 Hz ,H-7") ,7.65(1H ,s ,H-3) ,7.47(2H ,d ,J = 8.4 Hz ,H-2" ,6") ,6.81(2H ,d ,J = 8.4 Hz ,H-3" ,5") ,6.37(1H ,d ,J = 15.9 Hz ,H-8") ,5.74(1H ,s ,H-4) ,5.43(1H ,m ,H-8) ,5.38(1H ,m ,H-10a) ,5.30(1H ,m ,H-10b) ,4.77(1H ,d ,J = 10.9 Hz ,H-4') ,4.73(2H ,d ,J = 7.9 Hz ,H-4' ,7a) ,4.34(1H ,dd ,J = 10.5 ,4.3 Hz ,H-7b) ,3.71-3.53(4H ,m ,H-3' ,5' ,6') ,3.37-3.28(1H ,m ,H-2') ,2.95(1H ,d ,J = 9.2 Hz ,H-9) ,1.91(1H ,td ,J = 13.6 ,4.8 Hz ,H-6a) ,1.75(1H ,d ,J = 14.2 Hz ,H-6b). ¹³C-NMR(CD₃OD, 125 MHz) δ: 99.2(d ,C-4) ,154.7(d ,C-3) ,109.0(s ,C-4) ,64.3(s ,C-5) ,33.7(t ,C-6) ,66.0(t ,C-7) ,133.8(d ,C-8) ,51.9(d ,C-9) ,121.3(t ,C-10) ,168.0(s ,C-11) ,100.2(d ,C-1') ,74.6(d ,C-2') ,75.5(d ,C-3') ,72.2(d ,C-4') ,76.7(d ,C-5') ,62.3(t ,C-6') ,127.1(s ,C-1") ,161.5(s ,C-4") ,116.9(d ,C-3" ,5") ,131.3(d ,C-2" ,6") ,147.4(d ,C-

7") ,114.7(d ,C-8") ,168.5(s ,C-9") . These data were identical with those of 4'-O-[(Z)-coumaroyl]-swertiamarin [9].

Compound 9 Pale yellow needles (CHCl₃-MeOH, 1:1) . ¹H-NMR(C₆D₅N, 400 MHz) δ: 12.34(1H ,s ,OH-1) ,11.58(1H ,s ,OH-8) ,7.54(1H ,d ,J = 8.4 Hz ,H-6) ,6.87(1H ,d ,J = 8.8 Hz ,H-7) ,6.52(1H ,d ,J = 2.2 Hz ,H-4) ,6.25(1H ,s ,H-2) ,3.65(3H ,s ,OCH₃) ; ¹³C-NMR(C₆D₅N, 100 MHz) δ: 163.3(s ,C-4) ,98.1(d ,C-2) ,167.7(s ,C-3) ,93.0(d ,C-4) ,158.2(s ,C-4a) ,144.7(s ,C-4b) ,138.8(s ,C-5) ,125.1(d ,C-6) ,110.3(d ,C-7) ,153.4(s ,C-8) ,108.4(s ,C-8a) ,103.1(s ,C-8b) ,185.2(s ,C-9) ,56.1(q ,OMe) . Compound 9 was identified as 1 ,5 ,8-trihydroxyl-3-methoxyxanthone by comparison of those data with the reported data [10].

Compound 10 Yellow powder, positive HR-ESI-MS *m/z* 503.1159 [M + Na]⁺ (calcd for 503.1160) . ¹H-NMR(DMSO-d₆, 400 MHz) δ: 12.9(s ,OH-1) ,6.76(1H ,s ,H-4) ,7.44(1H ,d ,J = 9.0 Hz ,H-6) ,7.17(1H ,d ,J = 9.0 Hz ,H-7) ,3.74(3H ,s ,OMe ,C-2) ,3.93(3H ,s ,OMe ,C-3) ,3.90(3H ,s ,OMe ,C-5) ,4.86(1H ,d ,J = 7.5 Hz ,H-4') ,3.38(1H ,m ,H-2') ,3.30(1H ,m ,H-3') ,3.34(1H ,m ,H-5') ,3.19(1H ,m ,H-4') ,3.71(1H ,m ,H-6'a) ,3.49(1H ,m ,H-6'b) . ¹³C-NMR(DMSO-d₆, 100 MHz) δ: 153.5(s ,C-1) ,131.3(s ,C-2) ,159.9(s ,C-3) ,91.0(d ,C-4) ,152.0(s ,C-4a) ,146.1(s ,C-4b) ,142.9(s ,C-5) ,117.5(d ,C-6) ,111.5(d ,C-7) ,150.2(s ,C-8) ,111.2(s ,C-8a) ,103.9(s ,C-8b) ,181.1(s ,C-9) ,102.7(d ,C-1') ,73.5(d ,C-2') ,76.2(d ,C-3') ,69.7(d ,C-4') ,77.4(d ,C-5') ,60.8(t ,C-6') ,60.1(q ,OMe ,C-2) ,56.7(q ,OMe ,C-3) ,56.4(q ,OMe ,C-5) . These data were identical with those of 8-O-β-D-glucopyranosyl-4-hydroxy-2,3,5-trimethoxyxanthone [11].

Compound 11 Yellow powder, positive HR-ESI-MS *m/z* 635.1559 [M + Na]⁺ (calcd for 635.1583) . ¹H-NMR(DMSO-d₆, 400 MHz) δ: 12.9(s ,OH-1) ,6.76(1H ,s ,H-4) ,7.44(1H ,d ,J = 9.1 Hz ,H-6) ,7.27(1H ,d ,J = 9.1 Hz ,H-7) ,3.70(3H ,s ,OMe ,C-2) ,3.93(3H ,s ,OMe ,C-3) ,3.91(3H ,s ,OMe ,C-5) ,4.82(1H ,d ,J = 7.6 Hz ,H-1') ,3.40(1H ,m ,H-

2') 3.27(1H ,m ,H-4') ,3.06(1H ,dd ,J = 12.7 ,8.5 ,H-5') ,4.00(1H ,d ,J = 9.4 Hz ,H-6'a) ,4.20(1H ,d ,J = 7.4 Hz ,H-4") ,3.00(1H ,m ,H-2") ,3.54(2H ,d ,J = 8.9 Hz ,H-3" ,6'b) ,3.17(1H ,dd ,J = 13.2 ,5.5 ,H-4") ,3.67(1H ,dd ,J = 11.2 ,5.2 Hz ,H-5'a) ,2.96(1H ,s ,H-5'b) . ^{13}C -NMR(DMSO- d_6 ,100 MHz) δ : 153.5(s ,C-4) ,131.3(s ,C-2) ,159.9(s ,C-3) ,91.0(d ,C-4) ,152.0(s ,C-4a) ,146.0(s ,C-4b) ,143.0(s ,C-5) ,117.8(d ,C-6) ,111.4(d ,C-7) ,150.1(s ,C-8) ,111.7(s ,C-8a) ,103.9(s ,C-8b) ,181.2(s ,C-9) ,102.6(d ,C-4') ,73.4(d ,C-2') ,76.0(d ,C-3') ,69.8(d ,C-4') ,76.7(d ,C-5') ,68.4(t ,C-6') ,104.0(d ,C-1") ,73.4(d ,C-2") ,76.2(d ,C-3") ,69.6(d ,C-4") ,65.7(t ,C-5") ,60.1(q ,OMe ,C-2) ,56.7(q ,OMe ,C-3) ,56.4(q ,OMe ,C-5) . Spectral data were coincident with those reported for 8-O-[β -D-xylopyranosyl-(1→6)- β -D-glucopyranosyl]-7,8-dihydroxy-3-methoxyxanthone^[12].

Compound **12** Yellow powder ,negative ESI-MS m/z 431 [M - H]⁻. ^1H -NMR(CD₃OD 500 MHz) δ : 7.82(2H ,d ,J = 8.6 Hz ,H-2' ,6') ,6.91(2H ,d ,J = 7.6 Hz ,H-3' ,5') ,6.77(1H ,s ,H-8) ,6.31(1H ,s ,H-3) ,4.87(1H ,d ,J = 9.9 Hz ,H-4") ,4.22(1H ,t ,J = 7.1 Hz ,H-2") ,3.91(1H ,dd ,J = 12.1 ,1.7 Hz ,H-6'a) ,3.77(1H ,dd ,J = 12.1 ,5.4 Hz ,H-6'b) ,3.61(1H ,dd ,J = 10.6 ,4.3 Hz ,H-5") ,3.52(2H ,m ,H-3" ,4") . ^{13}C -NMR(CD₃OD ,125 MHz) δ : 166.3(s ,C-

2) ,103.9(d ,C-3) ,184.1(s ,C-4) ,162.2(s ,C-5) ,109.4(s ,C-6) ,165.2(s ,C-7) ,95.3(d ,C-8) ,158.8(s ,C-9) ,105.2(s ,C-10) ,123.2(s ,C-4') ,117.2(d ,C-2' ,6') ,129.6(d ,C-3' ,5') ,163.0(s ,C-4') ,75.4(d ,C-1") ,72.6(d ,C-2") ,80.3(d ,C-3") ,71.9(d ,C-4") ,82.8(d ,C-5") ,63.0(t ,C-6") . Spectral data were coincident with those reported for isovitexin^[12].

Compounds **13-15** were identified as β -sitosterol (**13**) , daucosterol (**14**) and oleanolic acid (**15**) by comparing with authentic samples and spectral data , respectively.

Compounds **1-12** were evaluated for their anti-HBV activities(Table 1) , namely inhibiting the HBV surface antigen secretion(HBsAg) ,HBV e antigen secretion(HBeAg) , and HBV DNA replication in HepG 2.2.15 cells , as reported previously (tenofovir was used as the positive control)^[13] . The results showed that compound 9 and 12 exhibited significant inhibitory activity on HBV DNA replication with IC₅₀ values of 0.09 and 0.05 mmol · L⁻¹(SI of 10.89 and 19.97) , and showed potent activity against the secretion of HBeAg with IC₅₀ values of 0.35 and 0.23 mmol · L⁻¹ (SI of ≥ 2.80 and 4.34) , respectively. Also , the compounds **1** , **2** , **6** and **7** exhibited anti-HBV activities. However , other compounds showed no anti-HBV activity and cytotoxicity at the highest tested concentration.

Table 1 Anti-HBV activities of **1** , **2** , **6** , **7** , **9** and **12**^a from *Swertia delavayi*

Compounds	CC ₅₀ [mM]	HBsAg ^b		HBeAg ^c		HBV DNA ^d	
		IC ₅₀ [m mol · L ⁻¹]	SI ^e	IC ₅₀ [m mol · L ⁻¹]	SI	IC ₅₀ [m mol · L ⁻¹]	SI
1	1.76	1.30	1.4	1.14	1.5	0.76	2.3
2	1.62	3.57	<1	—	—	1.46	1.1
6	>1.73	>1.73	—	>1.73	—	>0.43	4.0
7	>1.34	1.83	<1	1.25	>1.1	—	—
9	>0.98	>0.98	—	0.35	>2.8	0.09	>10.9
12	0.99	1.51	<1	0.23	4.34	0.05	19.8
Tenofovir ^f	>1.39	1.25	>1.1	1.21	>1.15	0.000 46	>3 021.7

Note: ^a All values are the mean of two independent experiments; ^b HBsAg: HBV surface antigen; ^c HBeAg: HBV e antigen; ^d DNA: HBV DNA replication; ^e CC₅₀ = 50% cytotoxic concentration , IC₅₀ = 50% inhibition concentration , SI(selectivity index) = CC₅₀/IC₅₀; ^f Tenofovir , an antiviral agent used as a positive control. The other compounds exhibited no anti-HBV activity at the maximal concentration.

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