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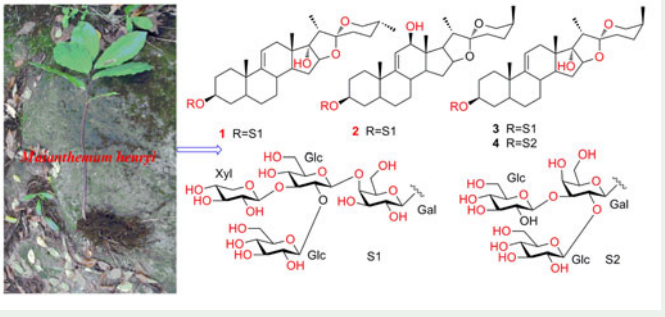
Cytotoxic steroidal saponins from the roots and rhizomes of *Maianthemum henryi*

Xin Zhang[a,b]*, Jing Sun[b]*, Xiaofei Zhang[b], Shuo Zhang[c], Xuan Zhang[d], Xuanji Xue[a,e] and Zengjun Guo[a,e]

[a]School of Pharmacy, Xi'an Jiaotong Univeristy, Xi'an, China; [b]School of Pharmacy, Shaanxi University of Chinese Medicine, Xianyang, China; [c]School of Traditional Chinese Medicine, Beijing University of Chinese Medicine, Beijing, China; [d]The Second Affiliated Hospital of Shaanxi University of Chinese Medicine, Xianyang, PR China; [e]Shaanxi key laboratory of "Qiyao" resources and anti-tumor acitivities, Xi'an, China

ABSTRACT

Henryiosides F and G (**1** and **2**), two new steroidal saponins along with two known analogues (**3** and **4**) were obtained from the roots and rhizomes of *Maianthemum henryi*. Their structures were determined by physicochemical properties and spectroscopic methods including 1D, 2D-NMR, IR and HR-ESI-MS data analysis. Cytotoxic activity in human HepG2 and SW620 tumour cells were evaluated by the MTT method and all of the saponins exhibited cytotoxicity with IC_{50} values ranging from 15.33 μM to 57.85 μM.



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1. Introduction

Maianthemum henryi (Baker) LaFrankie (synonym *Smilacina henryi* (bekev) Wang et Tang) is a perennial herb in the genus *Maianthemum* (Liliaceae) and is mainly distributed in Shaanxi, Hebei and Yunnan province in china (Qiao et al. 2019). Its roots, named Pian Tou Qi in the region of Qinba Mountains of Shaanxi province, are usually used as traditional Chinese medicines (TCMs) for the treatment of rheumatism,

CONTACT Jing Sun ph.175@163.com; Zengjun Guo guozj@mail.xjtu.edu.cn
*Xin Zhang and Jing Sun contribute equally to this work.
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traumatic injury and impotence (Lin et al. 2018; Zhang et al. 2013). Steroidal saponins, flavoids and nucleosides have been reported from the previous studies (Lin et al. 2018; Qiao et al. 2019; Zhang et al. 2013). In our research project of searching for the bioactive constituents from TCMs (Guo et al. 2014; Hui et al. 2012; Xu et al. 2017), an investigation of secondary metabolites of *M. henryi* was carried out and two new steroidal saponins (henryiosides F and G) along with two known analogues, henryioside A (**3**) (Zhang et al. 2013) and (25*S*)-5α-spirostan-9(11)-en-3β, 17α-diol-3-*O*-β-D-glucopyranosyl-(1→2)-[β-D-glucopyranosyl-(1→3)]-β-D-galactopyranoside (**4**) (Cui et al. 2018), were obtained. In addition, their cytotoxic activities in human HepG2 and SW620 tumour cells were also evaluated. Details of the isolation, structural elucidation and cytotoxic activities of the saponins are presented in this paper.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder, with $[\alpha]_D^{20}-29.6^\circ$ (*c* 0.3, MeOH). The HR-ESI-MS spectrum showed a negative molecular ion peak at *m/z* 1047.5007 $[M-H]^-$ corresponding to a molecular formula of $C_{50}H_{80}O_{23}$ (calculated 1047.5018 $[M-H]^-$). In the ^{1}H NMR spectrum of **1**, the characteristic proton signals were observed, such as four steroid methyl groups at δ_H 1.03 (3H, s, H-18), 0.78 (3H, s, H-19), 1.25 (3H, d, $J=6.8$ Hz, H-21) and 0.70 (3H, d, $J=7.1$ Hz, H-27), an olefinic proton at δ_H 5.40 (1H, d, $J=5.3$ Hz, H-11) and four anomeric protons at δ_H 4.86 (1H, d, $J=7.6$ Hz, Gal-H1), 5.21 (1H, d, $J=7.8$ Hz, Glc-H1), 5.26 (1H, d, $J=7.7$ Hz, Glc′-H1) and 5.59 (1H, d, $J=7.5$ Hz, Xyl-H1) were observed. The ^{13}C-NMR spectrum of **1** showed the signals a distinctive quaternary carbon signal of spirostane type steroids at δ_C 110.8 (C-22) (Song et al. 2015), a double bond carbons at δ_C 146.3 (C-9) and 116.8 (C-11) as well as four methyl carbons at δ_C 17.6 (C-18), 18.0 (C-19), 10.0 (C-21) and 17.4 (C-27) and four anomeric carbons at 102.5 (Gal-C1), 105.1 (Glc-C1), 104.9 (Glc′-C1) and 105.0 (Xyl-C1). These NMR data compared with the reported compound henryioside A (**3**) (Zhang et al. 2013) indicated the different spectroscopic features in the F ring. Detailed analysis of the ^{1}H-NMR spectrum of **1** showed the chemical shifts of the H-27 signals which transferred to upfield at δ_H 0.70 (<1.0) compared with that in henryioside A (δ_H 1.02 > 1.0, 25*S* absolutely configuration) (Zhang et al. 2011). This differences along with the IR absorptions observed at 979, 912, 894, 842 (912 < 894) cm^{-1} confirmed the 25*R* absolutely configuration of **1** (Cui et al. 2018; Song et al. 2015). Acid hydrolysis of **1** resulted in the products of D-galactose, D-glucose and D-xylose which were established by their optical rotation data (Gal: $[\alpha]_D^{20}+29.0^\circ$ in MeOH, Glc: $[\alpha]_D^{20}+48.8^\circ$ in MeOH and Xyl: $[\alpha]_D^{20}+33.6^\circ$ in MeOH) and R_f values (BuOH-AcOH-H_2O, 4:1:5 upper layer, Gal: 0.39, Glc: 0.36 and Xyl: 0.54) with the authentic sugar samples (Chai et al. 2014). Coupling constants of the anomeric proton signals ($J_{1,2}$ > 7.0 Hz) suggested the β-configurations for the D-galactose, D-glucose and D-xylose (Zhang et al. 2016). Accordingly, the structure of **1** was determined as (25*R*)-5α-spirostan-9(11)-en-3β, 17α-diol 3-*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside and named as henryioside F (Figure 1).

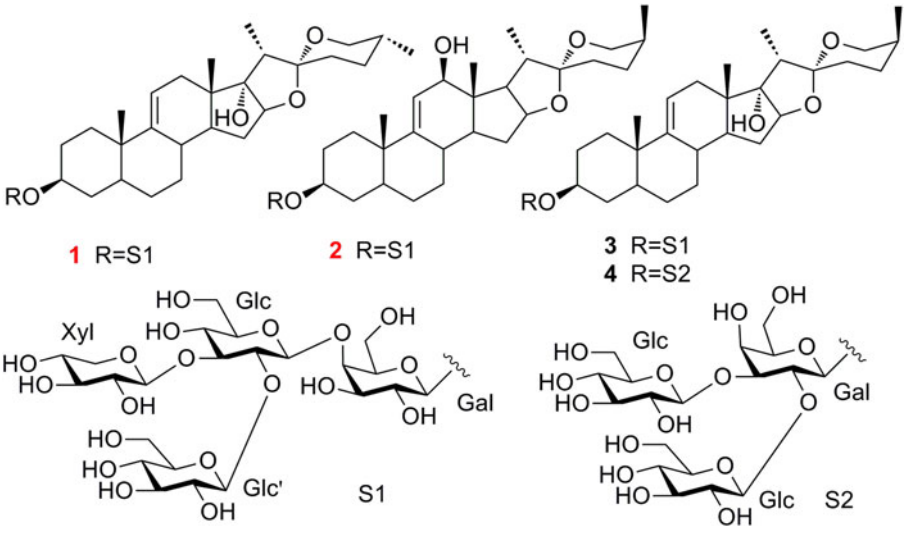

Figure 1. Structures of compounds **1–4**.

Compound **2** was obtained as a white amorphous powder, with $[\alpha]_D^{20}-26.1^\circ$ (*c* 0.3, MeOH). The HR-ESI-MS spectrum showed a negative molecular ion peak at *m/z* 1047.5006 $[M-H]^-$ corresponding to a molecular formula of $C_{50}H_{80}O_{23}$ (calculated 1047.5018 $[M-H]^-$). Four steroid methyl groups at δ_H 1.05 (3H, s, H-18), 0.82 (3H, s, H-19), 1.40 (3H, d, $J=6.8$ Hz, H-21) and 1.07 (3H, d, $J=7.1$ Hz, H-27) along with an olefinic proton 5.43 (1H, s, H-11) were observed in the ^{1}H-NMR spectrum of **1**. The ^{13}C-NMR spectrum displayed 50 carbon signals, in which a double bond carbons at δ_C 148.3 (C-9) and 123.1(C-11) and four methyl groups at δ_C 10.9 (C-18), 17.8 (C-19), 14.0 (C-21) and 16.3 (C-27) were observed. These NMR data compared with the literature (Qiao et al. 2019) supported the (25*S*)-5α-spirostan-9(11)-en-3β, 12β-diol aglycone of **1**. This inference was deduced from 2D-NMR data analysis including HSQC, HMBC, NEOSY and ^{1}H-^{1}H COSY experiments (Figure S16). The 3-*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside sugar chain in **2** was determined by the HMBC correlations observed from Gal-H1/C-3, from Glc-H1/Gal-C4, from Glc'-H1/Glc-C2 and from Xyl-H1/Glc-C3 and their NMR data which was in agreement with the literature (Liu et al. 2012; Zhang et al. 2016; Zhang et al. 2013). The β-configurations for the D-galactose, D-glucose and D-xylose in **2** were identified as the same method as **1**. Compound **2** was thus determined as (25*S*)-5α-spirostan-9(11)-en-3β, 12β-diol 3-*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-xylopyranosyl-(1→3)]-*O*-β-D-glucopyranosyl-(1→4)-β-D-galactopyranoside and named as henryioside G (Figure 1).

To the best of our knowledge, more than 30 cytotoxic steroidal compounds have been isolated from the genus *Maianthemum* (Lin et al. 2018; Liu et al. 2012; Qiao et al. 2019; Yang et al. 2009; Zhang et al. 2013; Zhang et al. 2006). In this paper, compounds **1**−**4** were assayed for their regarding cytotoxic activities in human HepG2 and SW620 tumour cells, the results (Table 1) showed that all of the saponins exhibitated inhibitory effects with IC_{50} values ranging from 15.33 μM to 57.85 μM. The comparison with the structures of **1**−**4** indicated that the 3-*O*-β-D-glucopyranosyl-(1→2)-*O*-[β-D-

Table 1. Cytotoxic activities of compounds **1**–**4** in HepG2 and SW620 tumour cells (IC_{50} μM).[a]

Compounds	1	2	3	4	5-FU[b]
HepG2	19.60 ± 1.76	24.06 ± 1.52	15.33 ± 1.09	57.85 ± 3.50	12.07 ± 0.82
SW620	27.41 ± 2.41	22.59 ± 0.85	16.06 ± 1.28	48.77 ± 4.68	15.84 ± 1.21

[a]IC_{50} values were expressed as mean ± SD (n = 3).
[b]5-FU = 5-fluorouracil was used as the positive control.

xylopyranosyl-(1→3)]-*O*-*β*-D-glucopyranosyl-(1→4)-*β*-D-galactopyranoside sugar chain is important to the cytotoxic activities.

3. Experimental

3.1. General experimental procedures

Optical rotation was measured using a Rudolph Autopol VI polarimeter (Rudolph, USA); IR spectra were recorded on a Nicolet iS10 instrument (Thermo Fisher Scientific, USA); 1D and 2D NMR spectra were recorded on a Bruker-Avance 400 instrument (Bruker Corp. Karlsruhe, Germany); Semipreparative HPLC was performed on Agilent infinity II system equipped with a UV detector and a YMC-Pack-ODS-A (10 mm × 250 mm, 5μm particles) column. The HR-ESI-MS spectra were taken on an Agilent Technologies 6650 Q-TOF (Agilent Technologies). Sephadex LH-20 gel and ODS C_{18} (5 μm) silica gel was purchased from GE Healthcare Bio-Sciences AB (Uppsala, Sweden). Silica gel was purchased from Qingdao Haiyang Chemical Group Corporation (Qingdao, China).

3.2. Plant material

The roots and rhizomes of *Maianthemum henryi* (Baker) LaFrankie (synonym *Smilacina henryi* (bekev) Wang et Tang) were collected on August in 2017 from Qinba Mountains in Shaanxi Province of China, and were authenticated by one of our co-authors Prof. Jing Sun (Shaanxi University of Chinese Medicine). A voucher specimen (herbarium No. SH-201708) is deposited in School of Pharmacy, Xi'an Jiaotong University, Xi'an 710061, China.

3.3. Extraction and isolation

The air-dried roots and rhizomes of *M. henryi* (6.6 kg) were extracted with 80% EtOH under reflux for three times (2h, 2h, 1h, successively). The concentrated residue was partitioned with petroleum ether (PE) and *n*-BuOH. The *n*-BuOH extract (130.2 g) was subjected to column chromatography (CC) on silica gel (1 kg), eluting with gradient solvent system (CH_2Cl_2-MeOH-H_2O, 100:0:0 − 60:40:10) to give six fractions (Fr.1 – Fr.6). Fr.4 (19.1 g) was subjected to CC on silica gel (200 g), eluting with (CH_2Cl_2-MeOH-H_2O, 100:10:0 − 80:20:5) to give six subfractions (Fr.4-1 − Fr.4-6). Fr.4-3 (3.1 g) was subjected to CC on Sephadex LH-20 gel (100 g) eluting with (CH_2Cl_2-MeOH 100:100) to give ten subfractions (Fr.4-3-1 − Fr.4-3-10). Fr.4-3-3 (61.1 mg) was purified by HPLC (YMC-Pack-ODS-A, 10 mm × 250 mm, 5 μm particles, flow rate: 1.5 mL/min) with MeCN-H_2O (70:30) as mobile phase to afford compound **4** (9.0 mg; t_R = 28 min); Fr.5 (23.8 g) was

subjected to CC on silica gel (200 g), eluting with (CH_2Cl_2-MeOH-H_2O, 100:10:0 – 70:30:5) to give five subfractions (Fr.5-1 – Fr.5-5). Fr.5-2 (2.6 g) was subjected to CC on Sephadex LH-20 gel (100 g) eluting with (CH_2Cl_2-MeOH 100:100) to give nine subfractions (Fr.5-2-1 – Fr.5-2-9). Fr.5-2-2 (45.2 mg) was purified by HPLC (YMC-Pack-ODS-A, 10 mm × 250 mm, 5 μm particles, flow rate: 1.0 mL/min) with MeCN-H_2O (74:26) as mobile phase to afford compound **2** (11.4 mg; t_R = 47 min); Fr.5-3 (4.8 g) was subjected to CC on Sephadex LH-20 gel (100 g) eluting with (CH_2Cl_2-MeOH 100:100) to give twelve subfractions (Fr.5-2-1 – Fr.5-3-12). Fr.5-3-3 (113.5 mg) was purified by HPLC (YMC-Pack-ODS-A, 10 mm × 250 mm, 5 μm particles, flow rate: 1.5 mL/min) with MeCN-H_2O (75:25) as mobile phase to afford compound **1** (14.3 mg, t_R = 58 min) and compound **3** (38.2 mg, t_R = 55 min).

3.4. Identification of structures

Henryioside F (**1**), a white amorphous powder; $[\alpha]_D^{20}$ −29.6° (*c* 0.3, MeOH); IR (KBr)ν_{max}: 3407, 2926, 1626, 1378, 1070, 1041, 979, 912, 894, 842 cm^{-1}; *m/z* 1047.5007 $[M-H]^-$ corresponding to a molecular formula of $C_{50}H_{79}O_{23}^-$ (calculated 1047.5018 $[M-H]^-$). ^{1}H-NMR (pyridine-d_5, 400 MHz, δ in ppm) : 1.19 (1H, m, H-1a), 1.52 (1H, m, H-1b), 1.63 (1H, m, H-2a), 2.07 (1H, m, H-2b), 3.94 (1H, m, H-3), 1.29 (1H, m, H-4a), 1.73 (1H, m, H-4b), 0.96 (1H, m, H-5), 1.11 (1H, m, H-6a), 1.15 (1H, m, H-6b), 0.84 (1H, m, H-7a), 1.63 (1H, m, H-7b), 2.03 (1H, m, H-8), 5.40 (1H, d, *J* = 5.3 Hz, H-11), 1.68 (1H, m, H-12a), 2.99 (1H, d, *J* = 17.0 Hz, H-12b), 2.01 (1H, m, H-14), 1.53 (1H, m, H-15a), 2.34 (1H, m, H-15b), 4.34 (1H, d, *J* = 7.3 Hz, H-16), 1.03 (3H, s, H-18), 0.78 (3H, s, H-19), 2.73 (1H, dd, *J* = 6.8, 14.1 Hz, H-20), 1.25 (3H, d, *J* = 6.8 Hz, H-21), 1.61 (1H, m, H-23a), 1.64 (1H, m, H-23b), 1.55 (1H, m, H-24a), 2.21 (1H, m, H-24b), 1.53 (1H, m, H-25), 3.71 (1H, d, *J* = 11.0 Hz, H-26a), 3.84 (1H, dd, *J* = 2.4, 11.0 Hz, H-26b), 0.70 (3H, d, *J* = 7.1 Hz, H-27), 4.86 (1H, d, *J* = 7.6 Hz, H-Gal-1), 4.38 (1H, m, H-Gal-2), 4.04 (1H, m, H-Gal-3), 4.57 (1H, m, H-Gal-4), 3.97 (1H, m, H-Gal-5), 4.14 (1H, m, H-Gal-6a), 4.62 (1H, m, H-Gal-6b), 5.21 (1H, d, *J* = 7.8 Hz, H-Glc-1), 4.38 (1H, m, H-Glc-2), 4.12 (1H, m, H-Glc-3), 3.78 (1H, m, H-Glc-4), 4.05 (1H, m, H-Glc-5), 4.01 (1H, m, H-Glc-6a), 4.48 (1H, d, *J* = 10.4 Hz, H-Glc-6b), 5.26 (1H, d, *J* = 7.7 Hz, H-Glc-1′), 4.06 (1H, m, H-Glc-2′), 3.88 (1H, m, H-Glc-3′), 4.15 (1H, m, H-Glc-4′), 4.10 (1H, m, H-Glc-5′), 4.33 (1H, m, H-Glc-6′a), 4.57 (1H, d, *J* = 10.3 Hz, H-Glc-6′b), 5.59 (1H, d, *J* = 7.5 Hz, H-Xyl-1), 4.01 (1H, m, H-Xyl-2), 4.07 (1H, m, H-Xyl-3), 4.08 (1H, m, H-Xyl-4), 3.65 (1H, t, *J* = 10.7 Hz, H-Xyl-5a), 4.19 (1H, m, H-Xyl-5b). ^{13}C-NMR (pyridine-d_5, 100 MHz, δ in ppm) : 35.8 (C-1), 29.9 (C-2), 77.3 (C-3), 34.9 (C-4), 43.3 (C-5), 28.8 (C-6), 33.6 (C-7), 36.7 (C-8), 146.3 (C-9), 38.2 (C-10), 116.8 (C-11), 34.2 (C-12), 44.4 (C-13), 50.5 (C-14), 33.6 (C-15), 90.1 (C-16), 89.0 (C-17), 17.6 (C-18), 18.0 (C-19), 46.1 (C-20), 10.0 (C-21), 110.8 (C-22), 27.2 (C-23), 27.1 (C-24), 30.7 (C-25), 70.0 (C-26), 17.4 (C-27), 102.5 (Gal-C1), 73.2 (Gal-C2), 75.6 (Gal-C3), 79.9 (Gal-C4), 75.3 (Gal-C5), 60.6 (Gal-C6), 105.1 (Glc-C1), 81.4 (Glc-C2), 86.8 (Glc-C3), 70.5 (Glc-C4), 77.6 (Glc-C5), 63.0 (Glc-C6), 104.9 (Glc-C1′), 76.2 (Glc-C2′), 77.7 (Glc-C3′), 71.0 (Glc-C4′), 78.8 (Glc-C5′), 62.5 (Glc-C6′), 105.0 (Xyl-C1), 75.1 (Xyl-C2), 78.7 (Xyl-C3), 70.8 (Xyl-C4), 67.4 (Xyl-C5), or see table S1 (Supplementary material).

Henryioside G (**2**), a white amorphous powder; $[\alpha]_D^{20}$ −26.1° (*c* 0.3, MeOH); IR (KBr) ν_{max}: 3414, 2928, 1642, 1373, 1067, 988, 919, 893, 849 cm^{-1}; *m/z* 1047.5006 $[M-H]^-$

corresponding to a molecular formula of $C_{50}H_{79}O_{23}^-$ (calculated 1047.5018 $[M-H]^-$). ^{1}H-NMR (pyridine-d_5, 400 MHz, δ in ppm) : 1.17 (1H, m, H-1a), 1.51 (1H, m, H-1b), 1.64 (1H, m, H-2a), 2.06 (1H, m, H-2b), 3.92 (1H, m, H-3), 1.29 (1H, m, H-4a), 1.73 (1H, m, H-4b), 0.96 (1H, m, H-5), 1.11 (1H, m, H-6a), 1.13 (1H, m, H-6b), 0.86 (1H, m, H-7a), 1.64 (1H, m, H-7b), 2.02 (1H, m, H-8), 5.43 (1H, s, H-11), 4.14 (1H, s, H-12), 3.31 (1H, m, H-14), 1.57 (1H, m, H-15a), 2.04 (1H, m, H-15b), 4.43 (1H, d, $J = 7.3$ Hz, H-16), 2.31(1H, t, $J = 7.3$ Hz, H-17), 1.05 (3H, s, H-18), 0.82 (3H, s, H-19), 2.13 (1H, m, H-20), 1.40 (3H, d, $J = 6.8$ Hz, H-21), 1.41 (1H, m, H-23a), 1.94 (1H, m, H-23b), 1.53 (1H, m, H-24a), 2.04 (1H, m, H-24b), 1.57 (1H, m, H-25), 3.38 (1H, d, $J = 11.0$ Hz, H-26a), 4.11 (1H, m, H-26b), 1.07 (3H, d, $J = 7.1$ Hz, H-27), 4.86 (1H, d, $J = 7.6$ Hz, H-Gal-1), 4.39 (1H, m, H-Gal-2), 4.04 (1H, m, H-Gal-3), 4.56 (1H, m, H-Gal-4), 3.99 (1H, m, H-Gal-5), 4.14 (1H, m, H-Gal-6a), 4.61 (1H, m, H-Gal-6b), 5.21 (1H, d, $J = 7.7$ Hz, H-Glc-1), 4.37 (1H, m, H-Glc-2), 4.13 (1H, m, H-Glc-3), 3.79 (1H, m, H-Glc-4), 4.04 (1H, m, H-Glc-5), 4.01 (1H, m, H-Glc-6a), 4.46 (1H, d, $J = 10.4$ Hz, H-Glc-6b), 5.26 (1H, d, $J = 7.7$ Hz, H-Glc-1′), 4.07 (1H, m, H-Glc-2′), 3.88 (1H, m, H-Glc-3′), 4.15 (1H, m, H-Glc-4′), 4.10 (1H, m, H-Glc-5′), 4.33 (1H, m, H-Glc-6′a), 4.57 (1H, d, $J = 10.3$ Hz, H-Glc-6′b), 5.58 (1H, d, $J = 7,5$ Hz, H-Xyl-1), 4.02 (1H, m, H-Xyl-2), 4.07 (1H, m, H-Xyl-3), 4.09 (1H, m, H-Xyl-4), 3.65 (1H, t, $J = 10.5$ Hz, H-Xyl-5a), 4.16 (1H, m, H-Xyl-5b). ^{13}C-NMR (pyridine-d_5, 100 MHz, δ in ppm) : 35.7 (C-1), 29.9 (C-2), 77.3 (C-3), 34.9 (C-4), 43.2 (C-5), 28.7 (C-6), 33.5 (C-7), 36.2 (C-8), 148.3 (C-9), 38.1 (C-10), 123.1 (C-11), 78.5 (C-12), 45.2 (C-13), 53.1 (C-14), 32.9 (C-15), 81.4 (C-16), 61.8 (C-17), 10.9 (C-18), 17.8 (C-19), 43.8 (C-20), 14.0 (C-21), 110.1 (C-22), 26.4 (C-23), 26.2 (C-24), 27.6 (C-25), 65.2 (C-26), 16.3 (C-27), 102.5 (Gal-C1), 73.2 (Gal-C2), 75.6 (Gal-C3), 79.9 (Gal-C4), 75.3 (Gal-C5), 60.6 (Gal-C6), 105.2 (Glc-C1), 81.4 (Glc-C2), 86.8 (Glc-C3), 70.5 (Glc-C4), 77.6 (Glc-C5), 63.0 (Glc-C6), 104.9 (Glc-C1′), 76.2 (Glc-C2′), 77.8 (Glc-C3′), 71.1 (Glc-C4′), 78.8 (Glc-C5′), 62.5 (Glc-C6′), 105.0 (Xyl-C1), 75.1 (Xyl-C2), 78.7 (Xyl-C3), 70.8 (Xyl-C4), 67.4 (Xyl-C5), or see table S2 (Supplementary material).

3.5. Acid hydrolysis assay

Solutions of **1** and **2** (5 mg each) were hydrolysed in 2 M hydrochloric acid (5 mL) at 80 °C for 2 h. After cooling, each solution was concentrated under vacuum, dissolved with water, and extracted twice with dichloromethane (CH_2Cl_2). The aqueous parts were subjected to CC on ODS C_{18} silica gel (10 g), eluting with (MeCN-H_2O, 5:95) to give three products. The D configurations of the galactose, glucose and the xylose moieties in **1** and **2** were confirmed through their optical rotation data (Gal: $[\alpha]_D^{20}$ +29.0°, MeOH, Glc: $[\alpha]_D^{20}$ +48.8°, MeOH and Xyl: $[\alpha]_D^{20}$ +33.6°, MeOH) and R_f values (BuOH-AcOH-H_2O, 4:1:5 upper layer, Gal: 0.39, Glc: 0.36 and Xyl: 0.54) with the authentic sugar samples.

3.6. Cytotoxicity assay

The cytotoxic activity assay in the human HepG2 and SW620 cell lines were measured by the MTT method (positive control: 5-fluorouracil, IC_{50} 12.07 μM). Briefly, 1×10^4 mL^{-1} cells were seeded into 96-well plates and allowed to adhere for 24 h. Compounds **1** and **2** were dissolved in DMSO and diluted with complete medium to 6

degrees of concentrations for inhibition rate determination. After incubation at 37.8 °C for 4 h, the supernatant was removed before adding DMSO (100 μL) to each well, the IC_{50} values were calculated (Table 1).

4. Conclusions

A total of four steroidal saponins were obtained from the roots and rhizomes of *M. henryi*, of which, henryiosides F and G (**1** and **2**) were determined as two new ones. Moreover, the isolated saponins (**1**−**4**) were assayed for their regarding cytotoxic activities in human HepG2 and SW620 tumour cells, the results (Table 1) showed that all of the saponins exhibited inhibitory effects with IC_{50} values ranging from 15.33 μM to 57.85 μM. This study enriched the chemical and pharmacological diversity of *M. henryi*.

Disclosure statement

No potential conflict of interest was reported by the authors.

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