



Chemical Constituents from the Whole Plant of *Cuscuta reflexa*

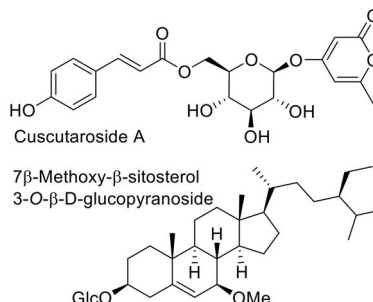
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Abstract

Two new 2*H*-pyran-2-one glucosides, cuscutarosides A (**1**) and B (**2**), and one new steroidal glucoside, 7 β -methoxy- β -sitosterol 3-*O*- β -glucopyranoside (**3**), together with 12 known compounds (**4–15**) were isolated from the whole plant of *Cuscuta reflexa* (Convolvulaceae) collected from Myanmar. The chemical structures of these new compounds were elucidated based on extensive spectroscopic analysis. The antiobesity activity of these isolates was evaluated using porcine pancreatic lipase (PPL), and the antiplatelet aggregation activity was screened using rabbit platelets induced by thrombin, platelet-activating factor (PAF), arachidonate (AA), or collagen. 7 β -Methoxy- β -sitosterol 3-*O*- β -glucopyranoside (**3**) showed weak PPL inhibitory activity. Cuscutaroside A (**1**), its acetylated derivative (**1a**), and scrophenoside B (**8**) showed weak inhibitory activity against rabbit platelet aggregation induced by collagen. Compound **1a** also showed inhibitory activity against rabbit platelet aggregation induced by AA.

Graphic Abstract



Keywords *Cuscuta reflexa* · Porcine pancreatic lipase · Platelet aggregation · 2*H*-pyran-2-one glucosides · Steroidal glucosides

Tin Thu Thu Aung and Meng-Yuan Xia contributed equally to this work.

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1 Introduction

Cuscuta reflexa Roxb. (Convolvulaceae), a twining parasitic plant, is distributed in China, Afghanistan, India, Indonesia, Malaysia, Myanmar, Nepal, Pakistan, Sri Lanka, and Thailand [1]. In Nujiang Prefecture, Yunnan Province, China, the Lisu people call it mu-gua-zhua and use its whole plant or seeds to treat soreness and weakness of the waist and knees, erectile dysfunction, spermatorrhea, diabetes, dizziness, hypopsia, and threatened abortion [2]. Named shwe-new or shwe-nwe-pin (Hsay) in Myanmar, the whole plant of *C. reflexa* is used to treat inflammation, irregularities of the blood, and other diseases [3]. It is also medically used in India, Nepal, Bangladesh, and Pakistan [4]. Based on published studies, the major chemical constituents of *C. reflexa* include flavonoids, coumarins, phenylpropanoids, triterpenoids, and cardiac glycosides [4–7].

A previous study showed that extracts of *C. reflexa* possess an antiobesity effect [8], but no further studies clarified the active compounds responsible for the activity. Pancreatic lipase inhibition is one of the most widely studied mechanisms for antiobesity treatment [9]. Purified human lipase (HPL) and porcine pancreatic lipase (PPL) show similar specific activities [10]. Recently, the extract of *C. reflexa* was found to have in vitro thrombolytic activity [11]. This finding may somewhat explain its traditional use in the treatment of irregularities of the blood. However, no active constituents were reported in this research. In the current study, we report the structural elucidation of three new compounds from the whole plant of *C. reflexa* and the results of a bioassay for the inhibitory activities against PPL and rabbit platelet aggregation.

2 Results and Discussion

2.1 Structural Elucidation

Through chromatographic techniques, three new compounds (**1–3**, Fig. 1) and 12 known compounds (**4–15**, Supplementary Material, Fig. S1) were obtained from the EtOH extract of *C. reflexa*.

Based on ^{13}C NMR data (Table 1) and the HRESIMS ion peak at m/z 457.1106 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{21}\text{H}_{22}\text{NaO}_{10}$, 457.1111), the molecular formula of **1** was deduced to be $\text{C}_{21}\text{H}_{22}\text{O}_{10}$. The ^1H and ^{13}C NMR data (Table 1) indicated the presence of a *p*-coumaroyl moiety [δ_{H} 7.60 (1H, d, $J = 15.9$ Hz), 7.48 (2H, br d, $J = 8.7$ Hz), 6.79 (2H, br d, $J = 8.7$ Hz), and 6.36 (1H, d, $J = 15.9$ Hz); δ_{C} 169.0], one methyl group [δ_{H} 2.20 (3H, s); δ_{C} 19.8], and one β -glucopyranosyl group [δ_{H} 5.05 (d, $J = 7.4$ Hz)]. By comparing its NMR data with those of 4-(β -D-glucopyranosyloxy)-6-methyl-2H-pyran-2-one (**5**) [12], it was implied that compound **1** might be a *p*-coumaroyl derivative of 4-glucopyranosyloxy-6-methyl-2H-pyran-2-one. Based on the 2D NMR spectra of **1**, especially the HMBC correlations from H-1' to C-4 and H₂-6' to C-9'', the 4-glucopyranosyloxy-6-methyl-2H-pyran-2-one moiety was confirmed, and the *p*-coumaroyl group was located at 6'-OH through an ester bond. An acetylated derivative (**1a**) was obtained using pyridine and acetic anhydride. We tried to obtain a crystal of **1a**, but unsuccessful. After acidic hydrolysis of **1**, D-glucose was obtained. Thus, the chemical structure of **1** was determined as shown in Fig. 1 and named cuscutaroside A.

The molecular formula of cuscutaroside B (**2**) was determined to be $\text{C}_{17}\text{H}_{24}\text{O}_9$ based on the ^{13}C NMR data (Table 1) and the HRESIMS ion peak at m/z 395.1313 $[\text{M} + \text{Na}]^+$

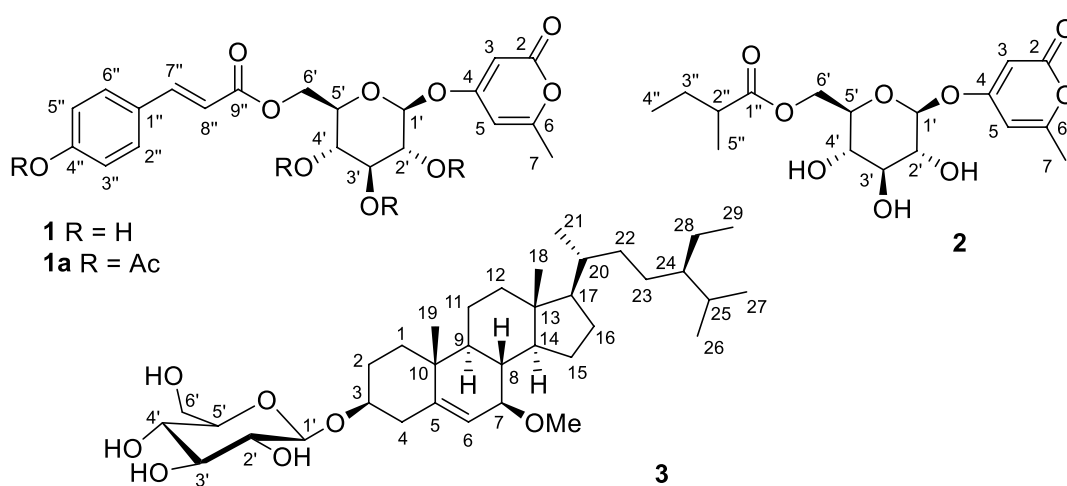
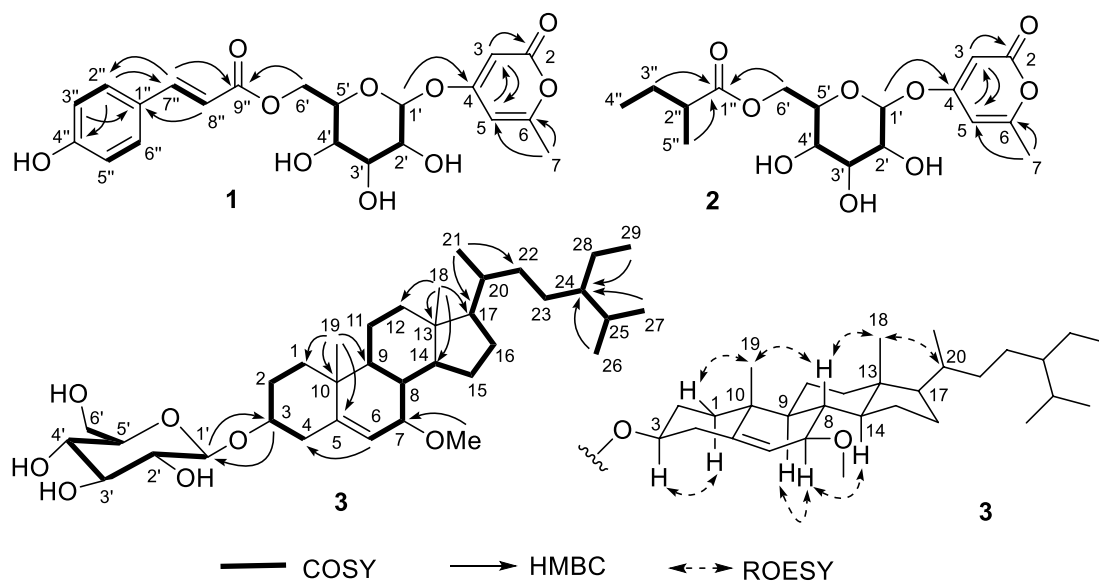


Fig. 1 Chemical structures of new compounds (**1–3**) and the acetylated derivative of **1**

Table 1 ^1H and ^{13}C NMR data of **1** and **2** in methanol- d_4 (δ in ppm, J in Hz)

No.	1		2	
	δ_{H} (500 MHz)	δ_{C} (125 MHz)	δ_{H} (600 MHz)	δ_{C} (150 MHz)
2		167.4, C		167.2, C
3	5.73 (d, 2.0)	91.9, CH	5.66 (d, 2.1)	91.6, CH
4		171.3, C		171.3, C
5	6.08 (m)	101.5, CH	6.10 (m)	101.5, CH
6		164.8, C		164.8, C
7	2.20 (s)	19.8, CH_3	2.25 (s)	19.7, CH_3
1'	5.05 (d, 7.4)	100.7, CH	5.05 (d, 7.5)	100.6, CH
2'	3.48 (m)	74.3, CH	3.43 (dd, 9.3, 7.5)	74.3, CH
3'	3.49 (m)	77.7, CH	3.47 (dd, 9.3, 8.7)	77.5, CH
4'	3.39 (dd, 9.4, 8.9)	71.5, CH	3.33 (dd, 9.7, 8.7)	71.5, CH
5'	3.78 (m)	76.0, CH	3.73 (m)	75.8, CH
6'	4.55 (dd, 12.0, 2.0) 4.24 (dd, 12.0, 7.3)	64.6, CH_2	4.43 (dd, 11.9, 2.1) 4.17 (dd, 11.9, 7.1)	64.5, CH_2
1''		127.1, C		178.2, C
2''	7.48 (br d, 8.7)	131.4, CH	2.43 (m)	42.3, CH
3''	6.79 (br d, 8.7)	116.9, CH	1.65 (m) 1.47 (m)	27.9, CH_2
4''		161.5, C	0.97 (t, 7.3)	11.9, CH_3
5''	6.79 (br d, 8.7)	116.9, CH	1.13 (d, 7.0)	17.0, CH_3
6''	7.48 (br d, 8.7)	131.4, CH		
7''	7.60 (d, 15.9)	147.0, CH		
8''	6.36 (d, 15.9)	114.7, CH		
9''		169.0, C		

**Fig. 2** Key 2D NMR correlations of compounds **1–3**

(calcd for $\text{C}_{17}\text{H}_{24}\text{NaO}_9$, 395.1318). The ^1H and ^{13}C NMR data of **2** (Table 1) indicated the presence of one 6-methyl-2*H*-pyran-2-one moiety (δ_{C} 171.3, 167.2, 164.8, 101.5, and 91.6) [12], one β -glucopyranosyl group [δ_{H} 5.05 (d,

$J=7.5$ Hz)], and one 2-methylbutyryl group (δ_{C} 178.2, 42.3, 27.9, 17.0, and 11.9) [13]. Compound **2** was deduced as a 2-methylbutyryl derivative of 4-(β -glucopyranosyloxy)-6-methyl-2*H*-pyran-2-one by the COSY and HMBC

Table 2 ^1H (800 MHz) and ^{13}C (200 MHz) NMR data of **3** and **4** in methanol- d_4 (δ in ppm, J in Hz)

No.	3		4	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1 β	1.88 (m)	38.2, CH ₂	2.00 (m)	39.9, CH ₂
1 α	1.05 (m)		1.25 (m)	
2	1.96 (m) 1.63 (m)	30.7, CH ₂	2.07 (m) 1.73 (m)	30.4, CH ₂
3	3.62 (m)	79.4, CH	3.76 (m)	78.5, CH
4 α	2.50 (ddd, 13.3, 4.7, 2.1)	39.4, CH ₂	2.68 (m)	39.6, CH ₂
4 β	2.30 (br t, 13.3)		2.47 (ddd, 13.5, 11.8, 2.2)	
5		145.7, C		168.8, C
6	5.48 (t, 1.7)	122.5, CH	5.69 (d, 1.2)	126.5, CH
7	3.44 (dt, 8.5, 2.0)	83.4, CH		204.7, C
8	1.52 (m)	38.3, CH	2.32 (dd, 12.7, 10.9)	46.6, CH
9	1.05 (m)	49.9, CH	1.52 (m)	51.5, CH
10		37.8, C		37.4, C
11	1.52 (m) 1.40 (m)	22.3, CH ₂	1.64 (m)	22.3, CH ₂
12 β	2.04 (dt, 12.8, 3.8)	41.0, CH ₂	2.08 (m)	40.1, CH ₂
12 α	1.16 (m)		1.16 (m)	
13		44.0, C		44.3, C
14	1.16 (m)	57.7, CH	1.32 (m)	51.5, CH
15	1.71 (m) 1.40 (m)	26.8, CH ₂	1.26 (m)	27.4, CH ₂
16	1.88 (m) 1.29 (m)	29.6, CH ₂	1.89 (m) 1.30 (m)	29.6, CH ₂
17	1.11 (m)	56.9, CH	1.12 (m)	56.1, CH
18	0.72 (s)	12.3, CH ₃	0.73 (s)	12.3, CH ₃
19	1.07 (s)	19.4, CH ₃	1.25 (s)	17.7, CH ₃
20	1.38 (m)	37.4, CH	1.38 (m)	37.5, CH
21	0.96 (d, 6.5)	19.4, CH ₃	0.97 (d, 6.6)	19.5, CH ₃
22	1.38 (m) 1.05 (m)	35.1, CH ₂	1.38 (m) 1.05 (m)	35.1, CH ₂
23	1.21 (m)	27.2, CH ₂	1.22 (m)	27.2, CH ₂
24	0.95 (m)	47.3, CH	0.96 (m)	47.3, CH
25	1.69 (m)	30.4, CH	1.68 (m)	30.4, CH
26	0.84 (d, 6.9)	19.4, CH ₃	0.84 (d, 6.9)	19.4, CH ₃
27	0.88 (d, 7.2)	20.2, CH ₃	0.87 (d, 7.2)	20.2, CH ₃
28	1.33 (m)	24.2, CH ₂	1.32 (m)	24.2, CH ₂
29	0.87 (t, 7.0)	12.3, CH ₃	0.90 (t, 7.1)	12.4, CH ₃
1'	4.39 (d, 7.8)	102.5, CH	4.40 (d, 7.8)	102.7, CH
2'	3.15 (dd, 9.1, 7.8)	75.1, CH	3.15 (dd, 9.0, 7.8)	75.1, CH
3'	3.36 (m)	78.1, CH	3.36 (dd, 9.0, 8.6)	78.1, CH
4'	3.27 (m)	71.7, CH	3.27 (m)	71.7, CH
5'	3.26 (m)	77.9, CH	3.27 (m)	78.0, CH
6'	3.85 (dd, 11.7, 1.5) 3.65 (dd, 11.7, 5.3)	62.8, CH ₂	3.86 (dd, 11.9, 1.7) 3.65 (dd, 11.9, 5.4)	62.8, CH ₂
7-OMe	3.28 (s)	54.9, CH ₃		

correlations (Fig. 2). Based on the HMBC correlations from H₂-6' to C-1'', the 2-methylbutyryl group was located at 6'-OH. Thus, the structure of **2** (cuscutaroside B) was elucidated to be 4- $[\beta$ -6-*O*-(2-methylbutyryl)-glucopyranosyloxy]-

6-methyl-2*H*-pyran-2-one. We were unable to calculate the ECD spectrum of **2**. The absolute configuration of C-2'' remains unknown.

Based on ^{13}C NMR (Table 2) and HRESIMS data, compound **3** was determined to have the molecular formula $\text{C}_{36}\text{H}_{62}\text{O}_7$. The ^1H and ^{13}C NMR data (Table 2) of **3** indicated the presence of one β -glucopyranosyl group [δ_{H} 4.39 (d, $J=7.8$ Hz)], one methoxy group [δ_{H} 3.28 (3H, s); δ_{C} 54.9], six methyl groups [δ_{H} 1.07 (3H, s), 0.96 (3H, d, $J=6.5$ Hz), 0.84 (3H, d, $J=6.9$ Hz), 0.88 (3H, d, $J=7.2$ Hz), 0.87 (3H, t, $J=7.0$ Hz), and 0.72 (3H, s)], and one trisubstituted double bond [δ_{H} 5.48 (t, $J=1.7$ Hz); δ_{C} 145.7 and 122.5]. Comparison of its NMR data with those of 7-oxo- β -sitosterol 3- O - β -D-glucopyranoside (**4**, Table 2) and 7 β -hydroxysitosterol-3- O - β -D-glucopyranoside indicated that compound **3** might be a 7-oxygenated derivative of daucosterol [14–16], which was confirmed by the COSY and HMBC correlations (Fig. 2). According to the HMBC correlations from the OMe group to C-7, H-3 to C-1', and H-1' to C-3, the methoxy group and the glucopyranosyloxy group were located at C-7 and C-3, respectively. The relative configuration of compound **3** was partially determined by ROESY correlations (Fig. 2) and coupling constants (Table 2). ROESY correlations of H-1 β /H₃-19, H₃-19/H-8, H-8/H₃-18, and H₃-18/H-20 were observed, indicating that these protons are cofacial, and thus, H-1 α and H-17 are α -oriented. H-7 and H-8 should be in a *trans* axial relationship because of a large coupling constant for H-7/H-8 ($J=8.5$ Hz), and thus, H-7 is α -oriented. The ROESY correlations of H-1 α /H-3, H-9/H-7, and H-7/H-14 indicated that H-3, H-9, and H-14 are also α -oriented. The configurations of C-20 and C-24 on the side chain could not be determined by the ROESY correlations. Because the NMR data of the side chain were highly consistent with those in the literature [14–17], the configurations of C-20 and C-24 were suggested to be the same as those in daucosterol. Finally, compound **3** was elucidated to be 7 β -methoxy- β -sitosterol 3- O - β -glucopyranoside.

The NMR data of 7-oxo- β -sitosterol 3- O - β -D-glucopyranoside (**4**) in pyridine- d_5 and CDCl_3 have been reported previously [14, 15]. Its NMR data in methanol- d_4 are presented in Table 2. The other known compounds, 4-(β -D-glucopyranosyloxy)-6-methyl-2*H*-pyran-2-one (**5**) [12], 4-hydroxyacetophenone (**6**) [18], piceoside (**7**) [19], scrophenoside B (**8**) [20], methyl 4,5-di- O -caffeoylquininate (**9**) [21], methyl 3,5-di- O -caffeoylquininate (**10**) [21], methyl 3,4-di- O -caffeoylquininate (**11**) [21], (6*S*,9*R*)-roseoside (**12**) [22], methyl *trans*-*p*-hydroxycinnamate (**13**) [23], ethyl *trans*-*p*-hydroxycinnamate (**14**) [24], and *N*-*trans*-feruloyltyramine (**15**) [25], were determined by comparing their NMR data with those in the literature.

2.2 Porcine Pancreatic Lipase and Platelet Aggregation Inhibition Assay

Compounds **1–15** were evaluated for their inhibitory activity against PPL. 7 β -Methoxysitosterol 3- O - β -glucopyranoside (**3**) showed weak PPL inhibitory activity

($\text{IC}_{50}=67.2\pm 1.7$ $\mu\text{g}/\text{mL}$) compared with the positive control orlistat ($\text{IC}_{50}=0.40\pm 0.02$ ng/mL). 7-Oxo- β -sitosterol 3- O - β -D-glucopyranoside (**4**) showed $12\pm 2\%$ inhibition at a concentration of 100 $\mu\text{g}/\text{mL}$. The other tested compounds were inactive, with inhibition values less than 10% at a concentration of 100 $\mu\text{g}/\text{mL}$.

Compounds **1–15** and **1a** were also evaluated for their inhibitory activity against rabbit platelet aggregation induced by thrombin (1 U/mL), platelet-activating factor (PAF, 0.4 $\mu\text{g}/\text{mL}$), arachidonate (AA, 0.5 mM), or collagen (4 $\mu\text{g}/\text{mL}$). Cuscutaroside A (**1**), **1a**, and scrophenoside B (**8**) showed weak inhibitory activity against rabbit platelet aggregation induced by collagen with IC_{50} values of 291.4 ± 47.9 $\mu\text{g}/\text{mL}$, 63.8 ± 4.4 $\mu\text{g}/\text{mL}$, and 180.5 ± 6.7 $\mu\text{g}/\text{mL}$, respectively, compared with aspirin ($\text{IC}_{50}=33.3\pm 1.3$ $\mu\text{g}/\text{mL}$). Compound **1a** also showed inhibitory activity against rabbit platelet aggregation induced by AA with an IC_{50} value of 72.6 ± 10.5 $\mu\text{g}/\text{mL}$ compared with aspirin (inhibition $88.1\pm 1.1\%$ at 40 $\mu\text{g}/\text{mL}$). The other tested compounds were inactive ($\text{IC}_{50}> 300$ $\mu\text{g}/\text{mL}$).

3 Experimental Section

3.1 General Experimental Procedures

Optical rotations were recorded using a JASCO P-1020 Polarimeter (Jasco Corp., Tokyo, Japan). UV spectra were obtained using a Shimadzu UV-2401 PC spectrophotometer (Shimadzu, Kyoto, Japan). ECD spectra were recorded on a Chirascan CD spectrometer (Applied Photophysics Ltd., Leatherhead, UK). ^1H and ^{13}C NMR spectra were collected on DRX-500, Avance III-600, and AscendTM 800 MHz spectrometers (Bruker Corp., Karlsruhe, Germany) with TMS as an internal standard. ESIMS and HRESIMS analyses were performed on an API QSTAR Pulsar 1 spectrometer (Applied Biosystems/MDS Sciex, Foster City, CA, USA). EIMS and HREIMS were performed on a Waters Auto-Spec Premier p776 spectrometer (Waters, Milford, MA, USA). Silica gel G (80–100 and 300–400 mesh, Qingdao Meigao Chemical Co., Ltd., Qingdao, China), C_{18} silica gel (40–75 μm , Fuji Silysia Chemical Ltd., Aichi, Japan), and Sephadex LH-20 (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) were used for column chromatography, and silica gel GF₂₅₄ (Qingdao Meigao Chemical Co., Ltd.) on precoated plates was used for preparative thin layer chromatography (TLC). TLC spots were visualized under UV light at 254 nm and by dipping them into 5% H_2SO_4 in alcohol followed by heating. Semipreparative high-performance liquid chromatography (HPLC) was performed with an Agilent 1200 series pump (Agilent Technologies, Santa Clara, USA) equipped with a diode array detector and an Agilent Zorbax

SB-C₁₈ column (5.0 μm , ϕ 9.4 \times 250 mm) and a Welch Ultimate AQ-C₁₈ column (5.0 μm , ϕ 4.6 \times 300 mm).

3.2 Plant Material

The plant material, growing on a *Bauhinia* plant, was collected near Golden Cave (20° 55' 44.25" N and 96° 38' 57.41" E) in Pindaya Township, Southern Shan State, Myanmar, in December 2016. It was identified as *Cuscuta reflexa* Roxb. by Dr. Jie Cai and Ms. Jun Yang at the Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (no. MMR631) was deposited at the Southeast Asia Biodiversity Research Institute, Chinese Academy of Sciences, Yezin, Nay Pyi Taw, Myanmar and a copy was placed at the Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences, China.

3.3 Extraction and Isolation

The air-dried powdered whole plant of *Cuscuta reflexa* (3.3 kg) was ultrasonically extracted for 30 min with 70% EtOH at 60 °C. The EtOH extract (519.0 g) was suspended in H₂O and further partitioned with petroleum ether, EtOAc, and *n*-butanol to the yield petroleum ether-soluble portion (6.4 g, A), the EtOAc-soluble portion (66.8 g, B), and the *n*-butanol soluble portion (276.6 g, C), respectively.

Part B was subjected to column chromatography (silica gel; CH₂Cl₂-MeOH, 50:1 \rightarrow 0:1, v/v) to yield four fractions (B1-B4). Fraction B1 was separated on an RP-C₁₈ silica gel column eluted with MeOH-H₂O (10% \rightarrow 100%) to yield three major subfractions. The 20% MeOH-eluted portion was purified by Sephadex LH-20 column chromatography (MeOH) and silica gel column chromatography (petroleum ether-EtOAc, 10:1) to yield **6** (8.9 mg). The 40% MeOH-eluted portion was purified by column chromatography (Sephadex LH-20, MeOH), preparative TLC (petroleum ether-acetone, 2:1), and semipreparative HPLC [Agilent Zorbax SB-C₁₈ column, MeCN-H₂O (containing 0.05% TFA), 35:65, 2 mL/min] to yield **13** (5.0 mg, t_{R} = 19.388 min) and **14** (2.0 mg, t_{R} = 32.159 min).

Fraction B2 was separated on an RP-C₁₈ silica gel column eluted with MeOH-H₂O (10% \rightarrow 100%) to yield one main subfraction. The 30% MeOH-eluted portion was isolated by Sephadex LH-20 (MeOH) and silica gel column chromatography (CH₂Cl₂-MeOH, 30:1) and further purified by semipreparative HPLC [Welch Ultimate AQ-C₁₈ column, MeCN-H₂O (containing 0.05% TFA), 20:80, 1 mL/min] to yield **15** (19.4 mg, t_{R} = 36.402 min).

Fraction B3 was separated on an RP-C₁₈ silica gel column eluted with MeOH-H₂O (10% \rightarrow 100%) to yield two main subfractions (B3-1 and B3-2). B3-1 (40% MeOH-eluted

portion) was separated by silica gel column chromatography (CH₂Cl₂-MeOH, 100:1 and 50:1) to yield B3-1-1 and B3-1-2. B3-1-1 was separated by Sephadex LH-20 (MeOH) and silica gel column chromatography (CH₂Cl₂-MeOH, 3:1) followed by semipreparative HPLC (Agilent Zorbax SB-C₁₈ column, MeOH-H₂O, 40:60, 2 mL/min) to yield **2** (3.0 mg, t_{R} = 24.052 min) and **8** (2.1 mg, t_{R} = 37.642 min). B3-1-2 was separated by Sephadex LH-20 (MeOH) and silica gel column chromatography (CH₂Cl₂-acetone, 3:1 \rightarrow 1:1) to yield **1** (571.5 mg) and a mixture. The mixture was further purified by semipreparative HPLC [Agilent Zorbax SB-C₁₈ column, MeCN-H₂O (containing 0.05% TFA), 28:72, 2 mL/min] to yield **9** (5.9 mg, t_{R} = 13.042 min), **10** (5.8 mg, t_{R} = 17.294 min), and **11** (2.6 mg, t_{R} = 19.588 min). B3-2 (90% MeOH-eluted portion) was separated by Sephadex LH-20 column chromatography (MeOH) and semipreparative HPLC (Welch Ultimate AQ-C₁₈ column, MeOH-H₂O, 87:13, 1 mL/min) to yield **4** (3.5 mg, t_{R} = 42.732 min) and **3** (2.0 mg, t_{R} = 64.797 min).

Fraction B4 was separated on an RP-C₁₈ silica gel column eluted with MeOH-H₂O (10% \rightarrow 100%) to yield three main fractions. The 10% MeOH-eluted portion was purified by silica gel column chromatography (CH₂Cl₂-MeOH, 30:1 and 20:1) and Sephadex LH-20 column chromatography (MeOH) to yield **5** (1.1 g) recrystallized from MeOH. The 15% MeOH-eluted portion was isolated by silica gel column chromatography (CH₂Cl₂-MeOH, 30:1 and 20:1) and Sephadex LH-20 column chromatography (MeOH) and further purified by semipreparative HPLC (Welch Ultimate AQ-C₁₈ column, MeOH-H₂O, 15:85, 1 mL/min) to yield **12** (6.4 mg, t_{R} = 36.095 min). The 20% MeOH-eluted portion was isolated by Sephadex LH-20 column chromatography (MeOH) and semipreparative HPLC (Agilent Zorbax SB-C₁₈ column, MeOH-H₂O, 15:85, 2 mL/min) to yield **7** (1.6 mg, t_{R} = 22.265 min).

3.4 Spectroscopic Data of Compounds

3.4.1 Cuscutaroside A (1)

White powder; $[\alpha]_{\text{D}}^{21}$ -46.2 (c = 0.19, MeOH); UV (MeOH) λ_{max} (log ϵ) 311 (4.42), 227 (4.11), 200 (4.49) nm; ¹H NMR and ¹³C NMR data see Table 1; ESIMS (negative) m/z 433 [M-H]⁻, 867 [2 M-H]⁻; HRESIMS (positive) m/z 457.1106 [M+Na]⁺ (calcd for C₂₁H₂₂NaO₁₀, 457.1111).

3.4.2 Cuscutaroside B (2)

Colorless solid; $[\alpha]_{\text{D}}^{21}$ -77.6 (c = 0.11, MeOH); UV (MeOH) λ_{max} (log ϵ) 285 (3.54), 198 (4.22) nm; ECD (c 0.011, MeOH) λ_{max} ($\Delta\epsilon$) 281 (-0.91), 199 (-4.06) nm; ¹H NMR and ¹³C NMR data see Table 1; ESIMS (positive) m/z 395

$[M + Na]^+$, 767 $[2M + Na]^+$; HRESIMS (positive) m/z 395.1313 $[M + Na]^+$ (calcd for $C_{17}H_{24}NaO_9$, 395.1318).

3.4.3 7 β -Methoxy- β -sitosterol 3-*O*- β -glucopyranoside (3)

Colorless solid; $[\alpha]_D^{21} - 25.2$ ($c = 0.11$, MeOH); 1H NMR and ^{13}C NMR data see Table 2; ESIMS (positive) m/z 629 $[M + Na]^+$; HRESIMS (positive) m/z 629.4388 $[M + Na]^+$ (calcd for $C_{36}H_{62}NaO_7$, 629.4393).

3.5 Acidic Hydrolysis of Compound 1

Compound **1** (20.0 mg, 0.046 mM) was dissolved in 15 mL of 6% aq. HCl and hydrolyzed under reflux (5 h) at 90 °C. Then, the acidic solution was evaporated in vacuo to dryness and separated by silica gel column chromatography eluted with $CHCl_3$ -MeOH (10:1) to yield D-glucopyranose (5.5 mg, 0.031 mM, 67% yield), which was identified based on its 1H NMR spectrum and optical rotation value of $[\alpha]_D^{20} + 40.0$ (c 0.09, H_2O) [26].

3.6 Acetylation of Compound 1

Compound **1** (20.6 mg, 0.048 mmol) was dissolved in 500 μ L of pyridine and 500 μ L of acetic anhydride and stirred for 24 h at room temperature. Then, water (2 mL) was added to the reaction mixture, followed by extraction with ethyl acetate (4 mL). The upper layer was dried under reduced pressure and purified by silica gel column chromatography (petroleum ether-EtOAc, 10:1) to yield **1a** (20.4 mg, 0.034 mmol, 71% yield). White solid; 1H NMR (500 MHz, $CDCl_3$) δ 7.68 (1H, d, $J = 16.0$ Hz, H-7''), 7.61 (2H, br d, $J = 8.6$ Hz, H-2'' and H-6''), 7.12 (2H, br d, $J = 8.6$ Hz, H-3'' and H-5''), 6.46 (1H, d, $J = 16.0$ Hz, H-8''), 5.80 (1H, br s, H-5), 5.63 (1H, d, $J = 2.2$ Hz, H-3), 5.28 (2H, m), 5.16 (2H, m), 4.37 (1H, dd, $J = 12.6, 2.3$ Hz, H-6'a), 4.27 (1H, dd, $J = 12.6, 6.2$ Hz, H-6'b), 3.94 (1H, m, H-5'), 2.30 (3H, s), 2.19 (3H, s), 2.06 (3H, s), 2.06 (3H, s), 2.03 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.2, 169.5, 169.3, 169.2, 168.1, 166.4, 164.4, 163.3, 152.4, 145.2, 132.0, 130.5, 129.7, 122.3, 117.1, 100.1, 96.9, 91.4, 73.0, 72.6, 70.7, 68.2, 62.1, 21.3, 20.7, 20.7, 20.7, 20.0; ESIMS (positive) m/z 625 $[M + Na]^+$; HRESIMS (positive) m/z 625.1528 $[M + Na]^+$ (calcd for $C_{29}H_{30}NaO_{14}$, 625.1533).

3.7 In vitro Porcine Pancreatic Lipase Inhibition Assay

For lipase inhibition tests of each compound, porcine pancreatic lipase was used. *p*-Nitrophenyl butyrate (*p*-NPB) was used as the substrate. First, 5 μ L of the lipase solution (40 U/mL) in Tris-HCl buffer (100 mM Tris-HCl, 5 mM $CaCl_2$;

pH 7.0) was added to a 96-well microtiter plate. Each compound in 1 μ L of DMSO and 184 μ L of Tris-HCl buffer were added and mixed with the enzyme buffer to start the reaction. After incubation at 37 °C for 15 min, 10 μ L of the substrate solution (10 mM *p*-NPB in dimethyl formamide) was added. The enzymatic reaction was carried out for 15 min at 37 °C. The hydrolysis of *p*-NPB to *p*-nitrophenol was monitored at 400 nm using a spectrophotometer [27].

3.8 In Vitro Platelet Aggregation Assay

The inhibitory effects of the compounds against rabbit platelet aggregation induced by thrombin (1 U/mL), PAF (0.4 μ g/mL), AA (0.5 mM), or collagen (4 μ g/mL) were evaluated according to published methods [28–30].

4 Conclusion

Three new and 12 known compounds were isolated from the whole plants of *Cuscuta reflexa* (Convolvulaceae) collected from Myanmar. 7 β -Methoxy- β -sitosterol 3-*O*- β -glucopyranoside (**3**) showed weak PPL inhibitory activity ($IC_{50} = 67.2 \pm 1.7$ μ g/mL). Cuscutaroside A (**1**), its acetylated derivative (**1a**), and scrophenoside B (**8**) showed weak inhibitory activity against rabbit platelet aggregation induced by collagen (4 μ g/mL) with IC_{50} values of 291.4 ± 47.9 μ g/mL, 63.8 ± 4.4 μ g/mL, and 180.5 ± 6.7 μ g/mL, respectively. Compound **1a** also showed inhibitory activity against rabbit platelet aggregation induced by AA (0.5 mM) with an IC_{50} value of 72.6 ± 10.5 μ g/mL. These results support previous findings in pharmacological studies related to the traditional uses of the plant.

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Compliance with Ethical Standards

Conflicts of interest The authors declare that there are no conflicts of interest associated with this work.

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