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Bioactive glycosides from Salacia cochinchinensis

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Graphical Abstract



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Abstract

Four new triterpene glycosides, named salaciacochinosides A-D (1 - 4) were isolated from the 90% ethanol extract of *Salacia cochinchinensis*, together with five known compounds 2α , 3β , 23-trihydroxyurs-12, 18-dien-28-oic acid 28-*O*- β -D-glucopyranoside (5), racemiside (6), alangiplatanoside (7), acantrifoside E (8), and syringin (9). The structures of the four new triterpenoids were characterized by chemical methods and MS, IR, 1D and 2D NMR spectral analyses. The α -glucosidase inhibitory activities of the nine compounds were assessed, compounds 6 and 7 showed remarkable α -glucosidase inhibitory activities, with IC₅₀ values of 0.44 and 0.75 μ M, respectively. Compounds 1 - 5 exhibited moderate α -glucosidase inhibitory activity in our current experiments.

Keywords: Salacia cochinchinensis; triterpene glycosides; salaciacochinosides A-D; α-glucosidase

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Salacia cochinchinensis, a perennial shrub about one or two meters high, is mainly distributed in south-east Asia such as China, Vietnam, and Cambodia. In Xishuangbanna, China, the roots and leaves of S. cochinchinensis had been used for the treatment of diabetes and inflammation by the indigenes. In consideration that many plants of the Salacia genus had been reported to possess antidiabetic, antiobese and anti-inflammatory activity,¹⁻⁴ the S. cochinchinensis was also tested for the antidiabetic activity in our previous bioassay experiment. Results suggested the 90% ethanol extract of S. cochinchinensis possessed remarkable α -glucosidase inhibitory activity. As far as we know, there was no investigation on S. cochinchinensis reported. With the aim to find active chemicals from this medicinal plant, the S. cochinchinensis was phytochemically investigated and nine compounds were isolated from the title plant. By chemical methods and comprehensive analysis of the IR, MS, HRESIMS, 1D and 2D NMR spectral data, all the isolates were identified as 2α , 3α , 24-trihydroxyurs-12-en-28-oic acid 28-*O*- β -D-glucopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside (salaciacochinoside A, 1), 2α , 3α , 23-trihydroxyurs-12-en-28-oic acid 28-O- β -D-glucopyranosyl- $(1 \rightarrow 2)$ - β -D-glucopyranoside (salaciacochinoside B, 2), 2α,3β,23-trihydroxyurs-12-en-28-oic acid 28-O-β-D-glucopyranosyl- $(1\rightarrow 2)$ - β -D-glucopyranoside (salaciacochinoside C, **3**), $2\alpha_3\alpha_2$ 4-trihydroxylup-12,20(29)-dien-28-oic acid 28-*O*- β -D-glucopyranosyl-(1 \rightarrow 2)- β -D-glucopyranoside (salaciacochinoside D. 4), 2α , 3β , 23-trihydroxyurs-12, 18-dien-28-oic acid 28-*O*- β -D- glucopyranoside (5), ^{5,6} racemiside (6), ⁷ alangiplatanoside (7),⁸ acantrifoside E (8),⁹ syringin (9)¹⁰ (Figure 1). Compounds 1 - 4 were new triterpene glycosides. All compounds were assayed for their α -glucosidase inhibitory activities; results suggested compounds 6 and 7 showed rermarkable α -glucosidase inhibitory activities, with IC₅₀ values of 0.44 and 0.75 μ M, respectively, and compounds 1 - 5 possessed moderate α -glucosidase inhibitory activity. Herein we reported the isolation, structural elucidation and α -glucosidase inhibitory activity of all isolates.

Compound 1 was obtained as a white amorphous powder. Its molecular formula was deduced as $C_{42}H_{68}O_{15}$ by the HRESIMS at m/z 811.4488 [M-H]⁻ ($C_{42}H_{67}O_{15}$, calcd. 811.4480). The IR spectrum showed the absorption bands at 3348, 1735, 1647 cm⁻¹, suggesting the presence of hydroxyl, carbonyl and olefinic functions in compound 1. In the ¹H NMR spectrum (Table 1), an olefinic proton at δ 5.43 (1H, brs, H-12) was observed, besides a typical doublet methine at δ 2.50 (1H, d, J = 11.2 Hz, H-18), six methyl signals at δ 1.63 (3H, s, H-23), 1.08 (3H, s, H-27), 1.07 (3H, s, H-26), 0.97 (3H, s, H-25), 0.92 (3H, d, J = 6.4 Hz, H-29), 0.88 (3H, d, J = 6.2 Hz, H-30), suggesting that compound 1 was a urs-12-en-28-oic acid derivative. The anomeric proton signals at δ 6.16 (1H, d, J = 8.1 Hz, H-1') and 5.66 (1H, d, J = 7.7 Hz, H-1") indicated the presence of two β -linkaged sugar moieties in compound 1. The ¹³C NMR spectrum (Table 2) displayed 42 carbon signals, of which an ester carbonyl at δ 176.3 (C-28), two olefinic carbon signals at δ 125.8 (C-12), 138.7 (C-13) were clearly visible, besides two anomeric carbon signals at δ 93.6, 104.9. The NMR data of the aglycone of compound 1 was comparable to those of methyl 2α , 3α , 24- trihydroxyurs-12- en- 28- oate, ¹¹ indicating compound **1** also contained an aglycone of 2α , 3α , 24- trihydroxyurs-12- en- 28- oic acid. After hydrolysis of 1 with 8% HCl in MeOH, glucose was identified by a comparison with the authentic sugar sample on TLC (developed by n-BuOH/AcOH/H₂O 4:1:5, upper layer; and PhOH/H₂O 4:1). The absolute configuration of D-glucose was finally confirmed by GC analysis of the trimethylsilyl L-cysteine derivative. The identical ¹³C NMR data of the sugar region with those of asteryunnanoside B,¹² in combination with the HMBC correlations (Figure 2) between H-1' (δ 6.16) and C-28 (δ 176.3), H-1" (δ 5.66) and C-2' (δ 79.4), established the existence of β -D- glucopyranosyl-(1 \rightarrow 2)- β -D-glucopyranosyl at C-28. The 2α , 3α hydroxyl groups were also verified by the ROESY (Figure 3) correlations of

H-2/Me-25 and H-3/Me-25. The other HMBC, ¹H-¹H COSY (Figure 2) and ROESY (Figure 3) correlations allowed the full proton and carbon assignments of compound **1**. Consequently, the structure of compound **1** was determined as $2\alpha, 3\alpha, 24$ -trihydroxyurs-12-en- 28-oic acid $28-O-\beta$ -D-glucopyranosyl- $(1\rightarrow 2)$ - β -D-glucopyranoside and named as salaciacochinoside A (**1**).

Compound 2 was obtained as a white amorphous powder and assigned the molecular formula $C_{42}H_{68}O_{15}$ by the positive HRESIMS at m/z 835.4450 [M+Na]⁺ ($C_{42}H_{68}O_{15}Na^+$, calcd. 835.4456). Its IR spectrum showed hydroxyl, carbonyl and olefinic functions at 3440, 1735, and 1645 cm⁻¹, respectively. Hydrolysis of 2 with 8% HCl in MeOH liberated D-glucose, identified by a comparison with the authentic sugar sample and further confirmed by GC analysis. The ¹H NMR spectrum (Table 1) presented an olefinic proton signal at δ 5.41 (1H, br.s, H-12), a doublet methine at δ 2.48 (1H, d, J = 11.3 Hz, H-18), together with four singlet methyls [δ 1.10 (H-26), 1.05 (H-27), 0.95 (H-25), 0.77 (H-24)] and two doublet methyls [δ 0.89 (3H, d, J = 6.3 Hz, H-29); 0.85 (3H, d, J = 6.1 Hz, H-30)], suggesting compound 2 was also a urs-12-en-28-oic acid derivatives. The anomeric proton signals at δ 6.18 (1H, d, J = 8.2 Hz, H-1') and 5.72 (1H, d, J = 7.8 Hz, H-1") suggested there were two β -linked sugar units in compound 2. The ¹³C NMR spectrum (Table 2) of compound 2 exhibited 42 carbon signals. The carbon signals due to the sugar moieties were identical with those of 1, implying compound 2 contained the same sugar linkage as compound 1. Compound 2 differed from 1 mainly in the aglycone unit, where a hydroxy linked at C-23 instead of a hydroxyl at C-24 in compound 1. The structure of the aglycone was determined as $2\alpha_3\alpha_2$ 3-trihydroxyurs-12-en-28-oic acid by comparing the ¹³C NMR data with those of methyl 2α , 3α , 23-trihydroxyurs-12-en-28-oate, ¹³ and further verified by the ROESY (Figure 3) correlations of H-2/H-25, H-3/H-25, and H-23/H-5. Accordingly, the structure

of	compound	2	was	deduced	as	2a,3a,23-trihydroxyurs-12-en-28-oic	acid
28-0	D-B-D-glucopyr	anosvl	-(1→2)-l	B-D-gluconvi	anosid	e and named as salaciacochinoside B (2).	

Compound **3** was obtained as a white amorphous powder with a molecular formula of $C_{42}H_{68}O_{15}$ deduced by the HRESIMS at m/z 811.4476 [M-H]⁻ ($C_{42}H_{67}O_{15}$, calcd. 811.4480). The IR spectral showed almost the same absorptions as those of compounds **1** and **2**. In the ¹H NMR spectrum (Table 1), the characteristic proton signals for a 12-en ursane triterpene aglycone [δ 5.23 (1H, brs, H-12), 2.20 (1H, d, J = 11.2 Hz, H-18), 0.97 (3H, d, J = 6.1 Hz, H-30), 0.90 (3H, d, J = 6.1 Hz, H-29), 1.12, 1.05, 0.83, 0.70 (each 3H, all s, for H-27, 25, 26, 24, respectively)] were displayed. A comparison of the ¹³C NMR (Table 2) data of **3** with those of asiaticoside E¹⁴ indicated that both compounds shared a same aglycone of 2α , 3β ,23-trihydroxyurs-12-en-28-oic acid. Acidic hydrolysis of **3** liberated D-glucose, . The evidence that the NMR data ascribable to sugar moieties were essentially identical to those of compounds **1** and **2**, illustrated compound **3** also contained a (1-2) linkaged diglucopyranosyl at C-28. This deduction was further proved by the HMBC cross-peak between H-1' (δ 5.42) and C-28 (δ 178.0), H-1" (δ 4.76) and C-2" (δ 78.7). Consequently, the structure of compound **3** was deduced as 2α , 3β ,23-trihydroxyurs-12-en-28-oic acid 28-*O-β*-D-glucopyranosyl-(1-2)-*β*-D-glucopyranoside and named as salaciacochinoside C (**3**).

Compound **4** was obtained as a white amorphous powder and had the molecular formula of $C_{42}H_{66}O_{15}$ revealed by the HRESIMS at m/z 833.4292 [M+Na]⁺ ($C_{42}H_{66}O_{15}Na^+$, calcd. 833.4299). On hydrolysis of **4**, D-glucose was detected by a comparison with the authentic sugar sample. The ¹H NMR (Table 1) displayed three olefinic proton signals at δ 5.43 (1H, brs, H-12), 4.76 (1H, br.s, H_a-29), 4.72 (1H, br.s, H_b-29), besides five singlet methyls and two anomeric protons ascribable to β -D-glucose units. The ¹³C NMR spectrum showed 42 carbon resonances, of which two glucosyl anomeric carbon

signals at δ 93.7 and 104.8 were displayed. The characteristic terminal double bond at δ 153.4 (C-20, C), 105.1 (C-29, CH₂) suggested compound **4** might be a lupine-type triterpene derivative,^{6,15} which was finally corroborated by the HMBC correlations between H-29 and C-19, C-30; H-18 and C-20; H-21 and C-20. The ¹H-¹H COSY correlations (Figure 2) of H-18/H-19, H-19/H-21, H-21/H-22 also supported the presence of five member ring E in compound **4**. In consideration that the ¹³C NMR data of rings A-D in compound **4** were almost the same as compound **1**, it can be concluded that the compound **4** had an aglycone of $2\alpha_3\alpha_2$ 24-trihydroxylup-12-en-28-oic acid, which was ascertained by the HMBC and ¹H-¹H COSY correlations indicated in Figure 2. Compound **4** possessed the same diglucosyl connections as **1-3** by a comparison of the sugar NMR data with those of **1-3**. Lastly, the structure of compound **4** was elaborated as $2\alpha_3\alpha_2$ 24-trihydroxylup-12-en-28- oic acid $28-O-\beta$ -D-glucopyranosyl-(1→2)- β -D-glucopyranoside and named as salaciacochinoside D (**4**).

The other compounds were identified as $2\alpha, 3\beta, 23$ -trihydroxyurs-12,18-dien-28-oic acid 28-*O*- β -D-glucopyranoside (**5**),^{5,6} racemiside (**6**),⁷ alangiplatanoside (**7**),⁸ acantrifoside E (**8**),⁹ syringin (**9**)¹⁰ by comparison the NMR data with those reported in literatures.

All isolates were assayed for their α -glucosidase inhibitory activities. Results were shown in tables 3. Compounds 6 and 7 exhibited remarkable α -glucosidase inhibitory activities, with IC₅₀ values of 0.44 and 0.75 μ M, respectively. Compounds 1-5 possessed moderate α -glucosidase inhibitory activities, with IC₅₀ values ranging from 1.01 to 4.72 μ M, and compounds 8 and 9 showed no α -glucosidase inhibitory activity in our experiment. The above results suggested the lignan and triterpene glycosides might be the bioactive constituents. Further investigation on this plant is still ongoing.

In conclusion, nine glycosides, including four new triterpene glucosides, were isolated from S.

cochinchinensis. All chemicals were tested for their α -glucosidase inhibitory activities and two lignan glycosides were found to possess notable α -glucosidase inhibitory activity. The triterpene glycosides also showed moderate inhibiting α -glucosidase activity. Our results suggested further investigation on this medicinal plant is necessary.

1. Experimental

1.1 General experiment procedures

Column chromatography (CC): silica gel (200-300 mesh; Qingdao Marine Chemical Inc., China); Lichrospher Rp-18 gel (40-63 μ ; Merck, Germany); MCI gel CHP-20P (70-150 μ m, Mitsubishi Chemical Corporation, Tokyo, Japan). Semi-preparative HPLC was performed on an Agilent 1260 liquid chromatography with a Venusil XBP C18 (10 × 250 mm, 5 μ m) column. GC experiments were conducted on an Agilent 7890A equipment. Optical rotations were carried out on a HORIBA SEPA-300 High Sensitive Polarimeter. IR spectra were measured on a Bio-Rad FTS-135 spectrometer with KBr pellets, ν in cm⁻¹. HRESIMS data were obtained on an Agilent QTOF 6545 LC/MS. NMR spectra were recorded on a Bruker AM-400 (¹H/¹³C, 400 MHz/100 MHz) spectrometer, and chemical shifts were given in δ (ppm) with TMS as internal reference. The authentic sugar samples (D--glucose and L-glucose) and Acarbose (positive control) were obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 8% H₂SO₄ in EtOH.

1.2. Planta material

The branches and leaves of *S. cochinchinensis* were collected from Xishuangbanna in September, 2015, and were identified as *Salacia cochinchinensis* by Dr. Shi-Shun Zhou from Xishuangbanna Tropical Botanical Garden, Chinese Academy of Sciences. A voucher specimen (TSYJ-2015057) was deposited in the Key Laboratory of Chemistry in Ethnic Medicinal Resources, State Ethnic Affairs Commission & Ministry of Education, Yunnan Minzu University.

1.3. Extraction and isolation

The air-dried branches and leaves of *S. cochinchinensis* (10.0 kg) were powdered and extracted with 90% ethanol under reflux for three times, 2 hours each time. After concentrated in *vacuo*, the extract was suspended in water and successively partitioned with petroleum ether, EtOAc and n-BuOH to give petroleum ether (A). EtOAc (B), n-BuOH (C) and aqueous (D) fractions.

The n-BuOH (C) (150 g) fraction was then subjected to silica gel chromatography column (CC) and eluted with gradient CHCl₃/MeOH/H₂O (95:5:0, 90:10:0, 85:15:1, 80:20:2, 70:30:5) to give four fractions (Frs.C.1-4). The Fr.C.3 (18 g, eluted by CHCl₃/MeOH/H₂O 85:15:1) was subjected on a silica gel CC with a gradient elution of CHCl₃/MeOH/H₂O (90:10:0, 85:15:1) to afford four sub-fractions (Frs.C.3.1-3.4). The Fr.C.3.2 (4.1 g) was performed to a MCI CC (MeOH/H₂O 60:40 to 100: 0) and further purified by HPLC with an eluent of MeOH/H₂O (65:35, flow rate: 2ml/min) to yield compounds **6** (7.3 mg), **8** (28 mg) and **9** (22 mg). The Fr.C.3.3 (3.8 g) was separated on a MCI CC (MeOH/H₂O 40:60 to 100: 0) to yield three sub-fractions (Frs.C.3.3.1-3.3.3). Fr.C.3.3.1 (0.5 g) was purified by HPLC (MeOH/H₂O, 68:32, flow rate: 2 ml/min) to afford compounds **4** (18.7 mg), **5** (12 mg) and **7** (14.3 mg). Fr.C.3.3.2 (1.01 g) was successively performed on a silica gel CC and further purified by Rp-18 CC (MeOH/H₂O 60:40) to yield compounds **1** (10.5 mg), **2** (23 mg), **3** (15 mg).

1.4. Identification

Salaciacochinoside A (1): white amorphous powder; $[\alpha]_D^{21.3} + 10.5$ (*c* 0.20, MeOH); IR (KBr) cm⁻¹: 3348, 2996, 1735, 1647, 1068; ¹H and ¹³CNMR (C₅D₅N, 400 MHz and 100 MHz) data, see Tables 1 and 2; ESI (neg.): *m/z* 811 [M - H]⁻; HRESIMS (neg.): *m/z* 811.4488 [M - H]⁻, (calcd for C₄₂H₆₇O₁₅⁻,

811.4480).

Salaciacochinoside B (2): white amorphous powder; $[\alpha]_D^{15.5} + 18.4 (c \ 0.25, MeOH)$. IR (KBr) cm⁻¹: 3340, 2998, 1735, 1645, 1067; ¹H and ¹³C NMR (C₅D₅N, 400 MHz and 100 MHz) data, see Tables 1 and 2; ESI (pos.): m/z 835 [M+Na]⁺; HRESIMS (pos.): m/z 835.4450 [M+Na]⁺ (calcd for $C_{42}H_{68}O_{15}Na^+$, 835.4456).

Salaciacochinoside C (**3**): white amorphous powder; $[\alpha]_D^{21.6} + 25.7 (c \ 0.20, MeOH)$. IR (KBr) cm⁻¹: 3338, 1736, 1651, 1065; ¹H and ¹³C NMR (CD₃OD, 400 MHz and 100 MHz) data, see Tables 1 and 2; ESI (neg.): m/z 811 [M - H]⁻; HRESIMS (neg.): m/z 811.4476 [M - H]⁻ (calcd for C₄₂H₆₇O₁₅⁻, 811.4480).

Salaciacochinoside D (4): white amorphous powder; $[\alpha]_D^{21.0}$ +47.8 (*c* 0.21, MeOH). IR (KBr) cm⁻¹: 3380, 1734, 1646, 1062; ¹H and ¹³C NMR (C₅D₅N, 400 MHz and 100 MHz) data, see Tables 1 and 2; ESI (pos.): *m*/*z* 833 [M + Na]⁺; HRESIMS (+): *m*/*z* 833.4292 [M + Na]⁺ (calcd for C₄₂H₆₆O₁₅Na⁺, 833.4299).

1.5. Sugar identification

1.5.1. Acidic hydrolysis of compounds 1-4.

Each solution of compounds **1-4** (each 3 mg) in a mixture of MeOH (1.0 mL) and 8% HCl (1.0 mL) was stirred at reflux for 4 h. The hydrolysate was allowed to cool, diluted 2-fold with H₂O, and then extracted with EtOAc (3×2 mL). The aqueous layer was neutralised with 2 M ammonium hydroxide and concentrated *in vacuo* to give a residue in which D-glucose was identified by comparison with authentic sugar sample (*n*-BuOH/AcOH/H₂O 4:1:5, upper layer; PhOH/H₂O, 4:1) on TLC (sprayed with aniline phthalate reagent, followed by heating).

1.5.2. Determination of absolute configuration of D-glucose

After being dried over P_2O_5 for 48 h, the above-mentioned aqueous residue was dissolved in anhydrous pyridine (1.0 mL), and 5 mg of L-cysteine methyl ester hydrochloride was added. The mixture was stirred at 60 °C for 2 h. The reaction mixture was then concentrated *in vacuo* to furnish a dry residue. 0.5 mL of *N*-trimethylsilylimidazole was added, and the reaction mixture was heated at 60 °C for 1 h. Subsequently, the mixture was partitioned between *n*-hexane and H₂O (1:1; v/v) and the *n*-hexane extract was directly subjected to GC analysis under the following conditions: capillary column, HP-5 (30 × 0.25 mm, with a 0.25 µm film, Dikma); detection, FID; detector temperature, 280 °C; injection temperature, 250 °C; initial temperature 160 °C, raised to 280 °C at 5 °C·min⁻¹ with the final temperature being maintained for 10 min; N₂ gas as carrier. By comparing the retention times (Rt.) of the derivatives with those of authentic sugars [D-glucose (Rt. 19.8 min), and L-glucose (Rt. 20.7 min)] prepared in a similar way, D-glucose (Rt. 19.8 min) from **1** - **4** were detected.

1.6. α-Glucosidase inhibitory assay

The α -glucosidase inhibitory activity was determined using the procedure previous reported¹⁶ in 96-well plates with acarbose (purity > 99%, purchased from Sigma) as a positive control. Briefly, each well of the plates contained 40 μ L of 2 mM 4-nitrophenyl α -D-glucopyranoside (purchased from TCI) in 100 mM potassium phosphate buffer (pH 7.0) and 5 μ L of sample in DMSO. The reaction was initiated by the addition of 5 μ L of the enzyme solution (0.30 μ units/ml from *Bacillus stearothermophilus*). The plates were incubated at 37 °C for 20 min and the absorbance was measured with a Spectra Max Plus plate reader. The increased absorbance (ΔA) was compared with that of the control to calculate the inhibition.

Inhibition (%) =
$$(\Delta A_{\text{control}} - \Delta A_{\text{sample}}) / \Delta A_{\text{control}}$$

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Supplementary data

Supplementary data (HRESIMS, ¹H, ¹³C-NMR, HSQC, HMBC, ¹H-¹H COSY, ROESY spectra of compounds **1-4**) associated with this article can be found.

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position	1 ^a	2 ^a	3 ^b	4 ^a
1	1.92-1.94 (1H, m)	1.91-1.95 (1H, m)	1.93-1.98 (1H, m)	1.92-1.97 (1H, m)
1	1.83-1.85 (1H, m)	1.81-1.84 (1H, m)	1.24-1.32 (1H, m)	1.80-1.84 (1H, m)
2	4.43-4.46 (1H, m)	4.24-4.28 (1H, m)	3.68-3.73 (1H, m)	4.41-4.47 (1H, m)
3	4.61 (1H, brs)	4.15 (1H, m)	3.31-3.35 (1H, m)	4.62 (1H, m)
5	1.75-1.81 (1H, m)	2.01-2.04 (1H, m)	1.25-1.32 (1H, m)	1.75-1.82 (1H, m)
6	1.62-1.68 (1H, m)	1.55-1.59 (1H, m)	1.42-1.50 (1H, m)	1.61-1.68 (1H, m)
6	1.38-1.42 (1H, m)	1.24-1.31 (1H, m)		1.03-1.09 (1H, m)
7	1.60-1.63 (2H, m)	1.70-1.73 (1H, m) 1.59-1.62 (1H, m)	1.62-1.66 (1H, m) 1.28-1.35 (1H, m)	1.58-1.64 (2H, m)
9	1.85-1.89 (1H, m)	1.84-1.90 (1H, m)	1.64-1.68 (1H, m)	1.82-1.90 (1H, m)
11	2.03-2.05 (2H, m)	2.01-2.03 (2H, m)	1.96-2.02 (2H, m)	1.98-2.06 (2H, m)
12	5.43 (1H, brs)	5.41 (1H, brs)	5.23 (1H, brs)	5.43 (1H, brs)
15	2.39 (1H, m)	2.38-2.41 (1H, m)	1.04-1.09 (1H, m)	1.32-1.38 (1H, m)
15	1.30-1.34 (1H, m)	1.29-1.34 (1H, m)	0.96-1.02 (1H, m)	1.21-1.24 (1H, m)
16	2.26 (1H, brd, 11.7)	2.27-2.30 (1H, m)	1 82-1 88 (2H m)	2.27-2.35 (1H, m)
10	2.07 (1H, m)	2.01-2.04 (1H, m)	1.02 1.00 (211, 11)	2.21-2.25 (1H, m)
18	2.50 (1H, d, 11.2)	2.48 (1H, d, 11.3)	2.20 (1H, d, 11.2)	2.61 (1H, d, 11.8)
19	1.34-1.42 (1H, m)	1.32-1.37 (1H, m)	1.40 (1H, m)	2.35-2.41 (1H, m)
20	0.93-1.11 (1H, m)	0.84-0.90 (1H, m)	0.93-0.97 (1H, m)	-
21	1.34-1.40 (1H, m)	1.29-1.34 (1H, m)	1.50-1.54 (1H, m)	2.28-2.34 (1H, m)
21	1.0-1.2 (1H, m)	1.10-1.23 (1H, m)	1.28-1.32 (1H, m)	2.10-2.17 (1H, m)
22	1.75-1.80 (1H, m)	1.81-1.86 (1H, m)	1.78-1.82 (1H, m)	1.81-1.83 (1H, m)
22	1.83-1.90 (1H, m)	1.68-1.72 (1H, m)	1.59-1.64 (1H, m)	1.99-2.04 (1H, m)
23	1.63 (3H.s)	3.84 (1H, d, 10.8)	3.50 (1H, d, 11.1)	1.64 (3H, s)
		3.69 (1H, d, 10.8)	3.27 (1H, d, 11.1)	
24	4.08 (1H, m)	0.77 (3H, s)	0.70 (3H, s)	4.08 (1H, d, 9.1)
	3./1 (1H, d, 10.0)			3.71 (1H, d, 10.9)
25	0.97 (3H, s)	0.95 (3H, s)	1.05 (3H, s)	0.97 (3H, s)
26	1.07 (3H, s)	1.10 (3H, s)	0.83 (3H, s)	1.06 (3H, s)

Table 1. The ¹H-NMR (400 MHz) spectral data of compounds 1-4.

	11m	al P		$\mathbf{n}\mathbf{r}$	
				<u> </u>	

27	1.08 (3H, s)	1.05 (3H, s)	1.12 (3H, s)	1.06 (3H, s)
29	0.92 (3H, d, 6.4)	0.89 (3H, d, 6.3)	0.90 (3H, d, 6.1)	4.76 (1H, brs) 4.72 (1H, brs)
30	0.88 (3H, d, 6.2)	0.85 (3H, d, 6.1)	0.97 (3H, d, 6.1)	1.05 (3H, s)
Glc-				
1'	6.16 (1H, d, 8.1)	6.18 (1H, d, 8.2)	5.42 (1H,d,8.0)	6.15 (1H, d, 8.2)
2'	4.42-4.48 (1H, m)	4.46-4.52 (1H, m)	3.78-3.83 (1H, m)	4.41-4.47 (1H, m)
3'	4.28-4.34 (1H, m)	4.27-4.33 (1H, m)	3.33-3.39 (1H, m)	4.25-4.32 (1H, m)
4'	4.18-4.28 (1H, m)	4.22-4.30(1H, m)	3.35-3.43 (1H, m)	4.15-4.21 (1H, m)
5'	3.90-3.96 (1H, m)	3.93-3.98 (1H, m)	3.31-3.65 (1H, m)	3.90-3.95 (1H, m)
6'	4.41-4.46 (1H, m)	4.42-4.45 (1H, m)	3.75-3.81 (1H, m)	4.42-4.47 (1H, m)
	4.32-4.36 (1H, m)	4.35-4.39 (1H, m)	3.65-3.70 (1H, m)	4.34-4.38 (1H, dd, 11.7, 4.4)
Glc-				
1″	5.66 (1H, d, 7.7)	5.72(1H, d, 7.8)	4.76 (1H, d, 8.0)	5.64 (1H, d, 7.7)
2″	4.06-4.14 (1H, m)	4.12-4.15 (1H, m)	3.21-3.26 (1H, m)	4.05-4.14 (1H, m)
3″	4.22-4.28 (1H, m)	4.24-4.28 (1H, m)	3.33-3.39 (1H, m)	4.21-4.27 (1H, m)
4″	4.11-4.16 (1H, m)	4.11-4.15 (1H, m)	3.11-3.17 (1H, m)	4.10-4.15 (1H, m)
5″	3.99-4.04 (1H, m)	4.02-4.06 (1H, m)	3.33-3.39 (1H, m)	3.98-4.04 (1H, m)
<i>C</i> !!	4.63 (1H, brd, 11.4)	4.69 (1H, dd, 11.6, 2.7)	3.89 (1H, dd, 11.2, 2.1)	4.62-4.64 (1H, m)
0″	4.42-4.48 (1H, m)	4.37-4.43 (1H, m)	3.61-3.65 (1H, m)	4.41-4.47 (1H, m)
δ in ppm, J	in Hz. ^a Measured in C ₅ D ₅ N.	^b Measured in CD ₃ OD.		

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Table	$2. \text{ The } {}^{13}\text{C} \text{ NMR}$	$\frac{100 \text{ MHz}}{2^{a}}$	ta of compour	$\frac{1}{4^a}$			
Position	12 2(t)	$\frac{2}{12.8(t)}$	3 <u>18 0 (t)</u>	4 (12.2(t))			
1	45.3(l)	42.0(l)	40.0(l)	43.3(l)			
2	74.1(d)	70.0(d)	79.7(d)	74.2(d)			
3	/4.1(d)	/9.0(d)	78.2(0)	/4.2(d)			
4	45.2(8)	41.8(s)	44.1(S)	45.1(8)			
5	49.5(d)	45.4(d)	48.2(d)	49.5(d)			
0	18.8(t)	18.3(t)	19.0(t)	18.8(t)			
/	34.0(t)	33.2(t)	33.6(t)	34.0 (t)			
8	40.5(s)	40.3(s)	41.0(s)	40.4(s)			
9	48.2(d)	48.1(d)	49.0(d)	48.2(d)			
10	38.5(s)	38.3(s)	38.9(s)	38.5(s)			
11	24.0(t)	23.7(t)	24.5(t)	23.9(t)			
12	125.8(d)	125.7(d)	126.8(d)	126.2(d)			
13	138.7(s)	138.7(s)	139.4(s)	138.3(s)			
14	42.5(s)	42.5(s)	43.3(s)	42.5(s)			
15	29.3(t)	29.3(t)	29.9(t)	29.2(t)			
16	24.4(t)	24.3(t)	24.8(t)	24.4(t)			
17	48.3(s)	48.3(s)	49.5(s)	48.5(s)			
18	53.4(d)	53.3(d)	54.1(d)	55.5(d)			
19	39.3(d)	39.2(d)	40.3(d)	37.5 (d)			
20	39.2(d)	39.2(d)	40.3(d)	153.4(s)			
21	30.9(t)	30.8(t)	31.7(t)	32.5(t)			
22	36.5(t)	36.4(t)	37.3(t)	38.6(t)			
23	23.8(q)	71.2(t)	66.3(t)	23.8(q)			
24	65.1(t)	17.7(q)	13.9(q)	65.1(t)			
25	17.3(q)	17.2(q)	17.7(q)	17.3(q)			
26	17.5(q)	17.6(q)	17.9(q)	17.5(q)			
27	23.8(q)	23.8(q)	24.2(q)	23.6(q)			
28	176.3(s)	176.3(s)	178.0(s)	175.7(s)			
29	17.4 (q)	17.4(q)	17.6(q)	105.1 (t)			
30	21.3(q)	21.3(q)	21.6(q)	16.4(q)			
Glc-							
1'	93.6(d)	93.6(d)	93.9(d)	93.7(d)			
2'	79.4(d)	79.2(d)	78.7(d)	79.3(d)			
3'	78.9(d)	78.9(d)	78.5(d)	78.8(d)			
4'	70.8(d)	70.7(d)	70.8(d)	70.8(d)			
5'	79.1(d)	79.0(d)	78.7 (d)	79.1(d)			
6'	62.2(t)	62.0 (t)	62.3(t)	62.2(t)			
Glc-							
1″	104.9(d)	104.9(d)	103.9(d)	104.8(d)			
2″	76.0(d)	76.1(d)	75.8(d)	76.0(d)			

3″	78.3(d)	78.3 (d)	78.1(d)	78.4(d)
4″	72.7(d)	72.6(d)	72.5(d)	72.7(d)
5″	78.3(d)	78.3(d)	77.9 (d)	78.3(d)
6″	63.7(t)	63.6(t)	63.6 (t)	63.8(t)

Table 3. The α -glucosidase inhibitory activities of compounds 1-9.

Compounds	IC ₅₀ ±RSD ($n=3$)/(μ mol·L ⁻¹)
1	2.32±1.5%
2	3.46±2.1%
3	2.66±3.0%
4	1.01±1.6%
5	4.72±2.2%
6	$0.44{\pm}2.0\%$
7	$0.75{\pm}1.8\%$
8	>20
9	>20
Acarbose	0.12±2.5%
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Figure 1. The structures of compounds 1-9.





Figure 3. Key ROESY correlations of compounds 1 and 2.

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Highlights

1.Nine glycosides were isolated from the branches and leaves of S. cochinchinensis.

2. Four new triterpene glucosides were identified by chemical methods and MS, IR, 1D and 2D NMR spectral analyses.

3. All isolates were assayed for their α -glucosidase inhibitory activities.

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