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PHENOLIC GLYCOSIDES FROM THE STEMS OF *CLERODENDRUM INERME* GAERTN. COLLECTED IN VIET NAM[#]

Tran Thi Minh[*], Nguyen Thi Minh Thuong, Nguyen Hoai Thu, Nhu Trong Nghia, Ho Khanh Toan, Nguyen Thi Viet Thanh, Tran Thu Huong

Hanoi University of Science and Technology, 1 Dai Co Viet Road, Ha Noi, Viet Nam

[*]Email: *minh.tranthi@hust.edu.vn*

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Abstract. Five phenolic glycosides, clerodenoside A (**1**), seguinoside K (**2**), cistanoside D (**3**), verbascoside (**4**), and isoverbacoside (**5**) were isolated from the ethyl acetate soluble fraction of methanolic extract obtained from the stems of *Clerodendrum inerme* growing in Viet Nam. Their structures were determined by spectroscopic analysis and comparison of spectral data with those reported in the literature. Compound **3** was isolated from this plant for the first time.

Keywords: Clerodendrum inerme, stems, phenolic glycoside, cistanoside D.

*Classification numbers:*1.1.1, 1.2.1.

1. INTRODUCTION

Clerodendrum inerme Gaertn., belonging to the Verbenaceae family, is a mangrove shrub found extensively near the seashore from the north to the south of Viet Nam [1]. This plant has been used as a folk medicine in Thailand, India, China, and Viet Nam for treating skin diseases [2]. Previous phytochemical studies have shown that it contains flavonoids [3], diterpenoids [4, 5], phenylethanoid glycosides [6, 7], megastigmane and iridoid glycosides [8]. In a preceding paper, we have reported the isolation and structural determination of andrographolide and lupeol hexacosanoate from the ethyl acetate fraction of the methanol extract of *C.inerme* leaves [9]. This paper deals with the isolation and structural elucidation of five phenolic glycosides comprising clerodenoside A (**1**), seguinoside K (**2**), cistanoside D (**3**), verbascoside (**4**), and isoverbacoside (**5**) from the ethyl acetate soluble fraction of the methanol extract of *C. inerme* stems collected in Thai Binh province. The Cistanoside D (**3**) was isolated for the first time from this plant.

2. MATERIALS AND METHODS

2.1. General experimental procedures

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NMR spectra were obtained on a Bruker Avance 500 MHz spectrometer with tetramethylsilane (TMS) as a zero internal standard. ESI-MS were measured on an ESI-LC/MS/MS-Xevo TQMS spectrometer. Silica gel 60 (0.04 - 0.063 mm, Merck), RP-18 resins (150 μm, YMC) and Sephadex LH-20 (25 - 100 μm, Sigma-Aldrich) were used for column chromatography (CC). Thin layer chromatography (TLC) was performed on Merck pre-coated TLC DC-Alufolien silica gel $60F_{254}$ and RP-$18F_{254S}$. Chromatograms were visualized under UV light or by spraying with 1 % vanillin-H_2SO_4 in methanol followed by heating at 100 ^oC for 1-2 min.

2.2. Plant materials

The stems of *C. inerme* (Verbenaceae) were collected from the semi-mangrove areas of Thai Binh province in May 2018 and identified by Prof. Tran Huy Thai, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST). A voucher specimen has been kept in the laboratory of the Organic Department, Hanoi University of Science and Technology (HUST), Viet Nam.

2.3. Extraction and isolation

The dry powdered stems (5.0 kg) of the *C.inerme* were extracted three times with 80 % aqueous methanol at 50^oC using sonicator. After evaporation of the solvent under reduced pressure, the residue (185.0 g) was suspended in water (3 L) and defatted with *n*-hexane. The aqueous layer was further extracted with ethyl acetate (EtOAc) to give EtOAc (17.0 g) and water (95.5 g) residues, after removal of the solvents.

The ethyl acetate soluble fraction (17.0 g) was subjected to CC on a silica gel eluting with a gradient solvent system of dichloromethane and methanol with increasing concentration of methanol (0–100 %) to give 10 fractions (F.1-F.10). F.7 (2.0 g) was further separated on a sephadex LH 20 column eluting with methanol to afford five *sub*-fractions (*F.7.1-F.7.5)*. *F.7.4* was purified on a RP-18 CC eluting with methanol-water (1:1.5, v/v) to yield compound **1** (51.0 mg). F.9 (3.98 g) was further separated on a sephadex LH-20 column eluting with methanol to give five *sub*-fractions *(F.9.1-F.9.5)*. *F.9.2* was chromatographed on a RP 18 column eluting with methanol-water (1:2, v/v) to give two smaller *sub*-fractions (*F.9.2.1* and *F.9.2.2*). These fractions were purified on a silica gel column eluting with dichloromethane-methanol (9:1, v/v) to yield compounds **2** (25.7 mg) and **3** (11.4 mg), respectively. Purification of *F.9.4* by RP-18 CC eluting with methanol-water (1:1.5, v/v) yielded compound **4** (62.1 mg). Compound **5** (54.4 mg) was obtained from *F.9.5* by chromatography on a RP-18 column eluting with methanol-water (1:1, v/v).

Clerodenoside A (1): Yellow amorphous powder, ESI-MS (negative): *m/z* 735 $[M-H]^-$; ^{1}H-NMR (500 MHz, CD_3OD) δ: 6.75 (1H, d, *J* = 2.0 Hz, H-2), 6.83(1H, d, *J* = 8.0 Hz, H-5), 6.71(1H, dd, *J* = 8.0, 2.0 Hz, H-6), 4.08 (1H, m, H-*α*), 3.76 (1H, m, H-*α*), 2.84 (2H, m, H-*β*), 3.83 (3H, s, 3-OMe), 4.39 (1H, d, *J* = 8.0 Hz, H-1'), 3.47 (1H, m, H-2'), 3.86 (1H, m, H-3'), 4.99 (1H, m, H-4'), 3.58 (1H, m, H-5'), 3.65 (1H, m, H-6'a), 3.55 (1H, m, H-6'b), 5.21 (1H, d, *J* = 1.5 Hz, H-1"), 5.37 (1H, m, H-2"), 3.43 (1H, m, H-3"), 4.97 (1H, m, H-4"), 3.79 (1H, m, H-5"), 1.16 (3H, d, *J* = 6.0 Hz, H-6"), 2.07 (3H, s, 2"-OAc), 1.98 (3H, s, 3"-OAc), 7.22 (1H, d, *J* = 2.0 Hz, H-2"'), 6.84 (1H, d, *J* = 8.0 Hz, H-5"'), 7.11 (1H, dd, *J* = 8.0, 2.0 Hz, H-6"'), 6.40 (1H, d, *J* = 16.0 Hz, H-*β*'), 7.70 (1H, d, *J* = 16.0 Hz, H-*γ*'), 3.91 (3H, s, 3"'-OMe); ^{13}C NMR (125

MHz, CD_3OD) δ: See Table 1.

Seguinoside K (2): white amorphous powder, ESI-MS (negative): *m/z* 583 $[M-H]^-$; ^1H NMR (500 MHz, DMSO-d_6) δ: 6.63 (1H, d, *J* = 2.5 Hz, H-2), 6.48 (1H, d, *J* = 8.5 Hz, H-5), 6.37 (1H, dd, *J* = 8.5, 2.0 Hz, H-6), 4.73 (1H, d, *J* = 7.5 Hz, H-1'), 3.45 (1H, m, H-2'), 3.43 (1H, m, H-3'), 3.30 (1H, m, H-4'), 3.14 (1H, m, H-5'), 3.69 (1H, m, H-6'a), 3.43 (1H, m, H-6'b), 5.38 (1H, d, *J* = 5.5 Hz, H-1"), 3.83 (1H, m, H-2"), 4.15 (1H, d, *J* = 9.5 Hz, H-4"a), 3.81 (1H, m, H-4"b), 4.25 (1H, d, *J* = 11.0 Hz, H-5"a), 4.20 (1H, d, *J* = 11.0 Hz, H-5"b), 7.40 (1H, d, *J* = 2.0 Hz, H-2"'), 6.82 (1H, d, *J* = 8.0 Hz, H-5"'), 7.44 (1H, dd, *J* = 8.0, 2.0 Hz, H-6"'), 3.66 (3H, s, 3-OMe), 3.79 (3H, s, 3"'-OMe); ^{13}C NMR (125 MHz, DMSO-d_6) δ: See Table 1.

Cistanoside D (3): Yellow amorphous powder, ESI-MS (positive): *m/z* 675 $[M+Na]^+$; ^1H-NMR (500 MHz, DMSO-d_6) δ: 6.68 (1H, d, *J* = 2.0 Hz, H-2), 6.82 (1H, d, *J* = 8.5 Hz, H-5), 6.63 (1H, dd, *J* = 8.5, 2.0 Hz, H-6), 3.91 (1H, m, H-α), 3.64 (1H, m, H-α), 2.76 (2H, m, H-β), 3.72 (3H, s, 3-OMe), 4.36 (1H, d, *J* = 8.0 Hz, H-1'), 3.22 (1H, m, H-2'), 3.68 (1H, m, H-3'), 4.71 (1H, m, H-4'), 3.46 (1H, m, H-5'), 3.37 (1H, m, H-6'a), 3.34 (1H, m, H-6'b), 5.03 (1H, d, *J* = 1.0 Hz, H-1"), 3.67 (1H, m, H-2"), 3.29 (1H, m, H-3"), 3.11 (1H, m, H-4"), 3.39 (1H, m, H-5"), 0.98 (3H, d, *J* = 6.0 Hz, H-6"), 7.28 (1H, d, *J* = 2.0 Hz, H-2"'), 6.79 (1H, d, *J* = 8.0 Hz, H-5"'), 7.09 (1H, dd, *J* = 8.0, 2.0 Hz, H-6"'), 6.41 (1H, d, *J* = 16.0 Hz, H-β'), 7.54 (1H, d, *J* = 16.0 Hz, H-γ'), 3.80 (3H, s, 3"'-OMe); ^{13}C NMR (125 MHz, DMSO-d_6) δ: See Table 1.

Verbascoside (4): Yellow amorphous powder, ESI-MS (positive): *m/z* 647 $[M+Na]^+$; ^1H-NMR (500 MHz, CD_3OD) δ: 6.72 (1H, d, *J* = 2.0 Hz, H-2), 6.70 (1H, d, *J* = 8.0 Hz, H-5), 6.59 (1H, dd, *J* = 8.0, 2.0 Hz, H-6), 4.07 (1H, m, H-α), 3.74 (1H, m, H-α), 2.82 (2H, t, *J* = 7.0 Hz, H-β), 4.40 (1H, d, *J* = 8.0 Hz, H-1'), 3.41 (1H, m, H-2'), 3.83 (1H, t, *J* = 9.0 Hz, H-3'), 4.94 (1H, t, *J* = 9.5 Hz, H-4'), 3.55 (1H, m, H-5'), 3.65 (1H, m, H-6'a), 3.55 (1H, m, H-6'b), 5.21 (1H, d, *J* = 2.0 Hz, H-1"), 3.93 (1H, m, H-2"), 3.60 (1H, m, H-3"), 3.30 (1H, m, H-4"), 3.60 (1H, m, H-5"), 1.12 (3H, d, *J* = 6.0 Hz, H-6"), 7.07 (1H, d, *J* = 2.0 Hz, H-2"'), 6.80 (1H, d, *J* = 8.0 Hz, H-5"'), 6.98 (1H, dd, *J* = 8.0, 2.0 Hz, H-6"'), 6.30 (1H, d, *J* = 16.0 Hz, H-β'), 7.63 (1H, d, *J* = 16.0 Hz, H-γ'); ^{13}C NMR (125 MHz, CD_3OD) δ: See Table 1.

Isoverbacoside (5): Yellow amorphous powder, ESI-MS (positive): *m/z* 647 $[M+Na]^+$; ^1H-NMR (500 MHz, CD_3OD) δ: 6.69 (1H, d, *J* = 2.0 Hz, H-2), 6.66 (1H, d, *J* = 8.0 Hz, H-5), 6.56 (1H, dd, *J* = 8.5, 2.0 Hz, H-6), 3.98 (1H, m, H-α), 3.76 (1H, m, H-α), 2.80 (2H, t, *J* = 7.0 Hz, H-β), 4.35 (1H, d, *J* = 8.0 Hz, H-1'), 3.34 (1H, m, H-2'), 3.55 (1H, m, H-3'), 4.00 (1H, m, H-4'), 3.57 (1H, m, H-5'), 4.52 (1H, m, H-6'a), 4.38 (1H, m, H-6'b), 5.20 (1H, d, *J* = 1.5 Hz, H-1"), 3.96 (1H, m, H-2"), 3.72 (1H, m, H-3"), 3.42 (1H, m, H-4"), 3.43 (1H, m, H-5"), 1.27 (3H, d, *J* = 6.0 Hz, H-6"), 7.05 (1H, d, *J* = 2.0 Hz, H-2"'), 6.80 (1H, d, *J* = 8.0 Hz, H-5"'), 6.56 (1H, dd, *J* = 8.0, 2.0 Hz, H-6"'), 6.32 (1H, d, *J* = 16.0 Hz, H-β'), 7.59 (1H, d, *J* = 16.0 Hz, H-γ'); ^{13}C NMR (125 MHz, CD_3OD) δ: See Table 1.

3. RESULTS AND DISCUSSION

The separation of ethyl acetate soluble fraction (17.0 g) of the *C. inerme* stems using chromatography methods yielded five compounds **1-5** (Figure 1). Their structures were identified by comparison of spectral data with those reported in the literature.

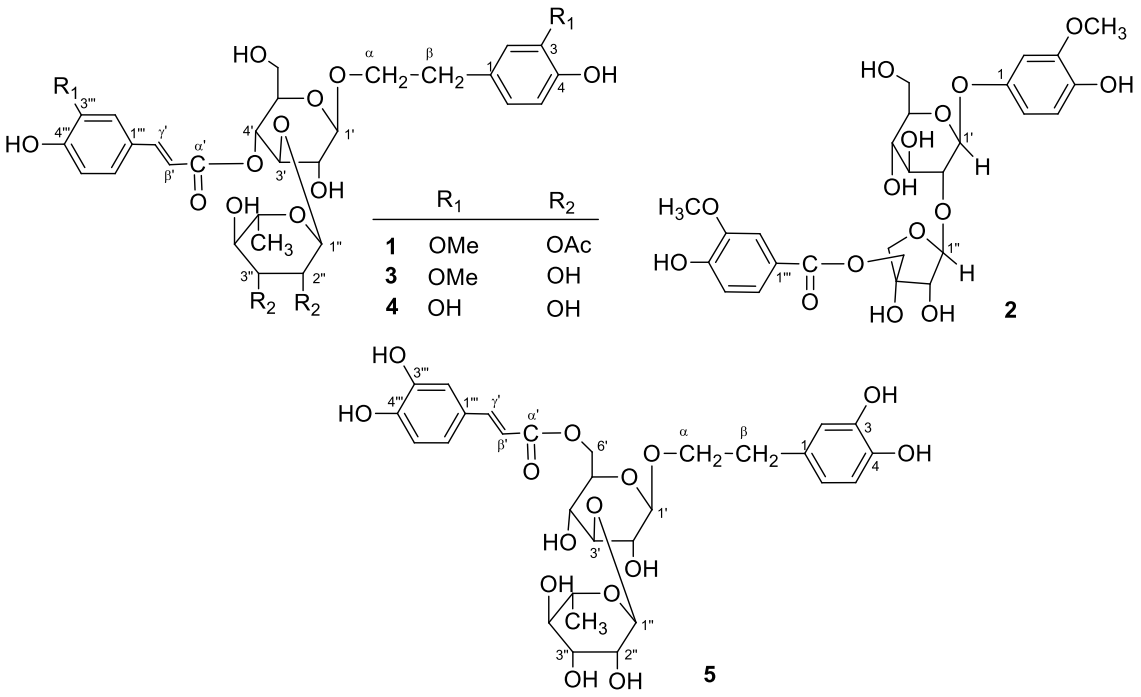

Figure 1. Structure of compounds **1** – **5**.

The compound **2** was isolated as a white amorphous powder. The molecular formula was determined as $C_{26}H_{32}O_{15}$ on the basis of the ESI-MS and NMR spectroscopic data. The ^{1}H NMR spectrum of **2** showed six aromatic proton signals of two sets of an ABX system [δ_H 6.63 (d, *J* = 2.5 Hz, H-2), 6.48 (d, *J* = 8.5 Hz, H-5), 6.37 (dd, *J* = 8.5, 2.0 Hz, H-6) and 7.40 (d, *J* = 2.0 Hz, H-2”’), 6.82 (d, *J* = 8.0 Hz, H-5”’), 7.44 (dd, *J* = 8.0, 2.0 Hz, H-6”’)], and two methoxy groups [δ_H 3.79 (s, 3”’-OMe) and 3.66 (s, 3-OMe)]. The ^{1}H NMR spectrum of **2** also indicated the presence of two sugar units with two anomeric proton signals [δ_H 4.73 (d, *J* = 7.5 Hz, H-1’)] and [δ_H 5.38 (d, *J* = 5.5 Hz, H-1”)] and other proton signals from 3.14 to 4.25 ppm. The ^{13}C NMR and DEPT spectra showed twenty six carbon signals comprising an ester carbonyl carbon at δ_C 165.3, twelve carbon signals of two aromatic rings, two methoxy signals at δ_C 55.3 and 55.6, and two sets of sugar signals with two anomeric carbon signals at δ_C 99.9 and 108.4. Two sugar units were identified as *β*-D-glucose and *β*-D-apiose by comparing the ^{1}H and ^{13}C-NMR data with those published [10]. The HMBC spectrum of **2** showed a cross peak between the anomeric proton signal of *β*-D-glucose at δ_H 4.73 and C-1 of aglycone at δ_C 150.4, indicating that the aglycone moiety (C-1) was attached to C-1’ of glucose unit via *O*-glycoside bond. The correlation peaks between the anomeric proton signal of *β*-D-apiose at δ_H 5.38 and C-2’ of D-glucose at δ_C 76.0, those between H_2-5” of apiose (δ_H 4.25 and 4.20) and the ester carbonyl carbon at δ_C 165.3 were also observed in HMBC of **2**, indicating that the apiosyl group was attached to C-2’ of glucose and the benzoyl group was attached at C-5” of apiose. The position of two methoxy groups at C-3 of aglycone moiety and C-3”’ of benzoyl moiety were indicated by the HMBC correlations of 3-OCH_3/C-3 and 3”’-OCH_3/C-3”’. Thus, by spectroscopic analysis of the compound **2** and comparison of its spectral data with those reported in the literature [11], the structure of the compound **2** was determined as seguinoside K. This compound was previously isolated from the *Myrsine seguinii* and *Millettia speciosa* [10, 11].

Table 1. ^{13}C-NMR chemical shifts of compounds **1**-**5**.

Carbon position	**1**[a]	**3**[b]	**4**[a]	**5**[a]	**2**[b]
Aglycone moiety					
1	132.9	131.0	131.5	131.4	150.4
2	117.1	116.2	117.1	117.1	102.1
3	147.4	146.0	144.7	144.6	147.7
4	147.5	146.2	146.8	146.1	141.3
5	116.5	112.3	116.3	116.3	115.0
6	121.2	119.3	121.2	121.2	107.7
α	72.1	70.0	72.2	72.4	-
β	36.5	34.9	36.5	36.6	-
3-OCH_3	56.5	55.7	-	-	55.3
Acyl moiety					
1’’’	127.6	125.5	127.7	127.1	120.4
2’’’	111.9	111.1	115.2	115.1	112.7
3’’’	149.4	147.8	146.1	146.7	147.3
4’’’	150.8	149.3	149.7	149.6	151.5
5’’’	112.9	115.5	116.5	116.5	115.0
6’’’	124.3	123.0	123.2	121.2	123.5
α’ (C=O)	168.1	165.7	168.3	169.1	165.3
β’	115.0	114.1	114.7	114.8	-
γ’	148.0	145.4	148.0	147.2	-
3’’’-OCH_3	56.4	55.6	-	-	55.6
Glucose moiety					
1’	104.2	102.2	104.2	104.4	99.9
2’	75.9	74.4	76.2	75.6	76.0
3’	82.0	79.0	81.6	84.0	76.9
4’	70.5	69.1	70.6	70.0	70.2
5’	76.0	74.5	76.0	75.4	77.0
6’	62.4	60.7	62.4	64.6	60.7
Rhamnose moiety					***Apiose***
1’’	100.3	101.1	103.0	102.7	108.4
2’’	71.4	70.5	72.3	72.3	76.7
3’’	71.1	70.3	72.0	72.2	77.4
4’’	73.2	71.6	73.8	74.0	73.9
5’’	70.4	68.7	70.4	70.4	67.1
6’’	18.4	18.0	18.4	17.8	-
2”-OAc	171.6(CO) 20.6 (CH_3)	-	-	-	
3”-OAc	172.2(CO) 20.8 (CH_3)	-	-	-	

[a] *Recorded in CD_3OD,* [b] *in DMSO-d_6*

The compound **1** was obtained as a yellow amorphous powder. The MS and NMR spectroscopic data suggested the molecular formula $C_{35}H_{44}O_{17}$. The ^{1}H NMR spectrum of **1** showed signals due to six aromatic protons of two sets of an ABX system [6.75 (1H, d, *J* = 2.0 Hz, H-2), 6.83 (1H, d, *J* = 8.0 Hz, H-5), 6.71 (1H, dd, *J* = 8.0, 2.0 Hz, H-6) and 7.22 (1H, d, *J* = 2.0 Hz, H-2’’’), 6.84 (1H, d, *J* = 8.0 Hz, H-5’’’), 7.11 (1H, dd, *J* = 8.0, 2.0 Hz, H-6’’’)], two *trans* olefinic protons (δ_H 7.70 and 6.40, *J* = 16.0 Hz), a rhamnose anomeric proton (δ_H 5.21, d, *J* = 1.5

Hz), a glucose anomeric proton (δ_H 4.39, d, J = 8.0 Hz), two acetoxyl groups (δ_H 2.07 and 1.98), two methoxy groups (δ_H 3.91 and 3.83), the protons of α-oximethylene and β-methylene of phenylethanoid moiety at (δ_H 4.08, 3.76, and 2.84), and a methyl group of rhamnose (δ_H 1.16, d, J = 6.0 Hz). All these proton signals and ^{13}C-NMR data suggested the presence of a *trans*-feruloyl moiety, a 3-methoxy-4-hydroxyphenylethanol moiety, a β-D-glucose unit and *a* α-L-rhamnose unit. The cross peaks between H-4'of glucose (δ_H 4.99) and C-α'of feruloyl (δ_C 168.1), between proton anomeric H-1" of rhamnose (δ_H 5.21) and C-3' of glucose (δ_C 82.0), between H-2" (δ_H 5.37), H-3" (δ_H 3.43) of rhamnose and two carbons of acetoxyl groups (δ_C171.6 and 172.2) were observed in the HMBC spectrum, indicating the linkage of rhamnosyl and feruloyl groups with C-3' and C-4' of the glucose moiety and the position of two acetoxyl groups at C-2" and C-3" of the rhamnose moiety. Thus, the structure of compound **1** was identified as clerodenoside A by comparison of its NMR spectral data with those published [6].

The compound **3** was isolated as a yellow amorphous powder. The molecular formula was determined as $C_{31}H_{40}O_{15}$ on the basis of the ESI-MS and NMR spectroscopic data. The ^{1}H and ^{13}C NMR data of **3** was very similar to that of phenylethanoid glycoside **1**, excepting the absence of two acetoxyl groups. On the basis of spectral analysis and comparison with the literature [12], the structure of compound **3** was determined to be cistanoside D.

The compound **4** was obtained as a yellow amorphous powder. The ESI-MS and NMR data suggested the molecular formula of this compound to be $C_{29}H_{36}O_{15}$. The NMR spectral data of **4** were very similar to those of **3**, excepting the absence of two methoxy groups. The structure of compound **4** was identified as verbascoside when compared with published data [13].

The compound **5** was isolated as a yellow amorphous powder. It gave the same molecular formula ($C_{29}H_{36}O_{15}$) as the compound **4** from ESI-MS and NMR data. The NMR spectra of **5** were very similar to those of **4**, except that the low field shifts of two carbon signals in the ^{13}C NMR spectrum, one assigned to the C-3' of glucose, shifted to δ_C 84.0 (Δ + 2.4 ppm) and the other assigned to the C-6' of glucose, shifted to δ_C 64.6 (Δ + 2.2 ppm). The correlations between H_2-6'of glucose (δ_H 4.52 and 3.38) and C-α' of caffeic acid (δ_C 169.1), and those between proton anomeric H-1" of rhamnose (δ_H 5.20) and C-3' of glucose (δ_C 84.0) were also observed in the HMBC spectrum of **5**. Thus, the structure of **5** was shown to be isoverbascoside [14], in which the rhamnosyl and caffeoyl groups are attached to C-3' and C-6' of the glucose moiety, respectively.

This is the first report on isolation of the phenolic glycoside **3** from the *C. inerme*, whereas the compounds **1, 2, 4** and **5** were previously reported from the same plant growing in Thailand and China [6, 8].

4. CONCLUSIONS

From the ethyl acetate soluble fraction of the methanol extract of the *C.inerme* stems, five phenolic glycosides including clerodenoside A (**1**), seguinoside K (**2**), cistanoside D (**3**), verbascoside (**4**), and isoverbacoside (**5**) have been isolated and characterized. The Cistanoside D (**3**) was isolated from this plant for the first time.

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CRediT authorship contribution statement. Author 1: Methodology, Formal analysis, Supervision, Authors 2-6: Formal analysis, and Author 7: Supervision.

Declaration of competing interest. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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