Journal of Multidisciplinary Engineering Science and Technology (JMEST)
ISSN: 2458-9403
Vol. 4 Issue 9, September - 2017

Steroidal saponins from *Solanum procumbens* growing in Vietnam

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***Abstract*—The *Solanum procumbens* species growing in Vietnam is a traditional plant which is commonly used as anti-inflammatory and hepatoprotective agent. The methanol extract of whole plant showed significant activities both toward cytotoxic (HepG2 and LU-1 human cancer cell lines with IC_{50} range 8.31±1.11 - 8.32±1.15 µg/mL) and hepatoprotective (EC_{50} = 8.1±0.07 µg/mL) effects . Thus, it was subjected to clarify the chemical components. In present study, three steroidal saponins were isolated including (22*R*, 23*S*, 25*R*)-3*β*,6*α*,23-trihydroxy-5*α*-spinostane 6-*O*-*β*-D-xylopyranosyl-(1→3)-*O*-*β*-D-quinovopyranoside (1), torvoside J (2), and torvoside K (3). Their chemical structures were determined by analysis NMR spectra, and as well as comparison with reported literature. This is the first chemical study of *S. procumbens* species in Vietnam.**

***Keywords*—Solanum procumbens; steroidal saponin; torvoside J; torvoside K.**

I. INTRODUCTION

The *Solanum* genus has documented around 20 species. Most of them are widely traditional plants [1]. *Solanum procumbens* species commonly used as hepatoprotective agent in Vietnam. Chemical investigations of *Solanum* species have been reported rich of steroidal saponins and flavonoids which possess broad spectrum of biological activities, especially, cytotoxic, protective effects, antifungal, and antimicrobial activity [2-8]. But there are no chemical components have been carried out yet in Vietnam.

Our present study described the isolation and structural elucidation of three steroidal saponins from *S. procumbens*. This is the first report on chemical investigation of *S. procumbens* species.

II. MATERIALS AND METHODS

A. General experiment procedures

NMR spectra were acquired on a Bruker AM500 FT-NMR spectrometer (Bruker BioSpin, Bremen, Germany) using TMS as an internal standard. Column chromatography was performed using silica gel (Merck, Whitehouse Station, NJ, USA) and reverse phase C18 resins (YMC Ltd., Kyoto, Japan). Thin layer chromatography was carried out using pre-coated silica gel 60 F_{254} (0.25 mm, Merck) and RP-C18 F_{254S} plates (0.25 mm, Merck). Spots were visualized under UV radiation (254 and 365 nm) and sprayed with aqueous solution of H_2SO_4 (10%), heating with a heat gun.

B. Plant materials

Whole plant of *Solanum procumbens* were collected at Tien Hai, Thai Binh province, Vietnam, in August 2015. Its scientific name was identified by MSc Do Thanh Tuan, Thai Binh Medical University. A voucher specimen (TB17.2015) is deposited at the Herbarium of Mientrung Institute for Scientific Research, VAST.

C. Extraction and Isolation

The dried whole plant (1.5 kg) was powdered and ultrasonically extracted in methanol (3 times, each 5L in 60 minutes). After removal of methanol *in vacuo*, a crude of methanol extract (150 g) was obtained. This extract was then suspended in 1L of distilled water and separated with *n*-hexane, dichloromethane and ethyl acetate (each 1L) to give corresponding crude extract of *n*-hexane (SPH, 14.5 g), dichloromethane (SPD, 47.5 g), ethyl acetate (SPE, 16 g) and water layer (SPW). The water layer was passed through diaion HP-20 column chromatography (CC) and eluted with increasing methanol concentration in water (0-100% volume of methanol) to give four main fractions (SPW1-SPW4). Fraction SPW2 (5.0 g) was continuously separated on a RP-18 column and eluted with acetone/water (2/1, v/v) to get four sub-fractions, SPW2A-SPW2D. Sub-fraction SPW2B was subjected on a silica gel column and dichloromethane/methanol/water (1/2.5/0.2, v/v/v) using as an eluent to obtain compound **2** (12 mg). Compound **1** (10 mg) was obtained from sub-fraction SPW2D (0.6 g) using a silica gel column and dichloromethane/methanol/water (7/1/0.05, v/v/v) as an eluent. Fraction SPW3 (2.2 g) was divided into four smaller fractions, SPW3A-SPW3D, on a RP-18 column using acetone/water (1/1.5, v/v) as eluent. Fraction SPW3B (0.3 g) was purified on a silica gel column and eluted with dichloromethane/methanol/water (3/1/0.05, v/v/v) to yield compound **3** (8.0 mg)

- (22*R*, 23*S*, 25*R*)-3*β*,6*α*,23-trihydroxy-5*α*-spinostane 6-*O*-*β*-D-xylopyranosyl-(1→3)-*O*-*β*-D-quinovopyranoside (**1**): Molecular formula: $C_{38}H_{62}O_{13}$ (M 726); White amorphous powder; ^{1}H and ^{13}C-NMR data are given in the Table I and Table II.

TABLE I. ^{1}H-NMR DATA FOR COMPOUNDS **1-3**

	1		2	3		1		2	3
No.	**$\delta_H^{a,c}$ (mult., *J* in Hz)**	**No.**	**$\delta_H^{a,c}$ (mult., *J* in Hz)**	**$\delta_H^{a,c}$ (mult., *J* in Hz)**	**No.**	**$\delta_H^{a,c}$ (mult., *J* in Hz)**	**No.**	**$\delta_H^{a,c}$ (mult., *J* in Hz)**	**$\delta_H^{a,c}$ (mult., *J* in Hz)**
1	1.06 m 1.70**	1	1.06 (dd, 3.0, 13.5) 1.71**	-	21	1.14 (d, 6.5)	21	1.12 (d, 7.5)	1.13 (d, 6.5)
2	1.42 (brd, 13.5) 1.79 m	2	1.43 (brd, 12.5) 1.78 m	-	22	-	22	-	-
3	3.50 m	3	3.46 m	3.49 m	23	3.59 (t, 8.5)	23	3.56 (t, 2.5)	3.59 (t, 8.5)
4	1.31 m 1.99 m	4	1.19** 2.40 (dd, 4.5, 11.0)	-	24	1.52** 2.09 (dt, 5.0, 11.0)	24	1.63** 1.68 brs	-
5	1.19**	5	1.18**	-	25	1.69 m	25	2.06 (m)	-
6	3.42**	6	3.40**	3.43 m	26	3.34** 3.97 (dd, 3.5, 11.0)	26	3.45** 3.50 (dd, 6.0, 11.0)	3.34** 3.98**
7	1.02 (brd, 12.0) 2.18 (dt, 4.0, 12.5)	7	0.98 (dd, 2.0, 19.0) 2.20 m	-	27	1.22 (d, 7.0)	27	0.80 (d, 6.5)	1.22 (d, 6.0)
8	1.67 m	8	1.64 (brd, 13.0)	-	1′	4.33 (d, 8.0)	1′	4.30 (d, 8.0)	4.29 (d, 8.0)
9	0.71 (3.5, 12.0)	9	0.71 (3.5, 10.5)	0.71 (2.5, 10.5)	2′	3.29**	2′	3.31 (t, 9.0)	-
10	-	10	-	-	3′	3.45 (t, 9.0)	3′	3.43**	3.44**
11	1.31** 1.55**	11	1.33** 1.57 (brd, 10.5)	-	4′	3.08 (t, 9.0)	4′	3.06 (t, 9.0)	3.04 (t, 9.0)
12	1.16** 1.79**	12	1.16** 1.76**	-	5′	3.39**	5′	3.33**	-
13	-	13	-	-	6′	1.29 (d, 6.5)	6′	1.30 (d, 6.0)	1.29 (d, 6.0)
14	1.18 m	14	1.17 m	-	1″	4.50 (d, 7.5)	1″	5.16 (d, 1.5)	5.16 (d, 1.5)
15	1.90 m 2.42 m	15	1.30** 2.04 m	-	2″	3.38**	2″	3.72 (dd, 3.5, 9.5)	3.71 (dd, 3.5, 9.5)
16	4.51 m	16	4.49 (dd, 8.0, 13.5)	4.50 (ddd, 6,0, 8,0, 16,0)	3″	3.36**	3″	3.97 brs	3.96 brs
17	1.82**	17	1.72 (dd, 5.0, 8.0)	1.72**	4″	3.50 (dd, 5.0, 9.0)	4″	3.41**	-
18	0.84 s	18	0.84 s	0.85 s	5″	3.26 (t, 10.5) 3.92 (dd, 5.5, 11.5)	5″	4.01 (dd, 6.0, 9.5)	4.00 (dd, 6.0, 9.5)
19	0.90 s	19	0.92 s	0.89 s			6″	1.27 (d, 6.0)	1.27 (d, 6.0)
20	2.38 (t, 6.5)	20		-					

Measured in [a] MeOD, [c] 500 MHz, [**] Overlapped signals

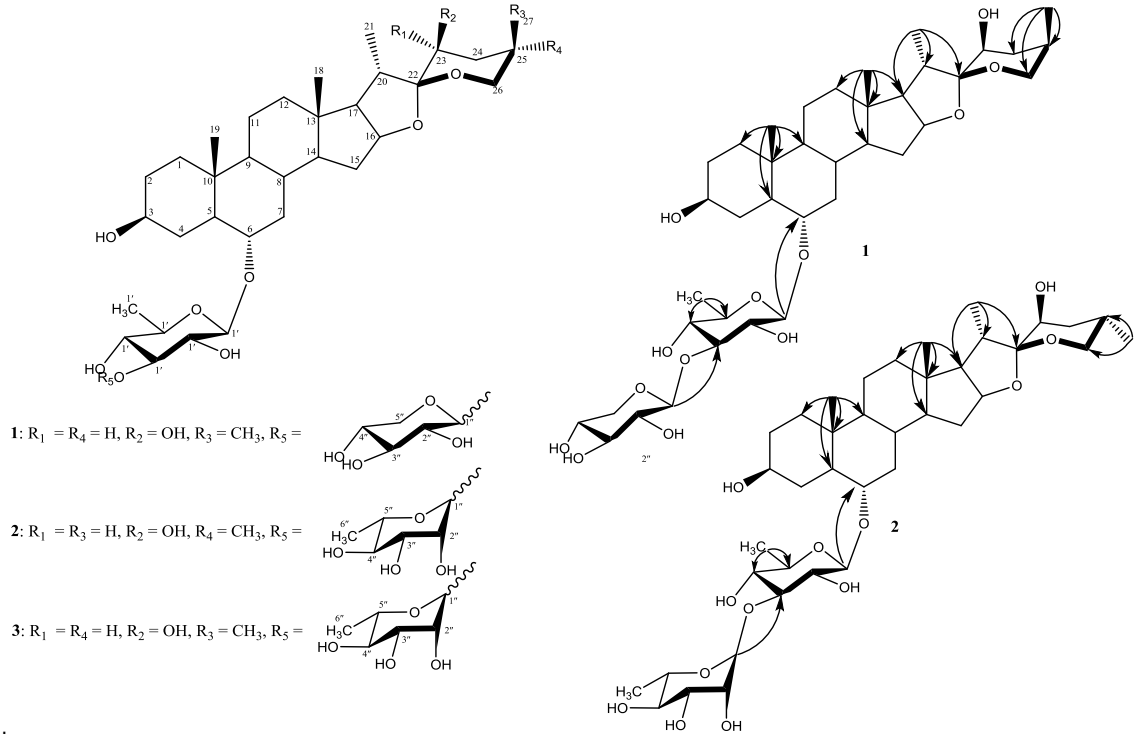

Fig. 1. *Chemical structures and key HMBC correlations of compounds **1-3***

Table II: ^{13}C-NMR DATA FOR COMPOUNDS **1-3**

	1			2		3	
C	δ_C[d]	δ_C[a,b]	C	δ_C[d]	δ_C[a,b]	δ_C[d]	δ_C[a,b]
1	37.8	38.5	1	37.8	38.5	38.5	38.5
2	32.2	31.9*	2	32.3	31.8*	31.8	31.8*
3	70.6	71.8	3	70.7	71.8	71.8	71.8
4	33.2	32.9*	4	33.2	32.6*	32.7	32.7*
5	51.4	51.8	5	51.4	51.7	51.8	51.8
6	79.5	80.4	6	79.5	80.4	80.4	80.4
7	41.1	41.6	7	41.1	41.6	41.6	41.6
8	34.5	35.3	8	34.4	35.3	35.3	35.3
9	53.9	55.1	9	54.0	55.0	55.1	55.1
10	36.8	37.6	10	36.8	37.6	37.6	37.6
11	21.2	22.0	11	21.1	22.0	22.0	22.0
12	39.9	40.9	12	39.9	40.7	40.8	40.8
13	41.2	42.1	13	41.2	42.0	42.1	42.1
14	56.5	57.5	14	56.5	57.5	57.5	57.5
15	32.4	32.7*	15	32.5	32.9*	32.9	32.9*
16	81.6	82.6	16	81.5	82.3	82.6	82.6
17	64.6	65.4	17	64.9	65.6	65.4	65.4
18	16.5	16.9	18	16.5	16.8	16.8	16.8
19	13.4	13.8	19	13.6	13.9	13.9	13.9
20	40.9	41.0	20	41.4	41.7	41.0	41.0
21	17.2	16.8	21	17.3	17.1	16.9	16.9
22	110.5	111.4	22	109.6	109.9	111.4	111.4
23	70.1	71.2	23	70.3	71.0	71.2	71.2
24	34.3	34.7	24	37.9	37.5	34.7	34.7
25	27.3	28.0	25	24.6	24.9	28.0	28.0
26	65.3	66.3	26	67.0	67.5	66.3	66.3
27	20.5	20.2	27	17.4	17.4	20.2	20.2
1′	105.3	104.9	1′	105.6	105.1	105.1	105.1
2′	74.9	75.2*	2′	76.2	76.3	76.4	76.3
3′	87.6	87.8	3′	83.7	84.4	84.4	84.4
4′	74.7	75.2*	4′	75.3	75.7	75.7	75.8
5′	72.3	72.7	5′	72.8	72.9	72.9	73.0
6′	18.6	18.3	6′	18.8	18.4	18.4	18.4
1″	106.5	106.3	1″	103.1	102.7	102.8	102.8
2″	75.4	75.1*	2″	72.7	72.2*	72.3	72.3*
3″	78.3	77.7	3″	72.8	72.3*	72.4	72.4
4″	70.9	71.0	4″	74.2	73.9	74.0	74.0
5″	67.4	67.1	5″	70.0	70.0	70.0	70.0
			6″	18.6	17.9	17.9	17.9

*Measured in [a] MeOD, [b] 125 MHz, [d] pyridine-d_5, * interchangable*;

- Torvoside J (**2**): Molecular formula: $C_{39}H_{64}O_{13}$ (M 740); White amorphous powder, ^{1}H and ^{13}C-NMR data are given in the Table I and Table II.

- Torvoside K (**3**): Molecular formula: $C_{39}H_{64}O_{13}$ (M 740); White amorphous powder; ^{1}H and ^{13}C-NMR data are given in the Table I and Table II.

III. Results and Discussion

Compound **1**, obtained as a white amorphous powder. The ^{1}H-NMR spectrum of **1** showed two signals of tertiary methyl group at δ_H 0.84 (3H, s), 0.90 (3H, s); three signals of secondary methyl group at δ_H 1.14 (3H, d, *J* = 6.5 Hz), 1.22 (3H, d, *J* = 7.0 Hz), 1,29 (3H, d, *J* = 6.5 Hz); and two anomeric signals at δ_H 4.33 (1H, d, *J* = 8.0 Hz), 4.50 (1H, d, *J* = 7.5 Hz) characterized for two glucoses with *β* orientation. The ^{13}C-NMR and DEPT spectra showed 38 carbon signals including 3 non-protonated carbons, 20 methine carbons (of these including 12 oxymethine carbons); 10 methylene carbons (of these including 2 oxymethylene carbons) and 5 methyl carbons.^{1}H-NMR and ^{13}C-NMR were assigned with the aid of HSQC analysis (Table I and II). In detail, a set of 11 carbons were charactesized for a dissacharide, *β*-D-xylopyranosyl-(1 → 3)- *β*-D-quinovopyranosyl moiety by comparing those already reported sugar residues [9]. The remaining 27 carbons were identical to (22*R*, 23*S*, 25*R*)-3*β*,6α,23-trihydroxy-5α-spinostane [7].The position of disaccharide moiety was identified with the HMBC correlations from anomeric proton (H-1′) to carbon (C-6) and from other anomeric proton (H-1″) to carbon (C-3′). Thus, chemical structure of **1** was elucidated as (22*R*, 23*S*, 25*R*)-3*β*,6α,23-trihydroxy-5α-spinostane 6-*O*-*β*-D-xylopyranosyl-(1→3)-*O*-*β*-D-quinovopyranoside [7].

Compound **2** also obtained as a white amorphous powder. The ^{1}H-NMR spectrum of **1** showed two signals of tertiary methyl group at δ_H 0.84 (3H, s), 0.92 (3H, s); four signals of secondary methyl group at δ_H 0.80 (3H, d, *J* = 6.5 Hz), 1.12 (3H, d, *J* = 7.5 Hz), 1.27 (3H, d, *J* = 6.0 Hz), 1,30 (3H, d, *J* = 6.0 Hz); and two anomeric signals at two anomeric signals at δ_H 4.30 (1H, d, *J* = 8.0 Hz), 5.16 (1H, d, *J* = 5.5 Hz) characterized for a glucose with *β* orientation and the remaining with α orientation. The ^{13}C-NMR and DEPT spectra showed 39 carbon signals including 3 non-protonated carbons, 20 methine carbons (of these including 12 oxymethine carbons); 10 methylene carbons (of these including 2 oxymethylene carbons) and 6 methyl carbons. Protons were assigned to corresponding carbons with the aid of HSQC analysis (Table I). The NMR spectra data of **2** were in good agreement to those of **1** except for the replacement of xylopyranosyl moiety by a rhamnopyranosyl moiety. The methyl signal appeared at δ_H 0.80 (H-27)/δ_C 17.4 (C-27) and a shielded methine carbon δ_C 29.4 (C-25) suggesting is to be equatorial. The NMR data of **2** were good agreement with those of 6-*O*-α-L-rhamnopyranosyl (1 → 3)-*β*-D-quinovopyranosy (22*R*,23*S*,25*S*)-3*β*-6α-23-trihydroxy-5α-spirostane (torvoside J) [10]. Consequently, compound **2** was determined as torvoside J.

Compound **3** was also isolated as white amorphous powder. The ^{13}C-NMR data for aglycone of **3** were good agreement with those of 1 while the data of disaccharide were similar to those of 2 (Table II). Based on NMR evidence, compound **3** was determined to be 6-*O*-α-L-rhamnopyranosyl (1 → 3)-*β*-D-quinovopyranosy (22*R*,23*S*,25*R*)-3*β*-6α-23-trihydroxy-5α-spirostane (torvoside K) in comparison with previous reported data [10].

In conclusion, chemical study of the *S. procumbens* was firstly carried out in Vietnam and lead to the isolation of three steroidal saponins including (22*R*, 23*S*,25*R*)-3*β*,6α,23-trihydroxy-5α-spinostane 6-*O*-*β*-D-xylopyranosyl-(1→3)-*O*-*β*-D-quinovopyranoside (1), torvoside J (2), and torvoside K (3). The chemical structures of isolated compounds were established with the aids of NMR spectroscopic technique and comparison with literature.

ACKNOWLEDGMENT

Author would like to thank MSc. Do Thanh Tuan, Thai Binh Medical University, Thai Binh province, Vietnam, for collection and taxonomic identification of the plant sample.

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