Vietnam Journal of Science and Technology ***59*** *(4) (2021) 498-506*

doi:10.15625/2525-2518/59/4/15733

CHEMICAL CONSTITUENTS FROM THE ROOTS OF *MYXOPYRUM SMILACIFOLIUM*

Nguyen Quoc Vuong*, Vu Van Chien, Nguyen Thi Hue, Pham Thi Hang, Tran Van Hieu, Nguyen Xuan Nhiem, Pham Van Cuong

Institute of Marine Biochemistry, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet Street, Cau Giay, Ha Noi, Viet Nam

*Emails: nguyenvuong@imbc.vast.vn.

Received: 6 December 2020; Accepted for publication: 5 March 2021

Abstract. *Myxopyrum smilacifolium* Blume (Oleaceae), is also called "Nhuong le kim cang" or "Sam xuyen da" in Viet Nam, is a well-known herbal medicine in tropical and subtropical regions of Asia. It was traditionally used for the treatment of cough, rheumatism, cephalalgia, notalagia and otopathy. This is the first time that it is from its roots, which were collected at Vi Xuyen district - Ha Giang province, were five compounds isolated. These compounds include two phenylethanoid glycosides, verbascoside (**1**), arenarioside (**2**); one iridoid glucoside, myxopyroside (**3**); one indole, 3-formylindole (**4**), and one furfural, 5-hydroxymethyl furfural (**5**). Their chemical structures were determined by ESI-MS, 1D-, 2D-NMR spectroscopic data analysis as well as in comparison with those reported in the literatures.

Keywords: Myxopyrum smilacifolium, iridoid glucoside, phenylethanoid glycoside.

Classification numbers: 1.1.1, 1.1.6.

1. INTRODUCTION

Myxopyrum smilacifolium (Wall.) Blume is one of the four species of *Myxopyrum* genus belonging to the family Oleaceae. Its Vietnamese name is Nhuong le kim cang [1, 5] or its local name is Sam xuyen da. *M. smilacifolium* is a large climbing shrub growing at high rock mountains (with altitude around 700 - 1000 m) of tropical and subtropical regions of Asia; it was a well-known herbal medicine traditionally used for the treatment of cough, rheumatism, cephalalgia, notalagia, and otopathy [2]. Phytochemical studies of *M. smilacifolium* showed the presence of terpenoids, flavones, anthraquinones, sugars, alkaloids, tannins and saponins [3, 4]; iridoid glucosides, myxopyroside (**3**) and its 6-O-acetyl-7-O-(E/Z)-p-methoxycinnamoyl esters (2/3) of dimethyl forsythide were also isolated from its leaf [5]. Initially investigations on biological activities of its root and leaf extracts were carried out. Experiments showed the leaves' extract exhibiting anti-inflammatory [2] and antimicrobial [3] activities, the roots' exhibiting cytotoxic effect [4]. So far, the studies on chemical constituents of its root have not been reported. However, in Viet Nam, *M. smilacifolium* is used widely among people for health promotion. Therefore, in this paper, we present the isolation and structural elucidation of compounds isolated from the roots of *M. smilacifolium*, which was collected at Vi Xuyen district - Ha Giang province. The obtained compounds include two phenylethanoid glycosides,

verbascoside (**1**) and arenarioside (**2**), one iridoid glucoside, myxopyroside (**3**), one indole, 3-formylindole (**4**), and one furfural, 5-hydroxymethyl furfural (**5**), from the roots of *M. smilacifolium.*

2. MATERIALS AND METHODS

2.1. General experimental procedures

The ^{1}H-, ^{13}C-, and 2D-NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer. Optical rotations were recorded on a JASCO P-2000 Polarimeter. HR-ESI-MS spectra were obtained using an AGILENT 6550 iFunnel Q-TOF LC/MS system. ESI-MS were measured on an Agilent 1100 Series LC/MSD Trap SL. Column chromatography (CC) was performed using a silica gel 60 (230 - 400 mesh, Merck) or RP-18 resins (30 - 50 μm, Fuji Silysia Chemical Ltd, Aichi, Japan). Thin layer chromatography (TLC) used percolated silica gel 60 F254 (Merck) and RP-18 F254S plates (Merck).

2.2. Plant material

The roots of *M. smilacifolium* (Wall.) Blume were collected at Vi Xuyen district, Ha Giang province in November 2019 and identified by Dr. Do Van Hai, Institute of Ecology and Biological Resources, VAST. A voucher specimen (DVH3692019) was deposited at the Institute of Ecology and Biological Resources, VAST.

2.3. Extraction and isolation

The dried roots of *M. smilacifolium* (5.0 kg) were pulverized then sonicated in 85 % MeOH (10 L × 4) at 50 °C (3 times × 2 h). The solvent was removed in reduced pressure to give a crude MeOH extract (500 g). The MeOH extract was suspended with water (1.5 L), then successively partitioned with *n*-hexane and ethyl acetate (EtOAc) to give *n*-hexane (MSH, 5.0 g) and EtOAc (MSE, 45.0 g) residues and a water layer (MSW).

The MSE residue was applied on a silica gel CC eluting with CH_2Cl_2/EtOAc/MeOH (4/2/1) to give five fractions (fr. MSE1–MSE5).

The fraction MSE1 (6.5 g) was chromatographed on an RP-18 column eluting with MeOH/H_2O (1/1) to give seven fractions (MSE1.1–MSE1.7). The fraction MSE1.5 (0.3 g) was chromatographed on an RP-18 column eluting with Me_2CO/H_2O (1/1.5, v/v) to give three fractions (MSE1.5.1–MSE1.5.3). The fraction MSE1.5.2 was purified further by a Sephadex LH-20 CC eluting with MeOH to give compound **1** (7.0 mg). The fraction MSE1.6 (1.3 g) was chromatographed on an RP-18 column eluting with Me_2CO/H_2O (1/1.5, v/v) to give three fractions (MSE1.6.1–MSE1.6.3). The fractions MSE1.6.2 was purified further by a Sephadex LH-20 CC eluting with MeOH to give compound **2** (19 mg).

The fraction MSE3 (10.5 g) was chromatographed on a silica gel column eluting with CH_2Cl_2/MeOH (9/1, v/v) to give three fractions (MSE3.1–MSE3.3). The fraction MSE3.2 (250 mg) was chromatographed on a silica gel column eluting with CH_2Cl_2/EtOAc/MeOH (4/2/1, v/v) to give three fractions (MSE3.2.1–MSE3.2.3). The fraction MSE3.2.2 (150 mg) was further purified on a Sephadex LH-20 CC eluting with MeOH to give compound **3** (35 mg).

The fraction MSE4 (3.5 g) was chromatographed on a silica gel column eluting with *n*-hexane/CH_2Cl_2/acetone (Me_2CO) (1/1/0.3, v/v) to give four sub-fractions (MSE4.1–MSE4.4).

The fraction MSE4.2 (0.5 g) was subjected to a silica gel CC eluting with *n*-hexane/CH_2Cl_2/Me_2CO (1/1/0.3, v/v) to give three fractions (MSE4.2.1–MSE4.2.3). The fraction MSE4.2.2 (120 mg) was chromatographed on a silica gel column eluting with CH_2Cl_2/ EtOAc (4/1, v/v) to give compound **4** (4.0 mg).

Table 1. ^{1}H, ^{13}C-NMR data of **1**, **2** and reference compounds (in CD_3OD).

C	**1**			**2**		
	δ_C [#]	δ_C [a]	δ_H [b] mult., *J* in Hz	δ_C [##]	δ_C [a]	δ_H [b] mult., *J* in Hz
Aglycone						
1	131.4	131.5	-	132.5	131.5	-
2	116.3	117.1	6.72 d, 2.0	117.3	117.2	6.74 d, 2.0
3	144.0	144.7	-	147.1	146.1	-
4	145.5	146.1	-	145.6	144.6	-
5	117.0	116.3	6.70 d, 8.0	118.1	116.4	6.71 d, 8.0
6	121.2	121.3	6.59 dd, 8.0, 2.0	122.3	121.3	6.60 dd, 8.0, 2.0
7	36.1	36.6	2.82 td, 7.0, 3.0	37.5	36.5	2.82 td, 8.0, 1.5
8	71.9	72.3	4.07 m; 3.76 m	73.3	72.4	4.06 m; 3.76 m
Caffeoyl						
1′	127.4	127.7	-	128.7	127.6	-
2′	114.5	115.2	7.07 d, 2.0	116.3	115.3	7.08 d, 2.0
3′	149.1	149.8	-	147.8	146.8	-
4′	146.2	146.9	-	150.7	149.8	-
5′	116.3	116.5	6.80 d, 8.0	117.5	116.5	6.81 d, 8.0
6′	123.1	123.2	6.98 dd, 8.0 2.0	124.2	123.3	6.98 dd, 8.0, 2.0
7′	147.8	148.0	7.61 d, 16.0	149.1	148.2	7.62 d, 16.0
8′	115.3	114.7	6.29 d, 16.0	115.7	114.6	6.33 d, 16.0
9′	168.2	168.3	-	169.3	168.4	-
Glc						
1″	103.7	104.2	4.40 d, 7.5	105.2	104.2	4.40 d, 8.0
2″	75.4	76.2	3.41 dd, 9.5, 7.5	77.1	76.1	3.42 dd, 9.5, 8.0
3″	81.5	81.6	3.84 t, 9.5	82.5	81.6	3.83 brt, 9.0
4″	70.1	70.6	4.94 t, 9.5	71.6	70.5	5.00 t, 9.5
5″	75.7	76.1	3.55 m	75.8	74.8	3.77 m
6″	62.1	62.4	3.65 dt, 10.0, 3.0; 3.56 m	70.3	69.3	3.60 m; 3.88 m
Rha						
1‴	102.6	103.0	5.21 d, 2.0	104.0	103.0	5.20 d, 1.5
2‴	71.9	72.1	3.94 dd, 3.0, 2.0	73.3	72.3	3.94 dd, 2.0, 1.5
3‴	71.9	72.4	3.60 dd, 9.5, 3.0	73.1	72.0	3.60 dd, 9.0, 3.0
4‴	73.6	73.8	3.31 brt, 9.5	74.8	73.8	3.31 brt, 9.0
5‴	70.1	70.4	3.57 m	71.4	70.4	3.58 m
6‴	18.2	18.4	1.11 d, 6.0	19.4	18.4	1.11 d, 1.5
Xyl						
1⁗				106.2	105.2	4.27 d, 7.0
2⁗				75.8	74.8	3.22 dd, 9.0, 7.0
3⁗				78.5	77.5	3.32 t, 9.0
4⁗				72.1	71.1	3.48 m
5⁗				67.8	66.8	3.17 dd, 10.0, 11.5
						3.85 brt, 11.5

[#]δ_C recorded at 22.5 MHz of verbascoside [6], [##]δ_C recorded at 125 MHz of arenarioside [8], [a]recorded at 125 MHz, [b]recorded at 500 MHz.

The fraction MSE4.3 (2.0 g) was chromatographed on a silica gel column eluting with CH_2Cl_2/MeOH (9/1, v/v) to give three fractions (MSE4.3.1–MSE4.3.3). The fraction MSE4.3.1 (120 mg) was purified further by a Sephadex LH-20 column eluting with MeOH to give compound **5** (20 mg).

Verbascoside (**1**): white solid; $[\alpha]_D^{25}$ −24.0 (*c* 0.1, MeOH); HR-ESI-MS (*m/z*): 623.1966 $[M-H]^-$ (cacld. for $C_{29}H_{35}O_{15}$, 623.1976); ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (125 MHz, CD_3OD) see Table 1.

Arenarioside (**2**): white solid; $[\alpha]_D^{25}$ −22.1 (*c* 0.1, MeOH); HR-ESI-MS (*m/z*): 791.2181 $[M+Cl]^-$ (cacld. for $C_{34}H_{44}ClO_{19}$, 791.2165); ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (125 MHz, CD_3OD) see Table 1.

Myxopyroside (**3**): white solid; $[\alpha]_D^{25}$ −10.5 (*c* 0.1, MeOH); HR-ESI-MS (*m/z*): 485.1058 $[M+Cl]^-$ (calcld. for $C_{18}H_{26}ClO_{13}$, 485.1062); ^{1}H-NMR (CD_3OD, 500 MHz) and ^{13}C-NMR (125 MHz, CD_3OD) see Table 2.

Table 2. ^{1}H, ^{I3}C-NMR data of **3** and reference compound.

Position	Myxopyroside [5]		**3**	
	$\delta_C{}^{a,c}$	$\delta_H{}^{b,c}$ mult., *J* in Hz	$\delta^{a,d}$	$\delta_H{}^{b,d}$mult., *J* in Hz
1	97.5	5.37 d, 5.2	98.0	5.23 d, 6.0
3	153.6	7.58 brd, 1.0	154.2	7.55 d, 1.0
4	110.1	-	110.4	-
5	38.0	3.09 brdd, 8.5, 6.5	39.4	3.08 brt, 8.0
6	78.9	3.98 dd, 6.5, 4.0	80.0	3.88 dd, 7.0, 4.0
7	73.3	4.41 dd, 5.5, 4.0	73.8	4.30 dd, 5.0, 4.0
8	49.4	3.14 dd, 8.5, 5.5	50.1	3.00 dd, 7.5, 5.5
9	38.9	3.02 dt, 8.5, 5.2	39.6	2.95 dt-like, 8.0, 6.0
10	174.3	-	173.5	-
11	170.7	-	170.4	-
1′	99.6	4.80 d, 8.0	100.3	4.65 d, 8.0
2′	73.3	3.32 dd, 9.5, 8.2	74.5	3.19 brt, 8.5
3′	76.4	3.54 t, 9.5	77.8	3.39 td, 9.0,1.0
4′	70.4	3.42 t, 9.5	71.5	3.29 t, 9.0
5′	77.1	3.51 m	78.3	3.33 m
6′	61.5	3.95 dd, 12.5, 6.0	62.8	3.91 dd, 11.5, 2.0
		3.77 dd, 12.5, 2.0		3.67 dd, 11.5, 5.0
10-OCH_3	53.4	3.81 s	52.5	3.75 s
11-OCH_3	52.8	3.81 s	52.2	3.77 s

^{a}125 MHz, ^{b}500 MHz, ^{c}recorded in D_2O, ^{d}recorded in CD_3OD

3-Formylindole (**4**): white solid; ESI-MS (*m/z*): 144 $[M-H]^-$; ^{1}H-NMR (CD_3OD, 500 MHz): δ_H 9.91 (1H, s, H-8), 8.18 (1H, dt, *J* = 7.5, 1.0 Hz, H-4), 8.11 (1H, s, H-2), 7.50 (1H, dt, *J* = 8.0, 1.0 Hz, H-7), 7.30 (1H, td, *J* = 7.0, 1.0 Hz, H-6), and 7.26 (1H, td, *J* = 7.5, 1.0 Hz, H-5); ^{13}C-NMR (CD_3OD, 125 MHz): δ_C 187.4 (C-8), 139.6 (C-2), 138.9 (C-7a), 125.7 (C-3a), 125.0 (C-6), 123.6 (C-5), 122.4 (C-4), 120.1 (C-3), and 113.1 (C-7).

5-Hydroxymethyl furfural (**5**): yellow oil; ESI-MS (*m/z*): 127 $[M+H]^+$; ^{1}H-NMR (CD_3OD, 500 MHz): δ_C 9.55 (1H, s, H-1), 7.40 (1H, d, *J*= 3.5 Hz, H-3), 6.60 (1H, d, *J* = 3.5 Hz, H-4) and 4.63 (2H, s, H-6); and ^{13}C-NMR (CD_3OD, 125 MHz): δ_C 179.4 (C-1), 163.2 (C-5), 153.9 (C-2), 124.8 (C-3), 110.9 (C-4), 57.6 (C-6).

3. RESULTS AND DISCUSSION

Compound **1** (Figure 1) was isolated as a white amorphous powder. Its molecular formula was determined as $C_{29}H_{36}O_{15}$ based on HR-ESI-MS ion at *m/z* 623.1966 $[M-H]^-$ (calcd. for $C_{29}H_{35}O_{15}$, 623.1976). The NMR spectrum of compound **1** (Table 1) revealed signals of a caffeoyl phenyl ethanoid glycoside [6, 7]. The 3,4-dihydroxyphenylethyl moiety includes an ABX aromatic system at 6.72 (1H, d, *J* = 2.0 Hz, H-2), 6.70 (1H, d, *J* = 8.0 Hz, H-5) and 6.59 (1H, dd, *J* = 8.0, 2.0 Hz, H-6)/117.1 (C-2), 116.3 (C-5), and 121.3 (C-6) and two methylenes at 2.82 (2H, td, *J* = 7.0, 3.0 Hz, H-7) and 4.07 (1H, m, Ha-8) and 3.76 (1H, m, Hb-8)/36.6 (C-7) and 72.3 (C-8). The caffeoyl group also includes an ABX aromatic system at 7.07 (1H, d, *J* = 2.0 Hz, H-2′), 6.98 (dd, *J* = 8.0, 2.0 Hz, H-6′) and 6.80 (d, *J* = 8.0 Hz, H-5′)/115.2 (C-2′), 123.2 (C-6′), and 116.5 (C-5′) and two olefinic protons at 7.61 (1H, d, *J* = 16.0 Hz, H-7′) and 6.29 (1H, d, *J* = 16.0 Hz, H-8′)/148.0 (C-7′) and 114.7 (C-8′). The large coupling constant between H-7′ and H-8′ (*J* = 16.0 Hz) confirmed *trans*-configuration of double bond C-7′/C-8′. Two sugar moieties, one belongs to *β*-D-glucopyranosyl unit based on signals at δ_H 4.40 (1H, d, *J* = 7.5 Hz, H-1″), 3.41 (1H, dd, *J* = 9.5, 7.5 Hz, H-2″), 3.84 (1H, t, *J* = 9.5 Hz, H-3″), 4.94 (1H, t, *J* = 9.5 Hz, H-4″), 3.55 (1H, m, H-5″), 3.65 dt, *J* = 10.0, 3.0 Hz, Ha-6″), 3.56 (1H, m, Hb-6″)/δ_C 104.2 (C-1″), 76.2 (C-2″), 81.6 (C-3″), 70.6 (C-4″), 76.1 (C-5″), and 62.4 (C-6″). The second sugar unit was identified to be *α*-L-rhamnopyranosyl moiety by signals at δ_H 5.21 (1H, d, *J* = 2.0 Hz, H-1‴), 3.94 (1H, dd, *J* = 3.0, 2.0 Hz, H-2‴), 3.60 (1H, dd, *J* = 9.5, 3.0 Hz, H-3‴), 3.31 brt, *J* = 9.5, Hz, H-4‴), 3.57 (1H, m, H-5‴), 1.11 (3H, d, *J* = 6.0 Hz, H-6‴)/ δ_C 103.0 (C-1‴), 72.1 (C-2‴), 72.4 (C-3‴), 73.8 (C-4‴), 70.4 (C-5‴), and 18.4 (C-6‴). The HMBC correlations were observed between H-7 and C-8/C-2/C-6/C-1, between H-1″ and C-8, between H-1‴ and C-3″/C-5‴/C-2‴/C-3‴, between H-4″ and C-9′ (δ_C 168.3)/C-6″/C-2″/C-5″/C-3″ revealed the *β*-D-glucopyranosyl moiety attached at C-8 of 3,4-dihydroxyphenylethyl moiety and the *α*-L-rhamnopyranosyl moiety at C-3″ and the caffeoyl at C-4″ of *β*-D-glucopyranosyl unit. From above observation, the structure of **1** was determined as verbascoside [6, 7].

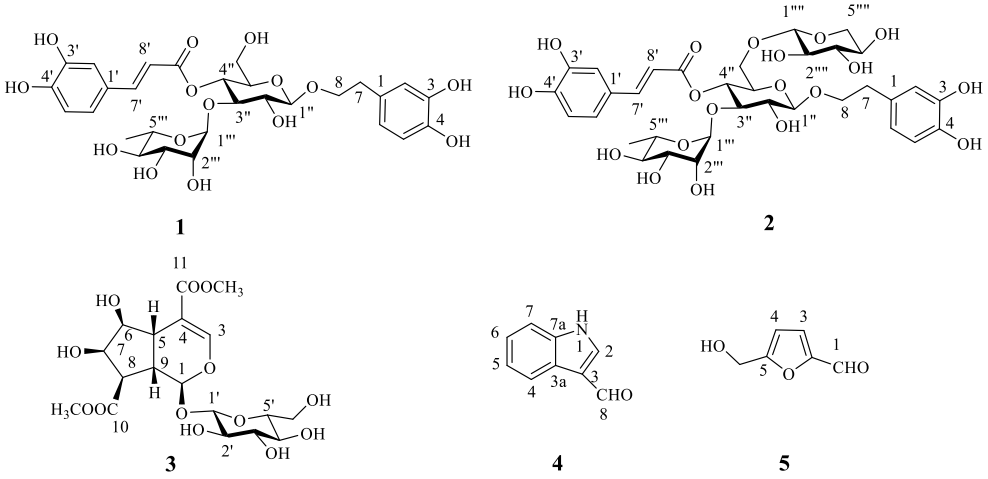

Figure 1. The chemical structures of compounds **1**–**5**.

Compound **2** was isolated as a white amorphous powder, $[\alpha]_D^{25}$ −22.1 (*c* 0.1, MeOH) and its molecular formula was determined as $C_{34}H_{44}O_{19}$ by HR-ESI-MS ion at *m/z* 791.2181 $[M+Cl]^-$ (calcd. for $C_{34}H_{44}ClO_{19}$, 791.2165). The NMR spectrum of **2** (Table 1) was similar to those of **1**

except for the addition of *β*-D-xylopyranosyl unit. The *β*-D-xylopyranosyl unit was determined by signals at δ_H 4.27 (1H, d, *J* = 7.0 Hz, H-1‴), 3.22 (1H, dd, *J* = 9.0, 7.0 Hz, H-2‴), 3.32 (1H, t, *J* = 9.0 Hz, H-3‴), 3.48 (1H, m, H-4‴), 3.17 (dd, *J* = 10.0, 11.5 Hz, H_a-5‴) and 3.85 (1H, brt, *J* = 11.5 Hz, H_b-5‴)/δ_C 105.2 (C-1‴), 74.8 (C-2‴), 77.5 (C-3‴), 71.1 (C-4‴), and 66.8 (C-5‴). The large coupling constant of H-1‴ and H-2‴ (*J* = 7.0 Hz) confirmed *β*-configuration of xylose. The COSY correlations were indicated between H-1‴/H-2‴/H-3‴/H-4‴/H-5‴/H-6‴; and the HMBC correlations between H-1‴ (δ_H 4.27) and C-6″ (δ_C 69.3) confirmed the position of *β*-D-xylopyranosyl unit at C-6″. From above evidence, the structure of **2** was determined as arenarioside [8].

Compound **3** was isolated as a white amorphous powder, its molecular formula was determined as $C_{18}H_{26}O_{13}$ (M = 450.1373) by HR-ESI-MS ion at *m/z* 485.1058 $[M+Cl]^-$ (calcd. for $C_{18}H_{26}O_{13}Cl^-$, 485.1062). The NMR spectrum of compound **3** revealed signals of an iridoid and one *β*-D-glucopyranosyl moiety with the typical of one anomeric proton at δ_H 4.65 (1H, d, *J* = 8.0 Hz, H-1′) on the ^{1}H-NMR spectrum and six typical signals at δ_C 100.3 (C-1′), 74.5 (C-2′), 77.8 (C-3′), 71.5 (C-4′), 78.3 (C-5′), 62.8 (C-6′) on the ^{13}C-NMR spectrum. The remaining signals were assigned to the aglycone to be a dihydroxy-substituted iridoid forsythide dimethyl ester [9]. The structure of this aglycone includes two carboxyl methoxy groups at δ_H 3.75 (3H, s, 10-OC$\underline{H_3}$)/δ_C 52.5 (10-O$\underline{C}$H$_3$) and 173.5 (C-10), and 3.77 (3H, s, 11-OC$\underline{H_3}$)/δ_C 52.2 (11-O$\underline{C}$H$_3$) and 170.4 (C-11); one dioxymethine at δ_H 5.23 (1H, d, *J* = 6.0 Hz, H-1)/δ_C 98.0 (C-1); one tri-substituted double bond at δ_H 7.55 (1H, d, *J* = 1.0 Hz, H-3)/δ_C 154.2 (C-3) and 110.4 (C-4); three methines at δ_H 3.08 (1H, brt, *J* = 8.0 Hz, H-5)/δ_C 39.4 (C-5), 3.00 (1H, dd, *J* = 7.5, 5.5 Hz, H-8)/δ_C 50.1 (C-8) and 2.95 (1H, like dt, *J* = 8.0, 6.0 Hz, H-9)/δ_C 39.6 (C-9); and two oxymethine at δ_H 3.88 (1H, dd, *J* = 7.0, 4.0 Hz, H-6)/δ_C 80.0 (C-6) and 4.30 (1H, dd, *J* = 5.0, 4.0 Hz, H-7)/73.8 (C-7). The COSY correlations (Figure 2) include H-1′/H-2′, H-2′/H-3′, H-3′/ H-4′, H-4′/H-5′ and H-5′/H-6′ of glucosyl moiety; and H-5/H-6, H-6/H-7, H-7/H-8, H-8/H-9, H-9/H-1 and H-5/H-9 of aglycone moiety.

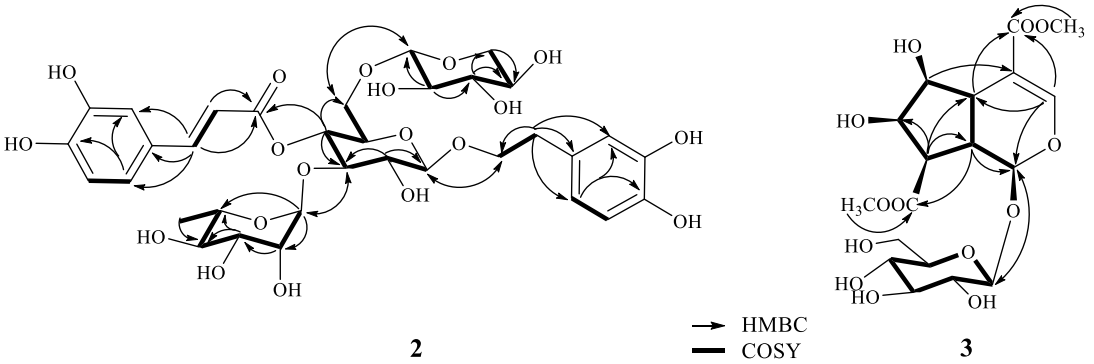

Figure 2. The key COSY and HMBC correlations of **2** and **3**.

The HMBC correlations between 11-OCH$_3$ and C-11; 10-OCH$_3$ and C-10; between H-1 and C-9/C-5/C-8/C-1′/C-3; between H-3 and C-5/C-1/C-4/C-11; between H-5 and C-9/C-8/C-1/C-4/C-3/C-11; between H-6 and C-4/C-7/C-5/C-9; between H-7 and C-5/C-9; between H-8 and C-5/C-9/C-7/C-1/C-10; between H-9 and C-5/C-8/C-7/C-1/C-4/C-10 revealed the forsythide dimethyl ester structure of aglycone moiety [4, 9, 10]. Additionally, the NOESY correlations between H-1 and H-6/H-8, between H-6 and H-8, and between H-7 and H-8/H-6 confirmed H-1, H-6, H-7, H-8 were at α-face of molecule due to an assumption of the usual iridoids displayed *cis-β* configuration at H-5 and H-9. The HMBC correlations between H-1′ and C-1 confirmed

the position of β-D-glucopyranosyl moiety at C-1 of iridoid. From above ESI-MS, 1D and 2D-NMR spectroscopic data analysis and comparisons of their spectral data with those reported in literature, compound **3** was identified as myxopyroside. This iridoid glucoside was isolated from a single leaf of herbarium sheets of *M. smilacifolium* collected in Thailand [5].

Compounds **4** and **5** were identified as 3-formylindole and 5-hydroxymethyl furfural, respectively by analysis of their ESI-MS, ^{1}H-, ^{13}C-NMR spectroscopic data analysis and comparison of the spectral data with those in literatures [11, 12].

Two caffeoyl phenylethanoid glycosides, verbascoside (**1**) and arenarioside (**2**), were well-known for possessing many significant bioactivities as antioxidant, anti-inflammatory, hepatoprotective, immunoregulation, and neuroprotective effects [13, 14]. Recently, both compounds have been reported to exhibit xanthine oxidase inhibitory activity with IC_{50} values of 115.1 ± 0.3 (for **1**), and 130.0 ± 2.2 μM (for **2**) [15]. In *in vivo* study on anti-inflammatory activity using the dextran sulfate sodium (DSS)-induced colitis model, in acute colitis, the histological score was 3.2 with verbascoside versus 5.2 with phosphate-buffered saline (PBS) ($P < 0.02$); and in chronic colitis, both 120 mg (3.3 versus 5.2) or 600 mg verbascoside (3.0 versus 5.2), significantly ameliorated colitis (both $P < 0.02$). Furthermore, the stimulated mesenteric lymph nodes from mice with chronic DSS-induced colitis treated with verbascoside showed a significant down-regulation of IFN-g secretion (195 pg/ml with 600 mg verbascoside versus 612 pg/ml with PBS, $P < 0.02$). The results showed that verbascoside reduced mucosal tissue damage in DSS colitis and could be a therapeutic alternative for inflammatory bowel disease treatment [16]. So far, bioactivities of myxopyroside (**3**) still have not been reported but iridoid metabolites have been evaluated to have neuroprotective, hepatoprotective, anti-inflammatory, antitumor, hypoglycemic, and hypolipidemic activities [17]. Two remain compounds, indole **4** was reported to exhibit antifungal activity [18] and fufural **5** was reported as a flavoring agent for food products [19].

4. CONCLUSIONS

From the roots of *Myxopyrum smilacifolium* (Wall.) Blume, five compounds including two phenylethanoid glycosides, verbascoside (**1**) and arenarioside (**2**), one iridoid glucoside, myxopyroside (**3**), one indole, 3-formylindole (**4**), one furfural, 5-hydroxymethyl furfural (**5**), were isolated. Their structures were elucidated by spectroscopic analysis including MS, 1D, 2D-NMR spectra, physical properties as well as by the comparison with reported data in literatures. This is the first study on the chemical constituents from the roots of *M. smilacifolium* in Viet Nam. In which compounds **1**, **2**, **4**, and **5** were reported from this plant for the first time.

Acknowledgements. The authors are grateful to Vietnam Academy of Science and Technology for financial support under grant number *NVCC38.05/20-20,* and Institute of Marine Biochemistry for financial support under grant number *HSB20-TN01*.

CRediT authorship contribution statement: Isolation, Nguyen Quoc Vuong, Vu Van Chien, Nguyen Thi Hue, Pham Thi Hang and Tran Van Hieu; research idea, Nguyen Quoc Vuong, Pham Van Cuong; Structure elucidation, Nguyen Quoc Vuong, Nguyen Xuan Nhiem, Pham Van Cuong; Writing, Nguyen Quoc Vuong, Nguyen Xuan Nhiem, Pham Van Cuong.

Declaration of competing interest: All authors have read and agreed to the published version of the manuscript.

REFERENCES

1. Chi V. V. - Dictionary of Vietnamese medicinal plants, Medicine Publishing House, Ha Noi, 2012, **2**, 369 (in Vietnamese).

2. Raveesha P. and Chandrasekhar K. B. - Pharmacognostical Investigation and Preliminary Phytochemical Screening of Leaves of Myxopyrum Smilacifolium B., *Pharmacognosy Journal* (2016), 8(2), 159-164.

3. Sudharmini D. and Ashalatha S. N. - Antimicrobial studies of triterpenoid fractions from *Myxopyrum smilacifolium* Blume, Ethnobotanical Leaflets **12** (2008) 912-915.

4. Praveen R. P., Ashalatha S N. - Study on cytotoxic effect of root and callus extracts of *Myxopyrum smilacifolium* Blume, International Journal of Pharmaceutical Sciences and Drug Research **7** (4) (2015) 345-348.

5. Franzyk H., Jensen S. R., Olsen C. E. - Iridoid glucosides from *Myxopyrum smilacifolium*, Journal of Natural Products **64** (2001) 632-633.

6. Miyase T., Koizumi A., Ueno. A., Noro T., Kuroyanagi M., Fukushima S., Akiyama Y. and Takemoto T. - Studies on the acyl glycosides from *Leucoseptrum japonicum* (MiQ.) Kitamura et Murata, Chemical of Pharmaceutical Bulletin **30** (1982) 2132-2137.

7. Andary C., Wylde R., Laffitte C., Privat G. and Winternitz F. - Structures of verbascoside and orobanchside, caffeic sugar esters from *Orobanche rapum-genistae*, Phytochemistry **21** (5) (1982) 1123-1127.

8. Rothenburgera J. and Haslinger E. - Caffeic acid glycoside esters from *Gratiola officinalis* L., Liebigs Ann. Chem. **1994** (1994) 1113 - 1115.

9. Damtoft S., Franzyk H., Jensen, S. R. Biosynthesis of iridoids in *Forsythia s*pp., Phytochemistry **37** (1994) 173-178.

10. Damtoft, S., Franzyk H., Jensen S. R. - Iridoid glucosides from *Picconia excelsa*, Phytochemistry **45** (1997) 743-750.

11. Luis S. M., Gómez J. F., Spadafora C., Guzmán H. M. and Gutiérrez M. - Antitrypanosomal alkaloids from the marine bacterium *Bacillus pumilus*, Molecules **17** (2012) 11146-11155.

12. Miyazawa M., Anzai J., Fujioka J. & Isikawa Y. - Insecticidal Compounds against *Drosophila melanogaster* from *Cornus officinalis* Sieb. Et Zucc., Natural Product Research **17** (5) (2003) 337-339.

13. Xue Z. and Yang B. - Phenylethanoid glycosides: Research advances in their phytochemistry, pharmacological activity and pharmacokinetics, Molecules **21** (2016) 991-1216.

14. Tai B. H., Jung B. Y., Cuong N. M., Linh P. T., Tung N. H., Nhiem N. X., Hương T. T., Anh N. T., Kim J. A., Kim S. K., and Kim Y. H. - Total peroxynitrite scavenging capacity of phenylethanoid and flavonoid glycosides from the flowers *of Buddleja officinalis*, Biol. Pharm. Bull. **32**(12) (2009) 1952-1956.

15. Cheng L. C., Murugaiyah V. and Chan K. N. - *In vitro* xanthine oxidase inhibitory studies of lippia nodiflora and isolated flavonoids and phenylethanoid glycosides as potential uric acid-lowering agents, Natural Product Communications **10** (6) (2015) 945-948.

16. Hausmann M, Obermeier F., Paper D. H., Balan K., Dunger N., Menzel K., Falk W., Schoelmerich J., Herfarth H. and Rogler G. - *In vivo* treatment with the herbal

phenylethanoid acteoside ameliorates intestinal inflammation in dextran sulfate sodium-induced colitis, Clinical and Experimental Immunology **148** (2007) 373-381.

17. Wang C. C., Gong X., Bo A., Zhang L., Zhang M., Zang E., Zhang C., and Li M. - Iridoids: Research advances in their phytochemistry, biological activities, and pharmacokinetics, Molecules **25** (2020) 287-310.
18. Brucker R. M., Harris R. N., Schwantes C. R., Gallaher T. N., Flaherty D. C., Lam B. A., Minbiole K. P. C. - Amphibian chemical defense: antifungal metabolites of the microsymbiont *Janthinobacterium lividum* on the salamander *Plethodon cinereus*, Journal of Chemical Ecology **34**(11) (2008) 1422-1429.
19. Lewkowski J. - Synthesis, chemistry and applications of 5-hydroxymethylfurfural and its derivatives, Arkivoc 2001 (i) 17-54.