Iridoid glycosides from the aerial parts of *Buddleja asiatica* Lour.

Truong Thi Thu Hien[1*], Nguyen Thanh Chung[1], Tran Hong Quang[2], Le Canh Viet Cuong[3], Nguyen Thi Thu Hien[4], Vu Kim Thu[4], Nguyen Xuan Nhiem[2], Phan Van Kiem[2]

[1]*Vietnam Military Medical University, 160 Phung Hung, Phuc La, Ha Dong, Hanoi, Viet Nam*

[2]*Institute of Marine Biochemistry, VAST, 18 Hoang Quoc Viet, Cau Giay, Hanoi, Viet Nam*

[3]*Mientrung Institute for Scientific Research, 321 Huynh Thuc Khang Street, Hue City, Viet Nam*

[4]*Hanoi University of Mining and Geology, Tu Liem, Hanoi, Viet Nam*

Received May 15, 2019; Accepted for publication August 2, 2019

Abstract

From the methanol extract of aerial parts of *Buddleja asiatica* five iridoid glycosides, litanthosalin (**1**), eurostoside (**2**), specioside (**3**), minecoside (**4**), and 6-*O*-caffeoylajugol (**5**) were isolated. Their chemical structures were identified based on 1D and 2D NMR spectroscopic analysis and comparison with the data reported in the literature. This is the first report of compounds **2**, **4**, and **5** from the genus *Buddleja*.

Keywords. *Buddleja asiatica*, Buddlejaceae, iridoid glycoside.

1. INTRODUCTION

Iridoids are cyclopenta[c] pyran monoterpenoid and present in almost of *Buddleja* species. They are active components in many medicinal plants, which were reported having valuable biological activities such as neuroprotective, hepatoprotective, cytotoxic, anti-inflammatory, and antioxidant activity, increasing immunity, reducing cholesterol, triglyceride, and glucose level in blood.[1] Some of iridoids isolated from *Buddleja* species such as aucubin, catalpol, methylcatalpol, ajugol, 6-*O*-vanilloyl ajugol, buddlejoside A4, biridoside, methylscutelloside exhibited interesting biological activities.[2-5]

In the present study, we describe the isolation and structural elucidation of five iridoid glycosides from the methanol extract of the aerial parts of *B. asiatica* including litanthosalin (**1**), eurostoside (**2**), specioside (**3**), minecoside (**4**), and 6-*O*-caffeoylajugol (**5**).

2. MATERIAL AND METHODS

2.1. Plant material

The aerial parts of *Buddleja asiatica* Lour. were collected in Sapa, Lao Cai province, Vietnam during September 2015. The scientific name was identified by Dr. Bui Van Thanh, Institute of Ecology and Biological Resources. A voucher specimen (NCCT- P03) was deposited at the Institute of Marine Biochemistry, VAST.

2.2. General experimental procedures

NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (500 MHz for ^{1}H-NMR and 125 MHz for ^{13}C-NMR). ESI mass spectra were recorded on an Agilent 6530 Accurate-Mass Q-TOF LC/MS system. Column chromatography was performed using a silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, Merck) or RP-18 resins (150 µm, Fuji Silysia Chemical Ltd.), thin layer chromatography (TLC) using a pre-coated silica-gel 60 F_{254} (0.25 mm, Merck) and RP-18 F_{254S} plates (0.25 mm, Merck).

2.3. Extraction and isolation

The dried and ground aerial parts of *B. asiatica* (1.9 kg) were extracted with MeOH (5L x 3 times) at room temperature for five days to give a MeOH extract. After concentration, a residue of the MeOH extract (BA, 140 g) was suspended in water (3.0 L) and partitioned with hexane, CH_2Cl_2, and EtOAc to give the hexane, CH_2Cl_2, EtOAc extracts, and water layer. The EtOAc fraction (BAE) was chromatographed on a silica gel column eluting with a stepwise gradient of MeOH/CH_2Cl_2 (1/10, 2/10, 5/10, 10/10, v/v) to get four subfractions (BAE1-BAE4). The BAE2 fraction was separated on a silica gel column, using CH_2Cl_2/MeOH/H_2O (5/1/0.1, v/v/v) as eluent to give

five subfractions (BAE2.1-BAE2.5). BAE2.1 was purified by silica gel column eluting with EtOAc-MeOH-H_2O (8/1/0.1, v/v/v) to obtain **1** (15 mg). BAE2.2 was chromatographed on a silica gel column eluting with EtOAc/MeOH/H_2O (20/1/0.1, v/v/v), and further purified by using reversed phase (RP) C18 column, eluting with ACN-H_2O (1/2, v/v) to give **2** (34 mg). BAE2.3 was chromatographed on a silica gel column eluting with CH_2Cl_2/MeOH/H_2O (5/1/0.1, v/v/v), and further purified by RP C18 column, using ACN-H_2O (1/2, v/v) as eluent to yield **3** (7 mg) and **4** (15 mg). The water layer was subjected to a Diaion HP-20 eluting with a stepwise gradient of MeOH 25 %, 50 %, 75 %, and 100 % in H_2O to give four fractions (BAW1 - BAW4). BAW2 was separated on a silica gel column, eluting with a stepwise gradient of MeOH (10-100 %) in CH_2Cl_2 to afford five subfractions (BAW2.1 – BAW2.5). Compound **5** (9 mg) was isolated from BAW2.3 by using a silica gel column and eluting with CH_2Cl_2/MeOH (7/1, v/v).

Lytanthosalin (**1**): White crystals, m.p. 98-100 ^oC; $[\alpha]_D^{25}$ -125 (c = 0.1, MeOH); ESI-MS *m/z* 477 $[M+H]^+$, $C_{24}H_{28}O_{10}$, M = 476; ^1H- and ^{13}C-NMR: see Table 1.

Eurostoside (**2**): White amorphous powder, m. p. 112-114 ^oC; $[\alpha]_D^{25}$ -128 (c = 0.1, MeOH); ESI-MS *m/z* 493 $[M+H]^+$, $C_{24}H_{28}O_{11}$, M = 492; ^1H- and ^{13}C-NMR: see table 1.

Specioside (**3**): White amorphous powder; m. p. 242-244 ^oC; $[\alpha]_D^{25}$ -205 (c = 0.1, MeOH); ESI-MS *m/z* 509 $[M+H]^+$, $C_{24}H_{28}O_{12}$, M = 508; ^1H- and ^{13}C-NMR: see table 2.

Minecoside (**4**): White crystals; m. p. 142-143 ^oC; $[\alpha]_D^{25}$ -192 (c = 0.1, MeOH); ESI-MS *m/z* 539 $[M+H]^+$, $C_{25}H_{30}O_{13}$, M = 538; ^1H- and ^{13}C-NMR: see Table 2.

6-*O*-caffeoylajugol (**5**): White amorphous powder, m. p. 153-155 ^oC; $[\alpha]_D^{25}$ -122 (c = 0.1, MeOH); ESI-MS *m/z* 511 $[M+H]^+$, $C_{24}H_{30}O_{12}$, M = 510; ^1H- and ^{13}C-NMR, see table 2.

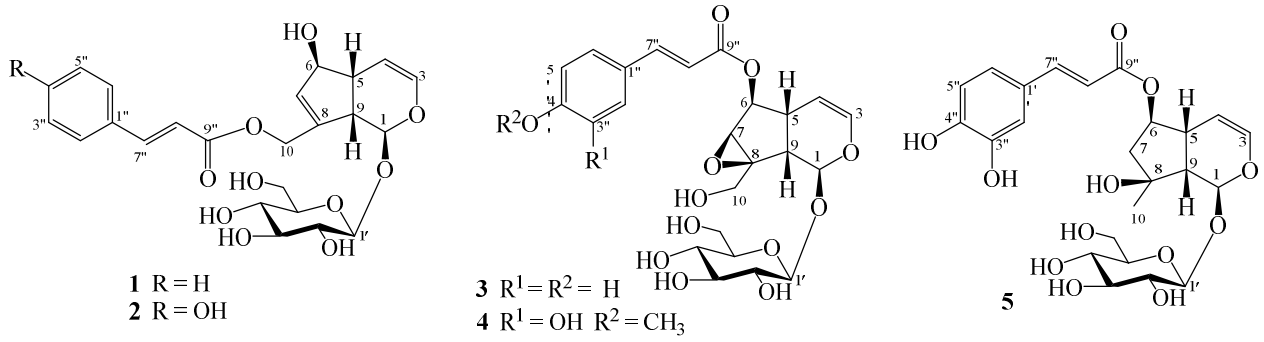

Figure 1: Chemical structures of compounds **1-5** from *B. asiatica*.

3. RESULTS AND DISCUSSION

Compound **1** was obtained as a white crystal. The ^1H-NMR spectrum of **1** showed the signals of two olefin protons at δ_H 6.60 (1H, d, J = 16.0 Hz), and 7.76 (1H, d, J = 16.0 Hz) which were recognized as *trans*-configured double bond by the large coupling value (J = 16 Hz); one phenyl ring at δ_H 7.43 (3H, m), and 7.64 (2H, m); a *cis*-configured double bond a δ_H 5.15 (1H, dd, J = 4.0, 6.0 Hz) and δ_H 6.37 (1H, dd, J = 1.5, 6.0 Hz) and one anomeric proton signal at δ_H 4.73 (1H, d, J = 8.0 Hz), revealing that **1** has a monosaccharide unit in the structure. The ^{13}C-NMR and HSQC spectra showed the the signals of 23 carbons, including three non-protonated carbons at δ_C 135.7, 142.6, and 168.3; eighteen methines at δ_C 46.3, 48.4, 71.5, 74.9, 77.9, 78.2, 82.9, 98.0, 100.2, 105.5, 118.6, 129.3 (x2), 130.0 (x2), 131.6, 132.7, 141.7, and 146.7; and two methylenes at δ_C 62.8, and 63.3. Based on this analysis and comparison of the ^1H- and ^{13}C-NMR data of **1** with those of the reported compounds in the literature, the structure of **1** was suggested to be an iridoid glycoside associated with a *trans*-cinnamoyl unit. The assignment of all the positions was done by detailed analysis of HSQC, and HMBC spectra. The location of the β-glucose at the C-1 position were deduced by HMBC correlation from δ_H 4.73 (H-1′) to δ_C 98.0 (C-1). The *trans*-cinnamoyl unit was located at C-10 as revealed based on an HMBC correlation from δ_H 4.90/5.04 (H-10) to δ_C 168.3 (C-9″). The relative configuration of **1** was determined by NOESY spectroscopic analysis. In the NOESY spectrum, NOE correlation between H-5 and H-9 revealed that H-5 and H-9 are located on the same face of the molecule. H-1 showed NOE cross-peak with H-6 but not with H-5, suggesting that H-1 and H-6 are α-oriented. Finally, the structure of **1** was established as lytanthosalin.[6]

The NMR spectra of **2** were almost similar to the corresponding spectra of **1**, except for the replacement of *trans*-cinnamoyl unit with *trans*-coumaroyl unit. Comparing the NMR data of **2** with those of **1** and acuminatuside[7] (acuminatuside has OH at C-4″ and OCH_3 at C-3″) identified that **2** to be

eurostoside, which was further confirmed by HSQC and HMBC spectra. To the best of our knowledge, this is the first report of the NMR data of eurostoside (table 1).

Table 1: ^{1}H-NMR and ^{13}C-NMR data for compounds **1** and **2**

C	1			2		
	δ_C [#, a]	δ_C [a]	δ_H [a] (mult., *J* = Hz)	δ_C [$, b]	δ_C [a]	δ_H [a] (mult., *J* = Hz)
1	97.8	98.0	4.99 (d, 7.5)	98.0	98.0	4.99 (d, 7.5)
3	141.5	141.7	6.37 (dd, 1.5, 6.0)	141.8	141.7	6.36 (d, 6.0)
4	105.4	105.5	5.15 (dd, 4.0, 6.0)	105.5	105.5	5.14 (dd, 4.0, 6.0)
5	46.0	46.3	2.72 (m)	46.4	46.3	2.71 (m)
6	82.6	82.9	4.49 (br d, 4.0)	82.9	82.9	4.49 (br s)
7	132.4	132.7	5.85 (br s)	132.5	132.5	5.83 (br s)
8	142.3	142.6	-	142.8	142.8	-
9	48.2	48.4	3.00 (t, 7.5)	48.5	48.5	2.98 (t, 7.5)
10	63.5	63.6	5.04 (d, 14.5)	63.4	63.4	5.02 (d, 15.0)
			4.90 (d, 14.5)			4.85 (d, 15.0)
1′	100.0	100.2	4.73 (d, 8.0)	100.2	100.2	4.72 (d, 8.0)
2′	74.4	74.9	3.28 (t, 9.0)	74.9	74.9	3.26 (m)
3′	77.9	78.2	3.32 (m)	78.0	78.0	3.40 (m)
4′	71.2	71.5	3.35 (m)	71.6	71.5	3.33 (m)
5′	77.6	77.9	3.41 (t, 9.0)	78.3	78.3	3.30 (m)
6′	62.6	62.8	3.89 (dd, 2.0, 12.0)	62.8	62.8	3.88 (brd, 12.0)
			3.69 (dd, 5.0, 12.0)			3.68 (dd, 5.0, 12.0)
1″	135.4	135.7	-	126.9	127.1	-
2″	129.9	129.3	7.64 (m)	111.7	131.3	7.50 (d, 8.5)
3″	129.2	130.0	7.43 (m)	151.4	116.9	6.83 (d, 8.5)
4″	131.5	131.6	7.43 (m)	149.6	161.4	-
5″	129.2	130.0	7.43 (m)	116.6	116.9	6.83 (d, 8.5)
6″	129.9	129.3	7.64 (m)	124.4	131.3	7.50 (d, 8.5)
7″	146.6	146.7	7.76 (d, 16.0)	147.4	147.0	7.68 (d, 16.0)
8″	118.4	118.6	6.60 (d, 16.0)	114.9	114.9	6.40 (d, 16.0)
9″	168.1	168.3	-	168.9	168.9	-

[a]Recorded in CD_3OD, [b]pyridine-d_5, [#] δ_C of lytanthosalin, [6] [$] δ_C of acuminatuside [7]

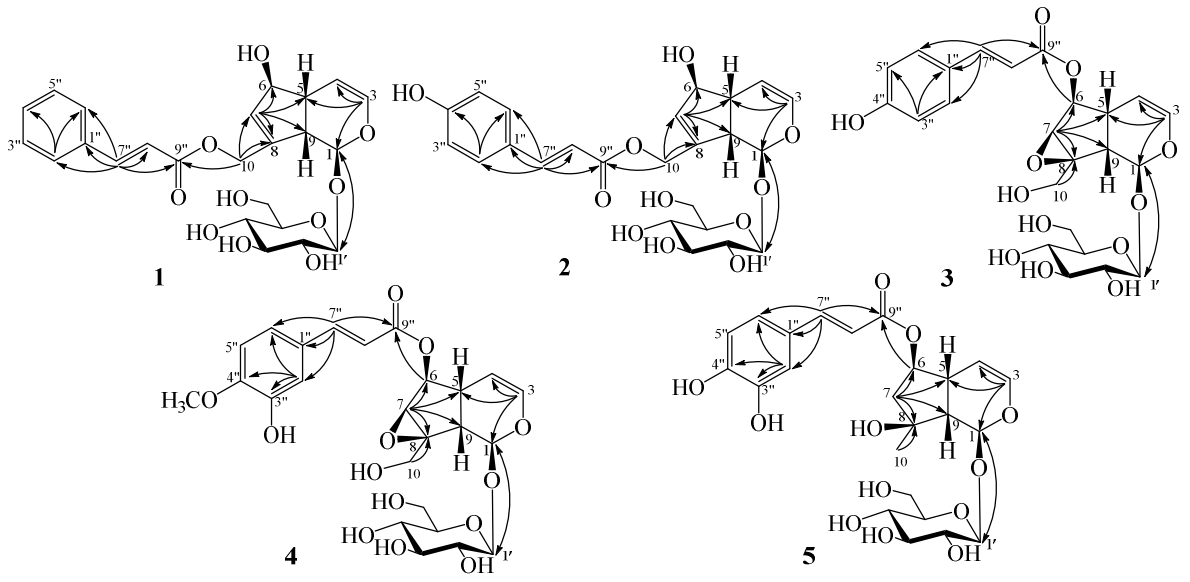

Figure 2: Selected HMBC and COSY correlations of compounds **1-5**

Table 2: ^{1}H and ^{13}C NMR data for compounds **3**, **4**, and **5**

C	**3**			**4**			**5**		
	δ_C[#, a]	**δ_C[a]**	**δ_H[a] (mult., *J* = Hz)**	**δ_C[$, b]**	**δ_C[a]**	**δ_H[a] (mult., *J* = Hz)**	**δ_C[@, a]**	**δ_C[a]**	**δ_H[a] (mult., *J* = Hz)**
1	95.2	95.1	5.18 (d, 9.0)	95.1	95.1	5.19 (d, 9.0)	93.4	93.5	5.52 (br s)
3	142.4	142.3	6.39 (d, 6.0)	142.4	142.4	6.39 (m)	141.0	141.1	6.24 (br d, 6.0)
4	103.0	103.0	5.01 (dd, 4.5, 6.0)	102.9	102.9	5.01 (m)	104.6	104.6	4.99 (br dd, 2.5, 6.0)
5	36.8	36.7	2.62 (m)	36.8	36.8	2.61 (m)	39.3	39.4	2.94 (br dd, 2.5, 9.0)
6	81.4	81.3	5.05 (br d, 8.0)	81.4	81.4	5.06 (d, 7.5)	80.3	80.3	4.94 (m)
7	60.3	60.3	3.73 (br s)	60.3	60.3	3.72 (d, 7.5)	47.8	47.9	2.02 (dd, 3.0, 14.0) 2.26 (dd, 6.5, 14.0)
8	66.9	66.8	-	66.9	66.9	-	77.9	79.2	-
9	43.3	43.2	2.65 (dd, 8.0, 9.0)	43.2	43.2	2.65 (m)	51.6	51.6	2.60 (br d, 9.0)
10	61.3	61.3	4.19 (d, 13.0) 3.86 (d, 13.0)	61.3	61.3	3.85 (d, 13.5) 4.19 (d, 13.5)	26.0	26.0	1.41 (s)
1′	99.9	99.7	4.82 (d, 8.0)	99.7	99.7	4.82 (d, 8.0)	99.3	99.4	4.69 (d, 8.0)
2′	74.9	74.8	3.31 (dd, 8.0, 9.0)	74.9	74.9	3.30 (m)	74.7	74.8	3.23 (dd, 8.0, 9.0)
3′	78.7	77.7	3.44 (t, 9.0)	78.7	77.7	3.43 (t, 8.5)	78.1	78.0	3.40 (dd, 9.0, 9.0)
4′	71.8	71.7	3.30 (m)	71.8	71.8	3.29 (m)	71.6	71.7	3.30 (dd, 9.0, 9.0)
5′	77.8	78.6	3.36 (m)	77.7	78.7	3.35 (m)	79.1	78.2	3.33 (m)
6′	63.0	62.9	3.95 (dd, 1.5, 11.5) 3.68 (dd, 6.5, 11.5)	63.0	62.9	3.67 (dd, 7.0, 12.0) 3.95 (dd, 1.5, 12.0)	62.8	62.9	3.69 (dd, 5.5, 11.5) 3.92 (br d, 11.5)
1″	136.8	126.7	-	128.8	128.8	-	127.7	127.8	-
2″	131.3	131.3	7.49 (d, 9.0)	114.9	114.9	7.12 (d, 1.5)	115.3	115.2	7.07 (s)
3″	117.0	117.1	6.83 (d, 9.0)	148.1	148.1	-	146.9	146.8	-
4″	161.7	162.1	-	151.7	151.7	-	149.5	149.6	-
5″	117.0	117.1	6.83 (d, 9.0)	112.6	112.6	6.97 (d, 8.0)	116.6	116.5	6.80 (d, 8.0)
6″	131.3	131.3	7.49 (d, 9.0)	114.9	115.6	6.40 (m)	122.9	123.0	6.97 (d, 8.0)
7″	147.3	147.3	7.69 (d, 16.0)	147.2	147.2	7.65 (d, 16.0)	146.7	147.0	7.58 (d, 16.0)
8″	114.5	114.3	6.38 (d, 16.0)	123.0	123.0	7.10 (d, 16.0)	115.1	115.4	6.31 (d, 16.0)
9″	161.8	169.0	-	168.7	168.8	-	169.0	169.1	-
4-CH_3				56.4	56.4	3.91 (s)			

[a]Recorded in CD_3OD, [#] δ_C of specioside,[8] [$]$\delta_C$ of minecoside,[7] [@]δ_C of 6-O-caffeoylajugol.[9]

The ^{1}H-NMR spectrum of **3** showed the signals of two olefin protons at δ_H 6.38 (1H, d, J = 16.0 Hz), and 7.69 (1H, d, J = 16.0 Hz); one phenyl ring at δ_H 6.83 (2H, d, J = 9.0 Hz), and 7.49 (2H, d, J = 9.0 Hz); a *cis*-configured double bond at δ_H 5.01 (1H, dd, J = 4.5, 6.0 Hz), and 6.39 (1H, d, J = 6.0 Hz). The ^{1}H-NMR spectrum further showed a signal for one anomeric proton δ_H 4.82 (1H, d, J = 8.0 Hz), revealing that **3** has a monosaccharide unit in the structure. The ^{13}C-NMR and HSQC spectra showed the the signals of 23 carbons, including four non-protonated carbons; seventeen methines; and two methylenes. The ^{1}H- and ^{13}C-NMR data of **3**, the structure of **3** was suggested to be an iridoid glycoside associated with a *trans*-coumaroyl unit, similar to those of specioside. [8] All NMR assignments of **3** were confirmed by detailed analyses of HSQC and HMBC spectra. The HMBC correlation between δ_H 4.82 (H- 1′) and δ_C 95.1 (C-1) suggested the positions of the β-glucose at the C-1. The *trans*-coumaroyl unit was located at C-6 as revealed based on an HMBC correlation from δ_H 5.05 (H-6) to δ_C 169.0 (C-9″). The epoxy ring at C-7 and C-8 were deduced by HMBC correlations from δ_H 3.73 (H-7) to δ_C 66.8 (C-8), and the ^{13}C-NMR chemical shifts of C-7 (δ_C 60.3) and C-8 (δ_C 66.8). Based on the above data, compound **3** was identified as specioside.

The NMR spectra of **4** were almost similar to the corresponding spectra of **3**, except for the additional methoxyl group at C-4″ and hydroxyl group at C-3″. This was confirmed by the HMBC correlation between H-2″ (δ_H 7.12) and C-3″ (δ_C 148.1)/C-4″ (δ_C 151.7), methoxyl group (δ_H 3.91) and C-4″ (δ_C 151.7). Thus, compound **4** was identified as minecoside. [7]

The NMR spectra of **5** were almost similar to the corresponding spectra of **3**, except for the additional hydroxyl group at C-3″, loss of hydroxyl group at C-10, and break epoxy ring at C-7 and C-8. This was

confirmed by the HMBC correlation between δ_H 2.02/2.26 (H-7) and δ_C 79.2 (C-8), δ_H 1.41 (H-10) and δ_C 79.2 (C-8), H-2″ (δ_H 7.07) and C-3″ (δ_C 146.8)/C-4″ (δ_C 149.6). Thus, compound **5** was identified as 6-*O*-caffeoylajugol.[9]

Acknowledgment. *This research was supported by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 104.01-2015.66.*

REFERENCES

1. R. Tundis, M. R. Loizzo, F. Menichini, G. A. Statti, F. Menichini. Biological and pharmacological activities of iridoids: recent developments, *Mini Rev Med Chem.*, **2008**, *8(4)*, 399-420.
2. N. Ding, S. Yahara, T. Nohara. Structure of mimengosides A and B, new triterpene glycosides from buddlejae flos produced in China, *Chem. Pharm. Bull.*, **1992**, *40*, 780-782.
3. M. M. El-Domiaty, M. Wink, M. M. Abdel Aa, M. M. Abou-Hashem, R. H. Abd-Alla. Antihepatotoxic activity and chemical constituents of *Buddleja asiatica* Lour., *Z Naturforsch C*, **2009**, *64(1-2)*, 11-19.
4. B. H. Tai, B. Y. Jung, N. M. Cuong, P. T. Linh, N. H. Tung, N. X. Nhiem, T. T. Huong, N. T. Anh, J. A. Kim., Y. H. Kim, S. K. Kim. Total peroxynitrite scavenging capacity of phenylethanoid and flavonoid glycosides from the flowers of *Buddleja officinalis*, *Biol. Pharm Bull.*, **2009**, *32(12)*, 1952-1956.
5. B. H. Tai, N. X. Nhiem, T. H. Quang, N. T. T. Ngan, N. H. Tung, Y. Kim, J. J. Lee, C. S. Myung, N. M. Cuong, Y. H. Kim. A new iridoid and effect on the rat aortic vascular smooth muscle cell proliferation of isolated compounds from *Buddleja officinalis*, *Bioorganic & Medicinal Chemistry Letters*, **2011**, *21*, 3462-3466.
6. R. K. Chaudhuri, O. Salama, O. Sticher. Iridoid and aryl glucosides from *Globularia nudicaulis* and *Globularia nana*, *Helvetica Chimica Acta*, **1981**, *64*(7), 2401-2404.
7. T. Vesper, K. Seifert, Iridoids and other glucosides from *Penstemon acuminatus*, *Phytochemistry*, **1994**, *37*(4), 1087-1089.
8. S. F. El-Naggar, R. W. Doskotch. Specioside: a new iridoid glycoside from *Catalpa speciosa*, *Journal of Natural Products*, **1980**, *43(4)*, 524-526.
9. R. Kasai, M. Rakotovao, K. Yamasaki. New iridoid and phenethyl glycosides from Malagasy medicinal plant, *Phyllarthron madagascariense*, *Natural Medicines*, **2001**, *55(4)*, 187-192.

Corresponding author: **Truong Thi Thu Hien**
Vietnam Military Medical University
160, Phung Hung, Phuc La, Ha Dong, Hanoi, Viet Nam
E-mail: truonghientruong@gmail.com; Tel: +84- 983652736