IDENTIFICATION OF COMPOUNDS FROM ETHYLACETATE OF *Leonotis nepetifolia* (L.) R.Br. (LAMIACEAE)

Do Thi My Lien[1], Nguyen Kim Phi Phung[2], Tran Ai Diem[1], Nguyen Thi Nhung[1], Le Cong Nhan[1], Nguyen Xuan Du[1], Nguyen Thi My Dung[1,*]

[1]*Sai Gon University, Ho Chi Minh City*

[2]*University of Science, VNU-HCM*

*Email: *nguyenthimydung@sgu.edu.vn*

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ABSTRACT

Phytochemical investigation of the aerial parts of *Leonotis nepetifolia* (L.) R.Br. (Lamiaceae) yielded five known iridoid glycosides including loganin (**1**), loganic acid (**2**), shanzhiside methyl ester (**3**), sweroside (**4**) and picconioside I (**5**), along with a benzenoid evofolin B (**6**). The structures of these compounds were elucidated on the basis of 1D and 2D NMR experiments. All of the obtained compounds were evaluated for *α*-glucosidase inhibitory activity, in which compounds **1**-**5** show moderate activity.

Keywords: Iridoid glycoside, evofolin B, *Leonotis nepetifolia* (L.) R.Br.

1. INTRODUCTION

The *Leonotis* genus belongs to the Lamiaceae family and consists of approximately 100 species [1, 2]. *Leonotis nepetifolia* R.Br., also known as Lion's Ear, is widely distributed throughout tropical Africa, southern India, and the tropical regions of America [3]. It is traditionally used in Caribbean folk medicine and Ayurvedic herbal medicine to treat a wide array of human diseases such as coughs, fever, stomachache, skin infections, rheumatism, bronchitis, and asthma [4-6]. Previous studies demonstrated that the crude extract or pure compounds of *L. nepetifolia* (L.) R.Br. exhibited anti-bacterial activity [7], anti-fungal [8, 9], anti-inflammatory [10], antispasmodic [11], antioxidant [4, 12, 13], and antiasthmatic [14] activities; however the evaluation of in vitro *α*-glucosidase inhibitory activities of this plant has not been elucidated. In Vietnam, this plant has not yet been chemically and biologically studied. From the aerial part of *Leonotis nepetifolia* (L.) R.Br., we isolated five iridoid glycosides including loganin (**1**), loganic acid (**2**), shanzhiside methyl ester (**3**), sweroside (**4**) and picconioside I (**5**), and a benzenoid evofolin B (**6**). This paper describes the structural elucidation of (**1**) – (**6**) and the *in vitro α*-glucosidase inhibitory activities of these compounds.

Figure 1. Flowers of *Leonotis nepetifolia* (L.) R.Br.

2. MATERIAL AND METHODS

2.1. Plant material

L. nepetifolia (L.) R. Br was collected at Long Hai City, Ba Ria Vung Tau province, Vietnam in December 2014. The material was authenticated by botanist Vo Van Chi. The voucher specimen (No US-A013) was deposited at the Herbarium of the Department of Organic Chemistry, Faculty of Chemistry, University of Science, National University-Ho Chi Minh City, Vietnam.

2.2. General procedures

NMR spectra were acquired on Bruker 400 AVANCE spectrometer (400 MHz for 1H and 100 MHz for ^{13}C). $CDCl_3$ and DMSO-d_6 were used both as a solvent and as an internal reference at δ_H 7.26, 2.50 and δ_C 77.2, 39.5. ESI MS spectra were recorded on Thermo Scientific – MSQ PLUS. TLC was carried out on precoated silica gel 60 F254 or silica gel 60 RP-18 F254S (Merck Millipore, Billerica, Massachusetts, USA). Gravity column chromatography was performed with silica gel 60 (0.040–0.063 mm) (HiMedia, Mumbai, India).

2.3. α-Glucosidase inhibition assay

The inhibitory activity of α-glucosidase was determined according to the modified method of Kim *et. al*. and 3 mM *p*-Nitrophenyl-α-*D*-glucopyranoside (25 μL) and 0.2 U/mL α-glucosidase (25 μL) in 0.01 M phosphate buffer (pH 7) were added to the sample solution (625 μL) to start the reaction [15]. Each reaction was carried out at 37 °C for 30 min and stopped by adding 0.1 M Na_2CO_3 (375 μL). Enzymatic activity was quantified by measuring absorbance at 401 nm. One unit of α-glucosidase activity was defined as amount of enzyme liberating *p*-nitrophenol (1.0 μM) per min. Acarbose, a known α-glucosidase inhibitor, was used as positive control.

2.4. Extraction and isolation

The air-dried stem bark (21.0 kg) was ground into powder and exhaustively extracted at room temperature with 95% (v/v) EtOH (5 × 35 L). The filtered solution was evaporated under reduced pressure to afford a residue (1.4 kg). This crude extract was suspended in H_2O and partitioned with *n*-hexane then EtOAc to yield an *n*-hexane extract (410.0 g), an EtOAc extract (390.0 g), and the remaining aqueous solution. The EtOAc extract was subjected to silica gel column chromatography using gradient elution with *n*-hexane/EtOAc (stepwise 80:20-0:10), EtOAc/MeOH (stepwise 10:0 – 50:50) and MeOH to give 10 fractions from EA01 to EA10.

EA08 fraction (14.6 g) was subjected to silica gel column chromatography eluted with EtOAc - MeOH (95:05) to give eight sub-fractions 8.1-8.8. Sub-fraction 8.2 (1.2 g) was applied to silica gel column chromatography eluted with EtOAc – MeOH (95:05) again and purified by a Sephadex LH-20 column with $CHCl_3$: MeOH (1:1) as eluent to afford **6** (17.9 mg). Sub-fraction 8.4 (3.5 g) was also applied to silica gel column chromatographed eluted with EtOAc – MeOH (90:10) and purified by a Sephadex LH-20 column with $CHCl_3$: MeOH (1:1) as eluent to afford **1** (23.7 mg), and **3** (20.1 mg).

EA09 fraction (20.0 g) was also applied to silica gel column chromatographed eluted with EtOAc – MeOH (90:10) to give six sub-fractions 9.1-9.6. Sub-fraction 9.1 (3.5 g) was chromatographed with RP-C18 silica gel eluted with H_2O - MeOH (60:40) to give **2** (7.4 mg), and **4** (10.5 mg). The same manner was applied to sub-fraction 9.4 (2.8 g) to yield **5** (8.6 mg).

Loganin (1): pale yellow oil, ESI-MS (negative mode) *m/z* 389.0 $[M-H]^-$, calcd. 389.4 for $[C_{17}H_{26}O_{10}-H]$, corresponding to the molecular formula of $C_{17}H_{26}O_{10}$. The ^1H and ^{13}C NMR (DMSO-d_6) data were presented in Table 1 and 2, respectively.

Loganic acid (2): pale yellow oil, ESI-MS (negative mode) *m/z* 375.2 $[M-H]^-$, calcd. 375.4 for $[C_{16}H_{24}O_{10}-H]$, corresponding to the molecular formula of $C_{16}H_{24}O_{10}$. The ^1H and ^{13}C NMR (DMSO-d_6) data were presented in Table 1 and 2, respectively.

Shanzhiside methyl ester (3): colorless oil, ESI-MS (positive mode) *m/z* 429.2 $[M+Na]^+$, calc. 429.4 for $[C_{17}H_{26}O_{11}+Na]$, corresponding to the molecular formula of $C_{17}H_{26}O_{11}$. The ^1H and ^{13}C NMR (DMSO-d_6) data were presented in Table 1 and 2, respectively.

Sweroside (**4**): white powder, ESI-MS (positive mode) *m/z* 378.9 $[M+Na]^+$, calc. 379.4 for $[C_{17}H_{24}O_8+Na]$, corresponding to the molecular formula of $C_{17}H_{24}O_8$. The ^1H and ^{13}C NMR (DMSO-d_6) data were presented in Table 1 and 2, respectively.

Picconioside I (**5**): pale yellow oil, ESI-MS (negative mode) *m/z* 731.1 $[M-H]^-$, calc. 731.7 for $[C_{33}H_{48}O_{18}-H]$, corresponding to the molecular formula of $C_{33}H_{48}O_{18}$. The ^1H and ^{13}C NMR (DMSO-d_6) data were presented in Table 1 and 2, respectively.

Evofolin B (**6**): pale brown oil, ESI-MS (negative mode) *m/z* 316.8 $[M-H]^-$, calc. 317.3 for $[C_{17}H_{18}O_6$ - H], corresponding to the molecular formula of $C_{17}H_{18}O_6$. ^1H NMR ($CDCl_3$, 500 MHz), δ_H (ppm) *J* (Hz), 7.52 (1H, *d*, *J* = 2.0, H-2), 6.84 (1H, *d*, *J* = 9.0, H-5), 7.53 (1H, *dd*, *J* = 8.0; 2.0, H-6), 4.65 (1H, *dd*, *J* = 10.0; 6.0, H-8), 4.23 (1H,*dd*, *J* = 14, 5.5, H-9a), 3.86 (1H, *m*, H-9b), 6.71 (1H, *d*, *J* = 2.0, H-2'), 6.86 (1H, *d*, *J* = 8.5, H-5'), 6.80 (1H, *dd*, *J* = 10.0; 2.0, H-6'), 3.88 (3H, *s*, 3-OCH_3) and 3.82 (3H, *s*, 3'-OCH_3), 6.12 (1H, *s*, -OH), 5.60 (1H, *s*, -OH). ^{13}C NMR ($CDCl_3$, 125 MHz) δ_C (ppm), 129.4 (C-1), 110.4 (C-2), 146.7 (C-3), 150.7 (C-4), 114.1 (C-5), 124.6 (C-6), 198.7 (C-7), 55.7 (C-8), 65.5 (C-9), 128.7 (C-1'), 110.8 (C-2'), 147.2 (C-3'), 145.3 (C-4'), 115.2 (C-5'), 121.8 (C-6'), 56.1 (3-OCH_3) and 56.1 (3'-OCH_3).

3. RESULTS AND DISCUSSION

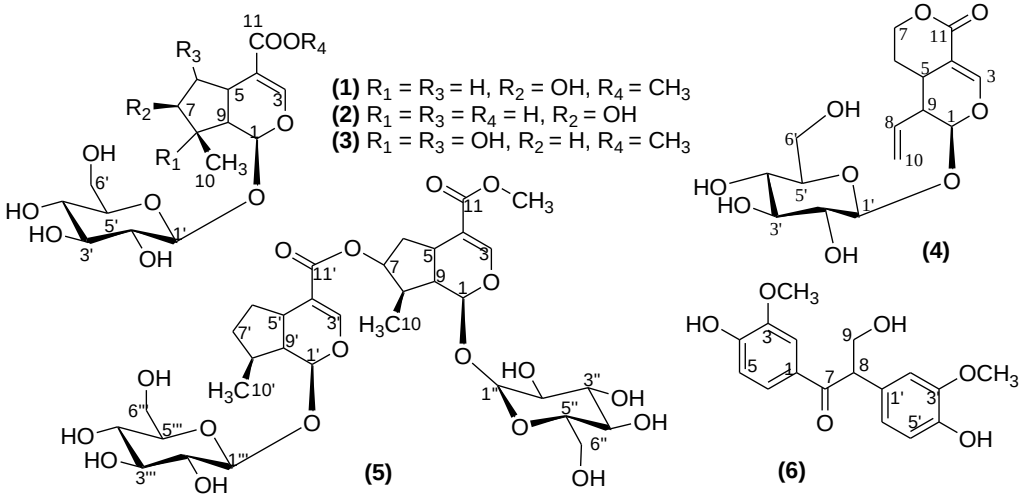

Figure 2. The structure of isolated compounds from *Leonotis nepetifolia* (L.) R.Br.

Table 1. ^{1}H NMR spectroscopic data for **(1) - (5)** in DMSO-d_6

N^o	(1)	(2)	(3)	(4)	(5)
1	5.12 (*d*, 4.8)	5.10 (*d*, 4,8)	5.47 (*d*, 2.0)	5.31 (*d*, 10.0)	5.19 (*d*, 6.5)
3	7.35 (*s*)	7.28 (*s*)	7.34 (*s*)	7.47 (*d*, 2.0)	7.40 (*s*)
5	2.98 (*m*)	2.96 (*m*)	2.80 (*dd*, 2.4, 9.6)	3.04 (*m*)	2.97 (*s*)
6	1.45 (*m*) 2.08 (*m*)	2.06 (*m*) 1.44 (*m*)	3.90 (*m*)	1.75 (*m*) 1.60 (*m*)	2.14 (*m*) 1.68 (*m*)
7	4.94 (*d*, 7.2)	3.15 (*m*)	1.67 (*dd*, 6.4,13.2) 1.83 (*dd*, 5.6, 13.2)	4.32 (*m*) 4.28 (*m*)	5.06 (*m*)
8	1.71 (*m*)	1.70 (*m*)	-	5.44 (*dd*, 16.6, 8.0)	1.87 (*dd*, 7.2, 6.8)
9	1.85 (*m*)	1.80 (*m*)	2.45 (*dd*, 1.6, 10.0)	2.66 (*d*, 3.0)	1.65 (*s*)
10	0.99 (*d*, 6.8)	0.97 (*d*, 6.8)	1.09 (*s*)	5.28 (*d*, 9.6) 5.24 (*dd*, 8.8, 1.2)	1.03 (*d*, 8.0)
1'	4.47 (*m*)	4.47 (*d*, 8.0)	4.44 (*d*, 8.0)	4.50 (*d*, 7.6)	5.09 (*s*)
2'	2.95 (*m*)	2,90 (*m*)	2.93 (*m*)	2.98 (*m*)	-
3'	3.16 (*m*)	3,02 (*s*)	3.14 (*m*)	3.16 (*ddd*, 8.4, 5.2, 3.2)	7.41 (*s*)
4'	3.04 (*m*)	3.04 (*s*)	3.05 (*m*)	3.04 (*t*, 7.0, 4.0)	-
5'	3.13 (*m*)	3.13 (*m*)	3.14 (*m*)	3.16 (*ddd*, 8.4, 5.2, 3.2)	2.80 (*m*)
6'	3.67 (*m*) 3.44 (*m*)	3.87 (*s*) 3.43 (*m*)	3.55 (*dd*, 5.6, 11.6) 3.86 (*dd*, 6.0, 10.8)	3.68 (*m*) 3.43 (*m*)	2.12 (*m*) 1.29 (*m*)
7'	-	-	-	-	1.81 (*m*) 1.13 (*m*)
8'	-	-	-	-	2.02 (*dd*, 6.4, 5.6)
9'	-	-	-	-	1.94 (*d*, 5.2)
10'	-	-	-	-	0.97 (*d*, 8.5)
1"	-	-	-	-	5.11 (*d*, 7.5)
2"	-	-	-	-	2.95 (*s*)
3"	-	-	-	-	3.17 (*m*)
4"	-	-	-	-	3.04 (*m*)
5"	-	-	-	-	3.14 (*m*)
6"	-	-	-	-	3.67 (*d*,11.2) 3.50 (*s*)
1"'	-	-	-	-	5.11 (*d*, 7.5)
2"'	-	-	-	-	2.95 (*s*)
3"	-	-	-	-	5.06 (*dd*, 9.2, 5.2)
4"	-	-	-	-	3.04 (*m*)
5"	-	-	-	-	3.14 (*m*)
6"	-	-	-	-	3.67 (*d*, 11.2) 3.50 (*s*)
6-OH	-	-	4.56 (*d*, 4.0)	-	-

Nº	(1)	(2)	(3)	(4)	(5)
7-OH	4.95 (*d*, 5.2)	-	-	-	-
8-OH		-	4.84 (*s*)	-	-
2'-OH	5.10 (*d*, 5.2)	-	4.95 (*d*, 5.2)	-	-
3'-OH	4.95 (*d*, 5.2)	-	4.98 (*d*, 5.2)	4.59 (*dd*, 6.0, 5.6)	-
4'-OH	4.93 (*d*, 7.2)	-	4.97 (*d*,5.2)	4.96 (*dd*, 4.0, 3.6)	-
6'-OH	4.99 (*d*, 5.2)	-	4.63 (*t*, 5.6)	5.00 (*d*, 4.8)	-
11-OCH_3	3.62 (*s*)	-	3.63 (*s*)	-	3.62 (*s*)

Chemical shifts (δ) are expressed in ppm, and *J* values are presented in Hz. recorded at 500 MHz for ^{1}H NMR

Compound **1** was obtained pale yellow oil. The ^{1}H NMR spectrum of **1** showed signals an olefinic proton at δ_H 7.35 (*s*, H-3)), two hemiacetal protons at δ_H 5.13 (*d*, *J* = 4.8 Hz, H-1) and 4.47 (*m*, H-1'), the protons of a methoxy group at δ_H 3.62 (*s*, 11-OCH_3), and a methyl group at δ_H 0.99 (*d*, *J* = 7.0 Hz, H-10). Additionally, the ^{13}C NMR spectrum of **1** displayed a total of 17 carbon signals including a carbonyl ester carbon at δ_C 166.9 (C-11), two olefinic carbons at δ_C 150.5 (C-3), and 112.1 (C-4), two hemiacetal carbon at δ_C 96.1 (C-1) and 98.6 (C-1'), an oxygenated methine carbon at δ_C 70.1 (C-7), together five signals of a glucose moiety at δ_C 73.2 (C-2'), 77.2 (C-3'), 71.1 (C-4'), 76.8 (C-5'), and 61.2 (C-6'), three methine carbon, a methylene carbon, a methyl carbon and a methoxyl carbon in the high field region from 13.4 to 50.9 ppm. These signals were also confirmed by HSQC and COSY spectra. These results indicated that compound **1** was the iridoid glycoside type. Detailed analysis of HMBC experiment of **1** showed the correlations of a methoxy group at δ_H 3.62 with the carbonyl ester at δ_C 166.9, of a methyl group at δ_H 0.99 with carbons at δ_C 72.2 (C-7), 40.5 (C-3), and 44.8 (C-9), of a hydroxyl group at δ_H 4.95 (1H, *d*, *J* = 5.2 Hz) with two methine carbons at δ_C 30.7 (C-5), 44.8 (C-9) confirmed the position of these substitute groups. The ESI-MS of **1** showed the pseudomolecular ion $[M-H]^-$ at *m/z* 389.0, and these spectroscopic data were compatible with the reported ones in the literature [16, 17] and therefore **1** was loganin.

Compound **2** was isolated pale brown oil. The ^{1}H and ^{13}C-NMR spectra data of **2** (Table 1) were similar to those of **1**, except for the lack of the signals of a methoxyl group. Additionally, the ESI-MS of **2** gave the pseudomolecular ion $[M-H]^-$ at *m/z* 375.2, calcd. 375.4 for [$C_{16}H_{24}O_{10}$-H], corresponding to the molecular formula of $C_{16}H_{24}O_{10}$. These data showed that compound **2** has also the iridoid glycoside skeleton. By comparing NMR data of **2** with those reported in the literature [18, 19], **2** was elucidated as loganic acid.

Compound **3** was isolated colorless oil. Its ESI-MS presented the pseudomolecular ion $[M+Na]^+$ at *m/z* 429.2 (calcd. 429.4 for [$C_{17}H_{26}O_{11}$+Na]), suggesting the molecular formula of $C_{17}H_{26}O_{11}$. The ^{1}H and ^{13}C-NMR spectra data of **3** (Table 1) were similar to those of **1**, except for more a signal of one hydroxyl group in **3**. Furthermore, the presence of signals of a hydroxyl group at δ_H 4.84 (1H, *s*, 8-OH) and a methyl group at δ_H 1.09 (3H, *s*, H-10) were correlated with a quaternary carbon at δ_C 77.3 (C-8), a methylene carbon at δ_C 49.1 (C-7), and a methine carbon at δ_C 50.1 (C-9) in HMBC experiment suggested the hydroxyl group and the methyl group same at C-8. Additionally, the proton of hydroxyl group at δ_H 4.56 (*d*, 4.0) was correlated with two methine carbons at δ_C 39.8 (C-5), and 75.2 (C-6) together a methylene carbon at δ_C 49.1 (C-7), which confirmed the position of this hydroxyl group at C-6. These spectroscopic data were compatible with the ones in the literature [20]. Thus, **3** was elucidated to be shanzhiside methyl ester.

Table 2. ^{13}C NMR spectroscopic data for **(1) - (5)** in DMSO-d_6

C	(1)	(2)	(3)	(4)	(5)
1	96.1	96.2	93.0	98.1	95.7
3	150.5	150.2	151.2	151.4	151.0
4	112.1	112.9	109.7	104.8	111.3
5	30.7	31.0	39.8	26.8	31.1
6	41.7	41.9	75.2	24.3	39.0
7	72.2	72.4	49.1	67.6	77.3
8	40.5	40.6	80.8	132.3	39.0
9	44.8	45.0	50.1	41.5	47.2
10	13.4	13.7	24.8	120.2	13.5
11	166.9	168.4	172.3	164.6	166.8
1'	98.6	98.5	98.2	95.6	96.1
2'	73.2	73.3	73.2	73.1	-
3'	77.2	77.3	77.1	77.3	151.0
4'	71.1	70.3	70.2	70.0	111.3
5'	76.8	76.9	76.8	76.4	33.6
6'	61.2	61.3	61.3	61.0	32.0
7'	-	-	-	-	32.6
8'	-	-	-	-	34.9
9'	-	-	-	-	45.3
10'	-	-	-	-	20.3
11'	-	-	-	-	166.2
1"	-	-	-	-	98.7
2"	-	-	-	-	73.2
3"	-	-	-	-	76.0
4"	-	-	-	-	70.1
5"	-	-	-	-	76.8
6"	-	-	-	-	61.2
1'"	-	-	-	-	98.8
2'"	-	-	-	-	73.2
3"	-	-	-	-	76.0
4"	-	-	-	-	70.1
5"	-	-	-	-	76.8
6"	-	-	-	-	61.2
11-OCH_3	50.9	-	51.3	-	51.1
Chemical shifts (δ) are expressed in ppm. Recorded at 500 MHz for ^{13}C NMR					

Compound **4** was obtained as white powder and the ESI-MS presented the pseudomolecular ion $[M+Na]^+$ at *m/z* 378.9 (calcd. 379.4 for $[C_{17}H_{24}O_8+Na]$). Comparison of ^{13}C NMR data of **2** and **4** revealed that **4** were structurally closely related to **2** except that the position at C-7 and C-8 were rearranged form. The HMBC experiment revealed the correlations of olefin proton at δ_H 5.44 (*dd*, *J* = 16.6; 8.0 Hz, H-8) with the hemiacetal carbon at δ_C 98.1 (C-1), the methine carbons at δ_C 26.8 (C-5), and 41.5 (C-9), of olefin protons at δ_H 5.28 (*d*, *J* = 9.6 Hz, H-10a), and 5.24 (*dd*, *J* = 8.8; 1.2 Hz, H-10b) with carbons at C-8 and C-9. But these olefin protons have no correlation with carbonyl ester carbon at δ_C 164.6 (C-11). At the same time, the signals of methylene group at δ_H 4.32 (H-7a), and 4.28 (H-7b) revealed the correlation with carbon C-11. These data suggested that the linkage C_7 - C_8 was broken in **2** and located the bridging ester bond between the hydroxyl group at C-7 with the carbonyl carbon (C-11) to performed **4**. The connectivity of ^1H and ^{13}C NMR signals was determined by HSQC and COSY spectra. Based on these NMR data as well as the comparison with the corresponding compound in the literature [21], **4** was suggested as sweroside.

Compound **5** was obtained pale yellow oil. The ^1H NMR spectrum (Table 1) showed two olefinic protons at δ_H 7.40 (*s*, H-3), and 7.41 (*s*, H-3'), four hemiacetal protons at δ_H 5.19 (*d*, *J* = 5.2 Hz, H-1), 5.11 (*d*, *J* = 6.0 Hz, H-1), 4.50 (*d*, *J* = 5.2 Hz, H-1"), and 4.48 (*d*, *J* = 5.2 Hz, H-1'"). The ^{13}C NMR spectrum (Table 1) showed signals of 33 carbon including a couple signal of two carbonyl ester carbon at δ_C 166.8 (C-11) and 166.2 (C-11'), two couple signals of olefinic carbons at δ_C 151.0 (C-3), 150.9 (C-3'), 111.3 (C-4) and 111.2 (C-4'), signals of two methyl groups at δ_C 20.3 (C-10) and 13.4 (C-10'), of a methoxyl group at δ_C 61.2 (11-OCH_3), together 12 signals of two glucoside units with two anomeric carbons at δ_C 98.7 (C-1") and 98.8 (C-1'"). Additionally, the ESI-MS presented the pseudomolecular ion $[M-H]^-$ at *m/z* 731.1 (calcd. 731.7 for $[C_{33}H_{48}O_{18}-H]$), suggesting the molecular formula of $C_{33}H_{48}O_{18}$. Comparison NMR data of **5** with those **1** suggested that the presence of an loganin moiety and a deoxyloganin [22] moieties in the molcules **5**. Interestingly, the proton signals at δ_H 7.41 (H-3'), 3.17 (H-7), and 2.80 (H-5') showed correlation with the same carbonyl ester carbon at δ_H 166.2 (C-11'). These data suggested that two iridoid glycoside moieties in **5** was linked by an ester the bridging ester bond between the hydroxyl group at C-7 of loganin unit and the carboxyl group (C-11') of deoxyloganin unit. These spectroscopic data were compatible with the ones in the literature [23]. Thus, **5** was suggested to be picconoside I.

Table 3. α-glucosidase inhibitory activities of the isolated compounds (**1**) – (**6**)

No	Compound	Percentage of cell growth inhibition (I%)
1	Loganin (**1**)	42.7 ± 1.3
2	Loganic acid **(2)**	38.5 ± 1.1
3	Shanzhiside methyl ester (**3**)	49.3 ± 2.4
4	Sweroside (**4**)	51.2 ± 2.9
5	Picconioside I (**5**)	63.8 ± 3.7
6	Evofolin B (**6**)	30.1 ± 0.8
7	Acarbose (possitive control)	95.1 ± 2.3

Compound **6** was isolated pale brown oil. The ESI-MS of **6** an $[M+H]^+$ ion at *m/z* 319, implying a molecular of $C_{17}H_{18}O_6$. The ^1H NMR spectrum of **6** showed two sets of characteristic ABX coupled aromatic protons at δ_H 7.52 (1H, *d*, *J* = 2.0, H-2), 6.84 (1H, *d*, *J* = 8.5, H-5), 7.53 (1H, *dd*, *J* = 8.0; 2.0, H-6) as well as signals at δ_H 6.71 (1H, *d*, *J* = 2.0, H-2'), 6.86

(1H, *d*, *J* = 10.0, H-5'), and 6.80 (1H, *dd*, *J* = 10.0; 2.0, H-6'), suggesting the existence of two 1,3,4 - trisubstituted benzene rings. Furthermore, the protons of two methoxy groups at δ_H 3.88 (3H, *s*, 3-OCH_3), 3.82 (3H, *s*, 3'-OCH_3), and a methine group at δ_H 4.65 (1H, *dd*, *J* = 10.0; 6.0, H-8) were found, while the protons of a methylene group at δ_H 4.23 (1H,*dd*, *J* = 14, 5.5, H-9a), 3.86 (1H, *m*, H-9b) were observed in the ^{1}H NMR and HSQC spectra. Moreover, the COSY spectrum showed correlations that indicated the presence of a partial –$CHCH_2OH$ structure. ^{13}C NMR spectrum of **6** showed 12 signals of two 1,3,4-trisubstituted benzene rings, a carbonyl carbon at δ_C 198.7 (C-7), a signal of two methoxy groups at δ_C 56.1 (3-OCH_3 and 3'-OCH_3), and two aliphatic carbons at δ_C 55.7 (C-8) and 65.5 (C-9). In the HMBC experiment, the signals at δ_H 7.52 (H-2) and 7.53 (H-6) correlated with the carbons at δ_C 198.7 (C-7) and 55.7 (C-8), the signals at δ_H 6.71 (H-2′), and 6.80 (H-6′) correlated with carbon at δ_C 55.7 (C-8). These results confirmed the location of two 1,3,4-trisubstituted benzene rings connect through carbonyl carbon (C-7) and aliphatic carbon (C-8). Furthermore, the ESI-MS of **6** an $[M+H]^+$ ion at *m/z* 319, implying a molecular of $C_{17}H_{18}O_6$. Based on these spectral data as well as the comparison with the corresponding compound in the literature [24], **6** was assigned as evofolin B.

Six isolated compounds were evaluated of the *in vitro* α-glucosidase inhibitory activities with acarbose as the positive control. The α-glucosidase inhibitory assay was adopted from the method of Kim *et al.* [15] Every sample was tested three times. The cytotoxic activity of these compounds expressed as a percentage of cell growth inhibition (I%). The results showed that **1-5** demonstrated moderate α-glucosidase inhibitory activities with I% from 30.1% to 63.8%, and **6** showed weak activity (Table 3).

4. CONCLUSION

Six known compounds including five known iridoid glycosides including loganin, loganic acid, shanzhiside methyl ester, sweroside and picconioside I, along with a benzenoid evofolin B were isolated for the first time from Vietnamese *Leonotis nepetifolia* (L.) R.Br. (Lamiaceae). The compounds **1-5** evaluated moderate α-glucosidase inhibitory activities and **6** showed inactive.

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TÓM TẮT

NHẬN DẠNG CÁC HỢP CHẤT CÔ LẬP TỪ CAO ETHYLACETATE
CỦA CÂY SƯ NHĨ *Leonotis nepetifolia* (L.) R.Br. (HỌ HOA MÔI LAMIACEAE)

Đỗ Thị Mỹ Liên[1], Nguyễn Kim Phi Phụng[2],
Trần Ái Diễm[1], Nguyễn Thị Nhung[1],
Lê Công Nhân[1], Nguyễn Xuân Dũ[1], Nguyễn Thị Mỹ Dung[1,*]
[1]Trường Đại học Sài Gòn, TP.HCM
[2]Trường Đại học Khoa học Tự nhiên, ĐHQG-HCM
**Email: nguyenthimydung@sgu.edu.vn*

Khảo sát thành phần hóa học của cây sư nhĩ *Leonotis nepetifolia* (L.) R.Br. (họ Hoa môi: Lamiaceae) đã cô lập được 6 hợp chất, trong đó có 5 hợp chất iridoid glycoside bao gồm loganin (**1**), loganic acid (**2**), shanzhiside methyl ester (**3**), sweroside (**4**) and picconioside I (**5**), và một hợp chất benzenoid là evofolin B (**6**). Cấu trúc của các hợp chất được xác định dựa trên dữ liệu phổ cộng hưởng từ hạt nhân. Kết quả thử nghiệm hoạt tính ức chế enzyme α-glucosidase của các hợp chất cô lập được cho thấy các hợp chất (**1**) – (**5**) thể hiện khả năng ức chế trung bình, hợp chất (**6**) không có hoạt tính.

Từ khóa: Iridoid glycoside, evofolin B, *Leonotis nepetifolia* (L.) R.Br.