Vietnam Journal of Science and Technology ***57*** *(2) (2019) 162-169*

doi:10.15625/2525-2518/57/2/13019

STUDY ON THE CHEMICAL COMPOSITION OF *URENA LOBATA* GROWING IN VIET NAM[#]

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Received: 29 August 2018; Accepted for publication: 15 December 2018

Abstract. *Urena lobata* L. is used in Vietnamese traditional medicine for the treatment of several diseases. The roots are used to treat rheumatism, dysentery, poor digestion, flu, tonsils, malaria, asthma, and goiter. The flowers are used to treat chickenpox, fever, and mental disorders. The branches, leaves or whole trees are used to treat injuries, bruises, rheumatism, mastitis, and bites. In this study, phytochemical investigation of the *n*-hexane and ethyl acetate extract of leaves and twigs of *Urena lobata* L. led to the isolation of *β*-sitosterol (**1**), *β*-sitosterol-3-O-*β*-D-glucopyranoside (**2**), 2-acetylamino-3-phenylpropyl 2-benzoylamino-3-phenylpropanoate (**3**), quercetin (**4**), and *trans*-tiliroside (**5**). Their chemical structures were determined by spectroscopic methods, including MS, 1D-, 2D- NMR, and by comparing their spectral data with those reported in previous papers. Compounds **3** and **5** were isolated for the first time from *Urena lobata* L.

Keywords: Urena lobata, flavonoids, 2-acetylamino-3-phenylpropyl 2-benzoylamino-3-phenylpropanoate, phytosterols.

Classification numbers: 1.1.1.

1. INTRODUCTION

The plant *Urena lobata* L. (called Ké hoa đào in Viet Nam) belongs to the Malvaceae family and is usually distributed in tropical countries of South America, Africa and Asia. In Vietnam, *Urena lobata* is mainly found in the provinces of Lang Son, Tuyen Quang, and Cao Bang [1, 2]. This plant is often used in traditional medicine. The roots are used to treat rheumatism, dysentery, poor digestion, flu, tonsils, malaria, asthma, and goiter. The flowers are used to treat chickenpox, fever, and mental disorders. The branches, leaves or whole plants are

Presented at the conference RDNP2018, Hanoi 10/2018

used to treat bruises, rheumatism, mastitis, and snake bites [2, 3]. Traditional medicine practiced in some countries such as the Philippines, Malaysia, India, and China uses different parts of the plant as an antidote and remedy against dysentery. It is also used in overdose for cold sores, colds, fever, pain, or edema, gonorrhea, vomiting, and bleeding [3].

As of 2017, studies outside Viet Nam on the chemical composition and biological activity of *U. lobata* indicated that there are many flavonoids in this species [4], and that it has antioxidant, antibacterial, anti-diabetic, and anti-cancer activities [5, 6]. Some flavonoids, glycosides, and terpenes isolated from this plant have interesting activities such as antioxidant, anti-inflammatory, antimicrobial, anti-cancer, anti-diabetic and anti-hyperlipidemic activities [7]. This paper presents the isolation and structure determination of five compounds from the *n*-hexane and ethyl acetate extract of the Vietnamese *Urena lobata*, which is still little investigated.

2. MATERIALS AND METHODS

2.1. Plant materials

The plant samples (leaves and twigs) were collected in October 2016 in Tuyen Quang province. It was identified as *Urena lobata* L. (Malvaceae) by Dr. Nguyen Quoc Binh - Department of Biology, Vietnam National Museum of Nature, Vietnam Academy of Science and Technology. A voucher specimen under sample number UL1016 was stored at the Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology.

2.2. General experimental procedures

ESI mass spectra were recorded on an Agilent 1100 series single quadrupole LC/MS systems. NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (Bruker, Billerica, MA, U.S.A.) using TMS as an internal Standard. Column chromatography (CC) was performed using Kieselgel 60, 70-230 mesh and 230-400 mesh (Merck, Darmstadt, Germany). Thin layer chromatography (TLC) used pre-coated silica gel 60 F254 (Merck, Darmstadt, Germany); compounds were visualized by spraying with aqueous 10 % H_2SO_4 and heating for 3-5 minutes.

2.3. Extraction and isolation

The powdered sample (3.0 kg) was extracted five times with ethanol (at room temperature). The combined extracts were evaporated in vacuum to give the ethanol residue. The residue was suspended in water and then partitioned with hexane, ethyl acetate to afford the corresponding extracts: hexane (**KH**: 48 g), ethyl acetate (**KE**: 123 g) and water (**KW**: 45 g).

The hexane extract (45 g) was subjected to chromatography on a silica gel column, eluting with hexane-ethyl acetate (gradients for hexane-ethyl acetate 0→100, v/v) to give 6 fractions. The fraction **KH2** was subjected to chromatography on silica gel column, eluting with hexane-ethyl acetate (30:1, v/v) and then recrystallized in *n*-hexane-ethyl acetate to obtain compound **1** (white crystals, 1.2 g). The fraction **KH3** (6.2 g) was subjected to chromatography on silica gel column two times, eluting with hexane-ethyl acetate (20:1 → 3:1, v/v) to give five fractions KH3.1 → KH3.5. The fraction KH3.3 was subjected to chromatography on silica gel column, eluting with hexane-ethyl acetate (10:1, v/v) and recrystallized in acetone to obtain compound **3** (white crystals, 53.1 mg). The fraction **KH6** was subjected to chromatography on silica gel

column two times, eluting with *n*-hexane-ethyl acetate (10:1 → 1:1, v/v) to give four fractions KH6.1 → KH6.4. Fraction KH6.3 was recrystallized in ethyl acetate to give compound **2** (amorphous powder, 15.1 mg).

The ethyl acetate extract (50 g) was subjected to column chromatography using silica gel, the column was eluted with CH_2Cl_2:MeOH (100:1→1:100, v:v) to obtain 08 fractions KE1→KE8. The fraction **KE2** (1.5 g) was subjected on column chromatography, eluted with CH_2Cl_2:MeOH (10:1 → 100 % MeOH, v/v) to give three fractions KE2.1→ KE2.3. The KE2.2 was subjected on column chromatography, eluted with CH_2Cl_2:MeOH (100 % CH_2Cl_2 → 9:1, v/v) to give compound **4** (yellow crystals, 7.3 mg). The fraction **KE3** (6.6 g) was subjected on column chromatography, eluted with CH_2Cl_2:MeOH (10:1 → 100 % MeOH, v/v) to give four fractions KE3.1→ KE3.4. The fraction KE3.2 (1.2 g) was subjected to column chromatography using Sephadex LH-20, eluted with methanol to give compound **5** (yellow powder, 8.6 mg).

***β*-sitosterol (1):** White crystals. ^{1}H-NMR (500 MHz, $CDCl_3$), δ (ppm): 0.68 (3H, s, CH_3-18); 1.01 (3H, s, CH_3-19); 0.81-0.88 (2×3H, d, J = 7.7 Hz, CH_3-26, and CH_3-27); 0.83 (3H, t, J = 7.32 Hz, 29-CH_3); 0.92 (3H, d, J = 10 Hz, CH_3-21); 3.52 (1H, m, H-3α); 5.35 (1H, d, J = 5 Hz, H-6). ^{13}C-NMR (125 MHz, $CDCl_3$), δ (ppm): 37.3 (C-1); 31.7 (C-2); 71.8 (C-3); 42.3 (C-4); 140.8 (C-5); 121.7 (C-6); 31.9 (C-7); 33.9 (C-8); 50.2 (C-9); 36.5 (C-10); 21.1 (C-11); 39.8 (C-12); 42.33 (C-13); 56.8 (C-14); 24.3 (C-15); 28.3 (C-16); 56.1 (C-17); 11.9 (C-18); 19.4 (C-19); 36.2 (C-20); 18.8 (C-21); 33.97 (C-22); 26.2 (C-23); 45.9 (C-24); 29.2 (C-25); 19.8 (C-26); 19.1 (C-27); 23.1 (C-28); 11.9 (C-29).

***β*-sitosterol-3-O-*β*-D-glucopyranoside (2):** White powder. ^{1}H-NMR (500MHz, DMSO-d_6); δ (ppm): 4.20 (1H, d, J = 8.0 Hz, H-1′); 2.8 ~ 3.1 (4H, m); 3.57 (1H, m, H-3); 5.34 (1H, br s, H-6); 0.65 (3H, s, H-18); 0.93 (3H, s, H-19); 0.94 (3H, d, J = 6.6 Hz, H-21); 0.83 (3H, d, J = 7.1 Hz, H-29); 0.85 (3H, d, J = 6.6 Hz, H-26); 0.80 (3H, d, J = 6.6 Hz, H-27). ^{13}C-NMR (125MHz, DMSO-d_6) ; δ (ppm): 37.6 (C-1); 29.9 (C-2); 76.8 (C-3); 39.1 (C-4); 140.6 (C-5); 121.3 (C-6); 32.2 (C-7); 32.3 (C-8); 50.7 (C-9); 37.1 (C-10); 21.4 (C-11); 40.2 (C-12); 42.7 (C-13); 57.2 (C-14); 23.9 (C-15); 28.5 (C-16); 56.5 (C-17); 12.0 (C-18); 19.2 (C-19); 35.6 (C-20); 19.0 (C-21); 34.4 (C-22), 26.7 (C-23); 46.4 (C-24); 29.7 (C-25); 19.9 (C-26); 19.5 (C-27); 23.5 (C-28); 12.1 (C-29); 100.9 (C-1′); 74.0 (C-2′); 76.2 (C-3′); 70.8 (C-4′); 76.9 (C-5′); 61.1 (C-6′).

2-acetylamino-3-phenylpropyl 2-benzoylamino-3-phenylpropanoate (3): White crystals. ^{1}H-NMR (500MHz, $CDCl_3$) and ^{13}C-NMR (125MHz, $CDCl_3$) see table 1. ESI-MS *m/z* 445 $[M+H]^+$.

Quercetin (4): Yellow powder. ^{1}H-NMR (500 MHz, DMSO-d_6), δ (ppm): 12.48 (1H, s, 5-OH); 10.79 (1H, s, 7-OH); 9.59 (1H, s); 9,36 (1H, s); 9,30(1H, s) 7.67 (1H, d, J = 2.5 Hz, H-2′); 7,54 (1H, dd, J = 2.0 và 8.5 Hz, H-6'); 6.87 (1H, d, J = 8.5 Hz, H-5′); 6.40 (1H, d, J = 1.5 Hz, H-8); 6.18 (1H, d, J = 2.0 Hz, H-6). ^{13}C-NMR (125 MHz, DMSO-d_6), δ (ppm): 146.78 (C-2); 135.71 (C-3); 175.82 (C-4); 160.70 (C-5); 98.16 (C-6); 163.86 (C-7); 93.33 (C-8); 156.11 (C-9); 102.99 (C-10); 121.93 (C-1'); 115.03 (C-2'); 145.04 (C-3'); 147.68 (C-4'); 115.58 (C-5'); 119.95 (C-6').

***trans*-tiliroside (5):** Yellow powder. ^{1}H-NMR (500 MHz, DMSO) and ^{13}C-NMR (125 MHz, DMSO) see table 2; Positive ESI-MS *m/z* 595 $[M+H]^+$

3. RESULTS AND DISCUSSION

The chemical structure of *β*-sitosterol (**1**) and *β*-sitosterol-3-O-*β*-D-glucopyranoside (**2**) were determined by 1D and 2D NMR experiments and comparison with spectral data of *β*-sitosterol and *β*-sitosterol-3-O-*β*-D-glucopyranoside in references [8, 9, 10, 11].

Compound **3** was obtained as white crystals, mp 194-196 ^{0}C. The ESI-MS of **3** provided a molecular ion peak at *m/z* 445 $[M+H]^+$, corresponding to the formula $C_{27}H_{28}N_2O_4$. The ^{1}H-NMR spectrum of **3** showed the presence of three monosubstituted phenyl groups with signals at δ_H 7.21-7.30 (5H, m); 7.07 (2H, m); 7.18 (2H, m); 7.14 (1H, m); 7.70 (2H, m); 7.42 (2H, m); 7.52 (1H, tt, 6.5, 1.5) and two secondary amino (NH-) groups with signals at δ_H 6.70 ppm (1H, d, J = 7.5 Hz) and δ_H 5.90 ppm (1H, d, J = 8.5 Hz). In addition, the ^{1}H-NMR of **3** showed the presence of an acetyl group with signals at δ_H 2.02 (3H, s); three methylene groups with signals at δ_H 3.93 (1Ha, dd, 11.5, 4.5) and 3.82 (1Hb, dd, 11.5, 4.0); 3.24 (Ha, dd, 14.0, 6.0) and 3.05 (Hb, dd, 14.0, 8.5), and 2.76 (2H, m), and two methine groups with signals at δ_H 4.75 (1H, m) and 4.34 (1H, m).

The ^{13}C-NMR and DEPT spectra of **3** exhibited signals of 27 carbons, including 6 quaternary, 17 methine, 03 methylene, and 01 methyl carbons. The ^{13}C-NMR spectrum revealed the signals of carbonyl carbons (C=O) at δ_C 170.76; 170.22 và 167.11. The ^{13}C-NMR and DEPT spectra of **3** showed the presence of three monosubstituted phenyls, six carbon signals of which were two times greater than the others at δ_C 129.30; 129.14; 128.78; 128.65; 128.59 and 127.04, together with 03 methine carbons with signals at δ_C 131.91; 127.16 and 126.76.

The HMBC spectrum of **3** showed correlations between the proton at δ_H 3.24 (1H, dd, 14.0, 6.0, H-7a) and 3.05 (1H, dd, 14.0, 8.5, H-7b) with C-1 (δ_C 136.64); C-2,6 (δ_C 129.3); C-8 (δ_C 55.01) and C-9 (δ_C 170.22), between the proton at δ_H 4.75 (1H, m, H-8) with C-1 (δ_C 136.7); C-7 (δ_C 38.42) and C-9 (δ_C 170.22), and between the proton at δ_H 6.7 (1H, d, 7.5, H-10) with C-8 (δ_C 55.01) and C-7'' (δ_C 167.11) indicating that the benzoyl group was connected with N-10.

Furthermore, the HMBC spectrum showed correlations between the proton at δ_H 2.76 (2H, m, H-7') with C-1' (δ_C 136.7); C-2',6' (δ_C 129.14); C-8' (δ_C 49.48) and C-9' (δ_C 64.59), between the proton at δ_H 4.34 (1H, m, H-8') with C-1' (δ_C 136.7); C-7' (δ_C 37.46) and C-9' (δ_C 64.59), between the proton at δ_H 3.93 (1H, dd, 11.5, 4.5, H-9a') and 3.82 (1H, dd, 11.5, 4.0, H-9b') with C-7' (δ_C 37.46); C-8' (δ_C 49.48). The HMBC spectrum showed correlations between the proton at δ_H 5.90 (1H, d, 8.5, H-10') with C-8' (δ_C 49.48) and carbon at δ_C (170.76, acetyl C=O) indicating that the benzyl and acetyl groups were connected with N-10'.

Based on these evidences and comparison with the reported values in literature [12, 13], **3** was assigned the structure shown in Figure 1. In the cited original papers, the chemical name given to **3** as α-acetylamino-phenylpropyl α-benzoylamino-phenylpropanoate was not quite correct. Thus, we hereby identified **3** as 2-acetylamino-3-phenylpropyl 2-benzoylamino-3-phenylpropanoate.

Compound **4** was obtained as yellow powder, mp 314-316 ^{0}C. The ESI-MS spectrum of **4** provided an molecular ion peak at *m/z* 303 $[M+H]^+$, corresponding to the formula $C_{15}H_{10}O_7$. The ^{1}H-NMR of **4** showed the presence of aromatic protons at δ_H 6.40 (1H, d, 1.5, H-8); 6.18 (1H, d, 2.0, H-6); 7.67 (1H, d, 2.5, H-2'); 6.87 (1H, d, 8.5, H-5'); and 7.54 (1H, dd, 2.0, 8.5, H-6'). Furthermore, the ^{1}H-NMR spectrum confirmed the presence of hydroxyl groups at δ_H 12.48 (1H, s, 5-OH), 10.79 (1H, s, 7-OH), 9.59 (1H, s), 9.36 (1H, s) and 9.30 (1H, s). The ^{13}C-NMR and DEPT spectra of **4** exhibited signals of 15 carbons, including 10 quaternary carbon and 05 methine carbons. The ^{1}H and ^{13}C NMR, and DEPT spectra of **4** suggested a flavonoid. Based on comparison with the reported values in literatures [14, 15], compound **4** was identified as quercetin.

Table 1. The ^{1}H (500 MHz, CD_3OD) and ^{13}C-NMR (125 MHz, CD_3OD) data of compound **3**.

C	$^{a}\delta_C$	δ_C	δ_H (mult, *J* = Hz)	HMBC (C→H)
1	136.6	136.64		H-2, H-6, H-7a, H-7b, H-8
2,6	129.3	129.30	7.21-7.30 (5H, m)	H-3, H-5, H-7a, H-7b
3,5	128.8	128.78		H-2, H-4, H-6
4	127.1	127.16		H-3, H-5
7	38.4	38.42	3.24 (Ha, dd, 14.0, 6.0) 3.05 (Hb, dd, 14.0, 8.5)	H-2, H-6, H-8
8	55.0	55.01	4.75 (1H, m)	H-7a, H-7b, H-10
9	170.2	170.22		H-7a, H-7b, H-8
10			6.70 (1H, d, 7.5)	
1'	136.7	136.7		H-2', H-6', H-7'a, H-7'b, H-8'
2', 6'	129.1	129.14	7.07 (2H, m)	H-3', H-5', H-7'a, H-7'b
3', 5'	128.6	128.65	7.18 (2H, m)	H-2', H-4', H-6'
4'	126.7	126.76	7.14 (1H, m)	H-3', H-5'
7'	37.4	37.46	2.76 (2H, m)	H-2', H-6', H-8', H-9'a, H-9'b
8'	49.4	49.48	4.34 (1H, m)	H-7', H-9'a, H-9'b, H-10'
9'	64.6	64.59	3.93 (1Ha, dd, 11.5, 4.5) 3.82 (1Hb, dd, 11.5, 4.0)	H-8'
10'	–	-	5.90 (1H, d, 8.5)	
CH_3**CO**		170.76		C**H$_3$**, H-9'a, H-9'b, H-10'
CH$_3$CO		20.79	2.02 (3H, s)	**CO**
1"	133.6	133.69		H-3"
2", 6"	127.0	127.04	7.70 (2H, m)	H-3", H-4", H-5"
3", 5"	128.6	128.59	7.42 (2H, m)	H-2", H-4", H-5", H-6"
4"	131.9	131.92	7.52 (1H, tt, 6.5, 1.5)	H-2", H-6",
7"	167.1	167.11		H-2", H-6", H-10, H-8

$^{a}\delta_C$ of α-acetylamino-phenylpropyl α-benzoylamino-phenylpropanoate recorded in $CDCl_3$ [12].

Compound **5** was obtained as yellow powder, mp. 265-267 °C. The ESI-MS of **5** provided an molecular ion peak at *m/z* 595 $[M+H]^+$, corresponding to the formula $C_{30}H_{50}O$. The ^{1}H and ^{13}C NMR, and DEPT spectra of **5** suggested the presence of a kaempferol glucoside derivative and a (E)-p-coumaroyl moiety.

The ^{1}H-NMR of **5** showed the presence of aromatic protons at δ_H 7.99 (2H, H-2′/6′); 6.86 (2H, H-3′/5′) and δ_H 7.37 (2H, H-2‴/6‴); 6.78 (2H, m, H-3‴/5‴) characterizing two AA′BB′ spin systems of the flavonoid B ring and the coumaroyl group. In addition, the signals at δ_H 7.32 (1H, d, *J* = 15.5 Hz, H-7‴) and 6.12 (1H, d, *J* = 15.5 Hz, H-8‴) of two *trans* olefinic protons were attributed to H-8‴ and H-7‴ of a (*E*)-*p*-coumaroyl moiety. The ^{1}H-NMR spectrum also supported the presence of one *β*-D-glucose moiety with an anomeric proton signal at δ_H 5.45 (1H, d, *J* = 7.5 Hz, H-1″), δ_H 3.17 ~ 3.3 (4H, m), and methylene protons at δ_H 4.28 (1H, dd, 2.0, 12.5, H-6″a) and 4.02 (1H, dd, 6.0, 12.5, H-6″b).

The ^{13}C-NMR and DEPT spectra of **5** exhibited signals of 30 carbons, including 12 quaternary, 01 methylene and 17 methine carbons. The ^{13}C-NMR spectrum revealed the signals of carbonyl carbons at δ_C 177.35 and 166.1 and oxygene bearing carbon at δ_C 156.37 (C-2);

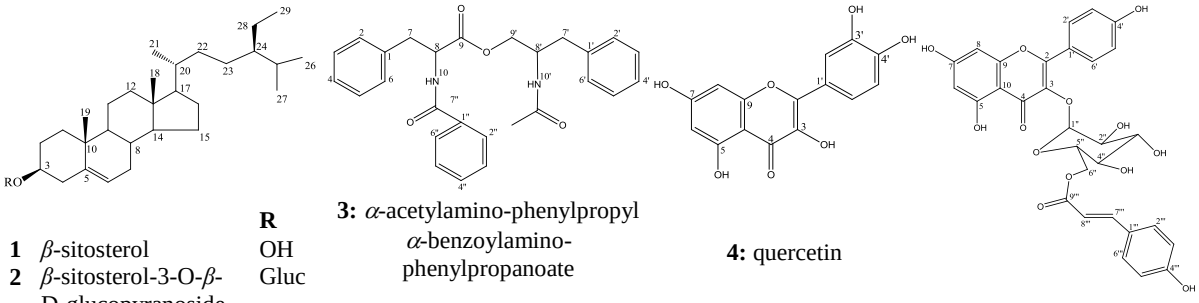
161.10 (C-5); 164.15 (C-7); 156.31 (C-9); 159.94 (C-4'); 159.73 (C-4'''). The ^{13}C-NMR spectrum also supported the presence of one *β*-D-glucose moiety with signals at δ_C 100.95 (C-1''); 74.08 (C-2''); 76.19 (C-3''); 69.92 (C-4''); 74.19 (C-5''); and 62.93 (C-6'').

The HMBC spectrum of **5** showed correlations between the proton at δ_H 5.45 (H-1'') with C-3 (δ_C 133.03), and between H-6'' (δ 4.02/4.28) and C-9''' (δ_C 166.1) indicating that the sugar unit is attached at the C-3 position and the (*E*)-*p*-coumaroyl group is linked to C6''. Furthermore, the HMBC spectrum also showed correlations between the proton at δ_H 6.12 (1H, d, *J* = 15.5 Hz, H-8''') and 7.32 (1H, d, *J* = 15.5 Hz, H-7''') with C-1''' (δ_C 127.09), C-2''', C-6''' (δ_C 130.07) and C-9''' (δ_C 166.1). Based on these evidences and comparison with the reported values in literatures [14, 16, 17], **5** was assigned the structure shown in Figure 1, and identified as *trans*-tiliroside.

Table 2. The ^{1}H (500 MHz, DMSO-*d6*) and ^{13}C-NMR (125 MHz, DMSO-*d6*) data of **5**.

C	[16]	**5**		C	[16]	**5**	
	[a]δ_C	δ_C	δ_H		[a]δ_C	δ_C	δ_H
2	156.4	156.37	-	1''	101.0	100.95	5.45 (1H, d, 7.5)
3	133.1	133.03	-	2''	74.1	74.08	3.20 m
4	177.4	177.35	-	3''	76.2	76.19	3.27 m
5	161.2	161.1	-	4''	69.9	69.92	3.17 m
6	98.8	98.71	6.15 (d, 2.0)	5''	74.2	74.19	3.30 m
7	164.2	164.15	-	6''	63.0	62.93	4.02 (1H, dd, 12.5, 6.0) 4.28 (1H, dd, 12.5, 2.0)
8	93.7	93.61	6.38 (d, 2.0)	1'''	124.9	124.88	-
9	156.3	156.31	-	2''', 6'''	130.2	130.07	7.37 (2H, m)
10	103.9	103.81	-	3''', 5'''	115.8	115.7	6.78 (2H, m)
1'	120.8	120.72	-	4'''	159.8	159.73	-
2', 6'	130.8	130.8	7.99 (2H, d, 9.0)	7'''	144.6	144.52	7.32 (1H, d, 15.5)
3', 5'	115.1	115.0	6.86 (2H, d, 9.0)	8'''	113.6	113.61	6.12 (1H, d, 15.5)
4'	160.0	159,9	-	9'''	166.2	166.1	-
		5-OH	12.56 s			4'-OH	11,5 brs

[a] δ_C of *trans*-tiliroside recorded in DMSO-*d6* [16].



1 *β*-sitosterol
2 *β*-sitosterol-3-O-*β*-D-glucopyranoside

R
OH
Gluc

3: *α*-acetylamino-phenylpropyl *α*-benzoylamino-phenylpropanoate

4: quercetin

5: *trans*-tiliroside

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

4. CONCLUSIONS

Using various chromatographic techniques, five compounds: *β*-sitosterol (**1**), *β*-sitosterol-3-O-*β*-D-glucopyranoside (**2**), 2-acetylamino-3-phenylpropyl 2-benzoylamino-3-phenylpropanoate (**3**), quercetin (**4**), and *trans*-tiliroside (**5**) were isolated from the *n*-hexane and ethyl acetate extracts of *Urena lobata* L.. Their chemical structures were elucidated by interpretation of their spectroscopic data as well as by comparison of those with literature data. To the best of our knowledge, this is the first time the compounds 2-acetylamino-3-phenylpropyl 2-benzoylamino-3-phenylpropanoate (**3**) and *trans*-tiliroside (**5**) have been isolated from the Vietnamese *Urena lobata* L.. We are continuing our studies on the isolation and structure elucidation of other components of this plant.

Acknowledgements. This work is supported by a research project (Code: 04/HĐ-KHCN) granted by Ministry of Agriculture & Rural Development.

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