Journal of Medicinal Materials, 2022, Vol. 27, No. 2 (pp. 67 - 71)

PHENOLIC COMPOUNDS AND A TRITERPENOID FROM THE STEM BARK OF *LIMONIA ACIDISSIMA* GROFF, RUTACEAE

Dang Thi Bao Tram, Nguyen Minh Tu, Tran Duy Hien, Nguyen Le Thanh Tuyen, Do Thi Hong Tuoi, Tran Thi Van Anh*

Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City

*Corresponding author: ttvananh@ump.edu.vn

(Received March 15th, 2022)

Summary

Phenolic Compounds and a Triterpenoid from the Stem Bark of *Limonia acidissima* Groff, Rutaceae

In the continuing search for active compounds from the stem bark of *Limonia acidissima*, six compounds were isolated from the petroleum ether fraction which showed cytotoxicity in RD-A cell line (IC_{50} = 42.49 µg/mL). They are ethyl divaricatinate (**1**), sekikaic acid (**2**), bergapten (**3**), auraptene (**4**), lupeol (**5**), and daucosterol (**6**). Their structures were identified based on 1D-NMR, 2D-NMR, ESI-MS and spectroscopic comparison with previous reports. Compounds **1** and **2** were isolated from *L. acidissima* for the first time. Compounds **4** and **5** showed cytotoxicity in RD-A cell line with IC_{50} values of 25.97 µM and 69.64 µM, respectively.

Keywords: *Limonia acidissima, Ethyl divaricatinate, Sekikaic acid, Bergapten, Lupeol.*

1. Introduction

Limonia acidissima Groff is a popular tree in the South of Vietnam. All parts of this plant (fruit, stem bark, leaf, and root) are used in folk medicine for the treatment of many diseases such as cough, asthma, constipation, dysentery, hepatitis [1],[2]. The fruit of *L. acidissima* was studied for the chemical constituents and antimicrobial activity [3],[4]. In the course of searching bioactive compounds from cytotoxic fractions of the stem bark of *L. acidissima*, we identified several compounds including atraric acid, four coumarins (auraptene, xanthyletin, xanthotoxin and isopimpinellin), a flavonoid ((2*S*)-5,3′-dihydroxy-4′-methoxy-6″,6″-dimethylchromeno-(7,8,2″,3″)-flavanone), a lignan ((7′*E*)-4-hydroxy-3,5′-dimethoxy-4′,7-epoxy-8,3′-neolig-7′-en-9,9′-diyil diacetate) and an alkaloid (4-methoxy-1-methyl-2-quinolone) from the dichloromethane fraction [5],[6]. Additionally, the petroleum ether fraction showed the cytotoxicity against the muscle rhabdomyosarcoma (RD-A) with an IC_{50} value of 42.49 µg/mL. As a part of our ongoing effort to study the bioactive compounds from *L. acidissima,* this study describes the isolation and structure elucidation of two phenolic derivatives, two coumarins, a triterpenoid and a sterol from petroleum ether fraction.

2. Material and methods

2.1. Plant material

The stem bark of *L. acidissima* were collected in Long Huu, Duyen Hai town, Tra Vinh province in April 2018 and authenticated by PhD. Tran Thi Van Anh (Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City). Voucher specimen of the plant (LACI0418) was deposited at the Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh city, Vietnam. Plant material was air-dried and ground to powder before further processing.

2.2. General experimental procedures

The NMR spectra were recorded by a Bruker Avance NEO 400 MHz using tetramethylsilane as an internal standard. The electrospray ionization mass spectrometry (ESI-MS) was obtained on a UPLC-ACQUITY QDa system. Column chromatography was performed with *silica gel* (40-63 µm, Merck) and Sephadex LH-20 (GE Healthcare Life). Thin-layer chromatography (TLC) was carried out on *silica gel* 60 F_{254} plates (Merck). Compounds were visualized by UV lamp and spraying with the vanillin - sulfuric acid reagent followed by heating to 105°C.

2.3. Extraction and isolation

The powder of *L. acidissima* stem bark (7.7 kg) was percolated with 96% EtOH at room temperature. The crude extract (280 g) was suspended to 1L of H_2O and extracted with petroleum ether (40-60°C) to get 16.8 g petroleum ether fraction.

The petroleum ether fraction (14.8 g) was subjected to a *silica gel* column chromatography using gradient solvent system of *n*-hexane-$CHCl_3$ (90:10 to 30:70, v/v) to obtain 23 fractions (A.1-

A.23). The fraction A.5 (760 mg) was separated by a Sephadex LH-20 column eluting with CH_2Cl_2-MeOH (9:1) to obtain 4 subfractions (A.5.1 - A.5.4). Fraction A.5.3 (20 mg) was purified through a *silica gel* column with *n*-hexane-CH_2Cl_2 (7:3, v/v) to obtain compound **1** (2.8 mg).

Fraction A.10 (3.11 g) was chromatographed on a *silica gel* column eluting with petroleum ether-$CHCl_3$-EtOAc (55: 40: 5, v/v/v) to yield 23 fractions (A.10.1-A.10.23). Sub-fraction A.10.9 (85.1 mg) was subjected to a *silica gel* column using *n*-hexane-EtOAc (8:2, v/v) as eluent to obtain compound **4** (17.0 mg). Fraction A.10.10 (292.2 mg) was separated by a *silica gel* column, eluting with petroleum ether-EtOAc (9:1, v/v) to give 10 fractions and then subfraction A.10.10.2 was further purified by a *silica gel* column with *n*-hexane-CH_2Cl_2 as mobile phase to yield compound **5** (37.7 mg). The precipitate was filtrated from fraction A.10.12 and then purified by a Sephadex-LH 20 column with MeOH to give compound **3** (5.2 mg). Fraction A.20 (463.5 mg) was chromatographed by a *silica gel* column, eluting with $CHCl_3$-MeOH (95:5, v/v) to give 6 fractions (A.20.1-A.20.6). Subfraction A.20.3 was purified by a Sephadex LH-20 column with $CHCl_3$-MeOH (9:1, v/v) to give compound **2** (12.1 mg). Compound **6** (29.3 mg) was obtained by filtration of the precipitate from the fraction A.21.

2.4. Cell viability assay of cytotoxic activity

The muscle rhabdomyosarcoma-A cell line (RD) was obtained from Pasteur Institute at Ho Chi Minh City (the origin from WHO). Cell viability was evaluated as a mitochondrial succinate dehydrogenase (SDH) activity, a marker of viable cells, using MTT test as the previous report [5]. Paclitaxel (Anzatax®, Mayne Pharma, New Zealand) was used as a reference compound.

Ethyl divaricatinate (1): pale yellow amorphous powder; ESI-MS *m/z* 239.29 $[M+H]^+$; ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H (ppm) 11.80 (1H, s, -OH), 6.33 (1H, d, J = 2.5 Hz, H-3), 6.28 (1H, d, J = 2.5 Hz, H-5), 4.40 (2H, q, J = 7.0 Hz, O-C**H$_2$**-CH_3), 3.80 (H, s, OCH_3), 2.85 (2H, m, H-1′), 1.59 (2H, m, H-2′), 1.44 (3H, t, J = 7.0 Hz, O-CH_2-**CH_3**), 0.96 (3H, t, J = 7.0 Hz, H-3′); ^{13}C-NMR (100 MHz, $CDCl_3$) δ_C (*ppm*): 104.9 (C-1), 165.8 (C-2), 99.0 (C-3), 164.0 (C-4), 110.9 (C-5), 147.9(C-6), 171.8 (C-7), 39.2 (C-1′), 25.2 (C-2′), 14.2 (C-3′), 61.4 (O-**CH_2**-CH_3), 14.3 (O-CH_2-**CH_3**), 55.4 (4-OCH_3).

Sekikaic acid (2): pale yellow amorphous powder; ESI-MS *m/z* 441.19 $[M+Na]^+$, 417.26 $[M-H]^-$; ^{1}H-NMR (400 MHz, CD_3OD): δ_H (ppm) 6.40 (1H, d, J = 2.5 Hz, H-5), 6.37 (2H, overlapped, H-3 and H-5′), 3.84 (3H, s, 4′-OCH_3), 3.83 (3H, s, 4-OCH_3), 3.13 (2H, m, H-7′), 2.95 (2H, m, H-7), 1.76 (2H, m, H-8), 1.65 (2H, m, H-8′), 1.00 (3H, d, J = 7.0 Hz, H-9′), 0.95 (3H, d, J = 7.0 Hz, H-9); ^{13}C-NMR (100 MHz, CD_3OD) δ_C (*ppm*): 107.3 (C-1), 164.9 (C-2), 99.9 (C-3), 165.4 (C-4), 111.0 (C-5), 149.2 (C-6), 39.3 (C-7), 26.1 (C-8), 14.6 (C-9), 55.8 (4-OCH_3), 113.3 (C-1′), 157.1 (C-2′), 126.1 (C-3′), 154.3 (C-4′), 105,0 (C-5′), 146.5 (C-6′), 38.9 (C-7′), 26.4 (C-8′), 14.7 (C-9′), 56.2 (4′-OCH_3), 169.9 (-COO-), 175.5 (-COOH).

Begrapten (3): colorless needles; ESI-MS *m/z* 217.21 $[M+H]^+$, ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H (ppm) 8.14 (1H, d, J = 9.5 Hz, H-4), 7.59 (1H, d, J = 2.5 Hz, H-1′), 7.13 (1H, s, H-8) 7.02 (1H, d, J = 2.5 Hz, H-2′), 6.27 (1H, d, J = 9.5 Hz, H-3), 4.26 (3H, s, 5-OCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ_C (*ppm*): 161.3 (C-2), 112.6 (C-3), 139.3 (C-4), 149.6 (C-5), 112.7 (C-6), 158.4 (C-7), 93.9 (C-8), 152.7 (C-9), 106.4 (C-10), 144.8 (C-1′), 105.1 (C-2′).

Aurapten (4): pale yellow scales; ESI-MS *m/z*: 299.39 $[M+H]^+$. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ_H (ppm) 6.42 (d, J = 9,5 Hz, H-3), 7.63 (d, J = 9.5 Hz, H-4), 7.35 (d, J = 8.5 Hz, H-5), 6.84 (dd, J = 8.5; 2.0 Hz, H-6), 6.81 (d, J = 2.0 Hz, H-8), 4.60 (d, J = 6.5 Hz, 2H, H-1′), 5.46 (m, H-2′), 2.11 (m, 2H, H-4′), 2.13 (m, 2H, H-5′), 5.08 (m, H-6′), 1.61 (s, 3H, H-8′), 1.66 (s, 3H, H-9′), 1.76 (s, 3H, H-10′). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ_C (ppm): 161.3 (C-2), 113.0 (C-3), 143.4 (C-4), 128.7 (C-5), 113.2 (C-6), 162.2 (C-7), 101.6 (C-8), 155.9 (C-9), 112.4 (C-10), 65.5 (C-1′), 118.5 (C-2′), 142.3 (C-3′), 39.5 (C-4′), 25.3 (C-5′), 123.6 (C-6′), 131.9 (C-7′), 17.7 (C-8′), 25.6 (C-9′), 16.8 (C-10).

Lupeol (5): white amorphous powder, ESI-MS *m/z*: 409.59 $[M-H_2O+H]^+$, ^{1}H-NMR (400 MHz, $CDCl_3$) δ_H (ppm) 4.69 (1H, d, J = 2.0 Hz, H-29); 4.57 (1H, m, H-29); 3.19 (1H, dd, J = 11.0, 2.0 Hz, H-3), 1.68 (3H, s, H-30), 1.03 (3H, s, H-26), 0.97 (3H, s, H-23), 0.94 (3H, s, H-27), 0.83 (3H, s, H-25), 0.79 (3H, s, H-28), 0.76 (3H, s, H-24); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ_C (ppm) 38.7 (C-1), 27.4 (C-2), 79.0 (C-3), 38.9 (C-4), 55.3 (C-5), 18.3 (C-6), 34.3 (C-7), 40.8 (C-8), 50.4 (C-9), 37.2 (C-10), 20.9 (C-11), 25.1 (C-12), 38.0 (C-13), 42.8 (C-14), 27.4 (C-15), 35.6 (C-16), 43.0 (C-17), 48.3 (C-18), 48.0 (C-19), 151.0 (C-20), 29.8 (C-21), 40.0 (C-22), 28.0 (C-23), 15.4 (C-24), 16.1 (C-25), 16.0 (C-26), 14.6 (C-27), 18.0 (C-28), 109.4 (C-29), 19.3 (C-30).

Daucosterol (6): white amorphous power. ^{1}H-NMR ($CDCl_3$ + CD_3OD, 500 MHz) δ_H (ppm) 5.36 (1H, d, J = 5.5 Hz, H-6); 4.40 (1H, d, J = 7.5 Hz, H-1′), 3.85 (1H, dd, J = 11.5, 3.0 Hz, H-6′), 3.75 (1H, d, J = 11.5 Hz, H-6′; 1.01 (3H, s, H-19); 0.93 (3H, d, J = 6.5 Hz, H-21); 0.83 (3H, overlapped, H-26), 0.81 (3H, d, J = 7.0 Hz, H-27), 0.83 (3H, overlapped, H-29), 0.68 (3H, s, H-18). Rf = 0.61 (TLC *silica gel*, EtOAc-MeOH-H_2O - acid formic (57:10:7:1), v/v/v/v.)

Results and discussion

Compound **1** was obtained as pale-yellow amorphous powder. The ESI-MS of **1** revealed a *quasi*-molecular peak (*m/z* 239.29 $[M+H]^+$) which suggested its molecular weight as 238 corresponding with its molecular formula as $C_{13}H_{18}O_4$. The ^{1}H-NMR of **1** showed a singlet signal of a hydroxyl proton at δ_H 11.80 (1H, s), two aromatic protons at δ_H 6.33 (1H, d, J = 2.5 Hz) and 6.28 (1H, d, J = 2.5 Hz), one *n*-propyl group [δ_H 2.85 (2H, m, H-1′), 1.59 (2H, m, H-2′) and 0.96 (3H, t, J = 7.0 Hz, H-3′), one methoxy group at δ_H 3.80 (3H, s) and ethoxy group [δ_H 4.40 (2H, q, J = 7.0 Hz), 1.44 (3H, t, J = 7.0 Hz). The ^{13}C-NMR of **1** showed a carbonyl resonance at δ_C 171.8, six aromatic signals, one methoxy carbon at δ_C 55.4, two methyl carbons at δ_C 14.3 and 14.2 and three signals of methylene carbons (δ_C 61.4, 39.2, 25.2). NMR data suggested compound **1** to be a tetrasubstituted benzene derivative. In HMBC spectrum, the correlation of the hydroxy proton at δ_H 11.8 with carbons at δ_C 104.9, 99.0 and 164.0 indicated the hydroxy group was attached to C-2 (δ_C 165.8) and hence the methoxy group was determined at C-4 (δ_C 164.0). The *n*-propyl group was connected to C-6 (δ_C 147.9) because the methylene protons at δ_H 2.85 (2H, m, H-1′) showed the correlation with C-5 (δ_C 110.9) and C-1 (δ_C 104.9). Additionally, the HMBC cross-peak of the oxygenated methylene proton at δ_H 4.40 with the carbonyl carbon at δ_C 171.8 revealed the ethoxy group at C-7 (**Fig. 1**). Analysis of the NMR data and comparison with those in literature [7] led to the identification of compound **1** as ethyl 2-hydroxy-4-methoxy-6-propylbenzoate (ethyl divaricatinate).

Compound **2** was obtained as a pale-yellow amorphous powder. The ESI-MS of **2** revealed a *quasi*-molecular peaks at [*m/z* 441.19 $[M+Na]^+$, 417.26 $[M-H]^-$] which suggested the molecular formula of **2** as $C_{22}H_{26}O_8$. The ^{1}H-NMR spectrum of **2** showed two triplet methyl signals [δ_H 0.95 (3H, t, J = 7.0 Hz) and 1.0 (3H, t, J = 7.0 Hz)], four methylene groups at δ_H 3.13, 2.95, 1.65 and 1.76 which suggested the presence of two *n*-propyl groups. Additionally, the ^{1}H-NMR spectrum of **2** showed three aromatic protons [δ_H 6.40 (1H, d, J = 2.5 Hz and 6.37 (2H, overlapped signal)] and two methoxy groups [δ_H 3.83 (3H, s) and 3.84 (3H, s]. The ^{13}C-NMR spectrum of **2** showed 22 carbon signals including two carbonyl carbons (δ_C 175.5 and 169.9), twelve aromatic carbons, two methoxy groups (δ_C 56.2 and 55.8) and six aliphatic carbons belonging to the two *n*-propyl groups. The characteristic NMR data suggested that **2** is a depside compound which composed by two aromatic units linked together by an ester bond. The ring A of **2** was established through the HMBC spectrum. The aromatic proton at δ_H 6.37 (H-3) correlated to signals at δ_C 107.3 (C-1), 111.0 (C-5), 165.4 (C-4) and 164.9 (C-2). The methylene proton δ_H 2.95 (H-7) correlated with signals of an *n*-propyl chain at δ_C 26.1 (C-8), 14.6 (C-9) and with signals of the aromatic carbons at δ_C 111.0 (C-5), 149.2 (C-6) and 107.3 (C-1). The HMBC spectrum also showed the correlation of the methoxy signal at δ_H 3.83 with the aromatic carbon at δ_C 165.4 (C-4) (**Fig. 1**). Therefore, the first unit of **2** was established as 2-hydroxy-4-methoxy-6-proylbenzoyl group. In the B ring, the HMBC of **2** showed the correlations of the aromatic proton δ_H 6.37 (H-5′) correlated to the methylene carbon δ_C 38.9 (C-7′) as well as to signal of the aromatic carbons at δ_C 126.1 (C-3′), 113.3 (C-1′), 154.3 (C-4′) and also to the carbonyl carbon at δ_C 175.5. The methylene proton at δ_H 3.13 (H-7′) correlated with signals at δ_C 26.4 (C-8′) 14.7 (C-9′), 105.0 (C-5′), 113.3 (C-1′) and 146.5 (C-6′). The HMBC spectrum also showed the correlation of the methoxy signal at δ_H 3.84 with the aromatic carbon at δ_C 154.3 (H-4′) (**Fig. 1**). Therefore, the B-ring of **2** was established as 2,3-dihydroxy-4-methoxy-6-propylbezoic. C-3′ and C-2′ of the B-ring are two oxygenated carbons but the chemical shift of C-3′ (δ_C 126.1) is more upfield than that of C-2′ (δ_C 157.1). Based on the above data the A-ring was linked to the B-ring through the ester bridge between C-1 and C-3′. Based on the analysis NMR data and comparison with the reported data [8], compound **2** was identified as sekikaic acid.

Compound **3** was obtained as colorless needles. The ESI-MS of **3** revealed a *quasi*-molecular peak at *m/z* 299.39 $[M+H]^+$; which suggested the molecular formula of **3** as $C_{12}H_8O_4$. The ^{1}H-NMR spectrum of **3** showed four doublet signals [δ_H 6.27 (d, J = 9.5 Hz), 8.14 (d, J = 9.5 Hz), δ_H 7.59 (1H, d, J = 2.5 Hz), and 7.02 (1H, d, J = 2.5 Hz)], an

aromatic proton [δ_H 7.13 (1H, s)], and a methoxy group [δ_H 4.26 (3H, s)]. The ^{13}C-NMR spectrum of **3** exhibited twelve signals including eleven aromatic carbons at the downfield region δ_C 161.3-105.1 and one methoxy group at δ_C 60.1. In the HMBC spectrum of **3**, the correlation of the proton at δ_H 8.14 with carbonyl carbon at δ_C 161.3 and oxygenated carbon δ_C 149.6; the correlation of the olefinic proton at δ_H 7.02 with oxygenated carbon at δ_C 158.4 and the correlation of the methoxy proton at δ_H 4.26 (s) to δ_C 149.6 revealed the structure of **3** as 6,7-furanocoumarin with the methoxy group at C-5 (**Fig. 1**). Based on the NMR analysis and comparison with the reported data [9], the structure of compound **5** was elucidated as bergapten.

Compound **4** was also obtained from dichloromethane fraction in the previous study and was identified as bergapten [5].

Compound **5** was obtained as a white amorphous powder. The ESI-MS spectrum revealed a *quasi*-molecular peak (*m/z* 409.59 [M-H_2O+H]$^+$),which suggested the molecular formula of **5** as $C_{30}H_{50}O$. The ^{1}H-NMR spectrum of **5** showed signals of seven methyl singlets at δ_H 1.68, 1.03 0.97, 0.94, 0.83, 0.79, 0.76, two olefinic methylene protons at δ_H 4.57 (1H, m) and 4.69 (1H, d, J = 2.0 Hz). The ^{13}C-NMR spectra showed 30 carbon signals including 2 olefinic carbons at δ_C 151.0 and 109.4, an oxymethine group at δ_C 79.0 and seven methyl groups. The HMBC showed the correlation of the olefinic protons at δ_H 4.57 and 4.69 with the methyl group at δ_C 19.3 and a methine carbon at δ_C 48.0. The NMR data identified **5** as lupeol by comparison with the published data [10].

Compound 6 was obtained as a white amorphous power. The ^{1}H-NMR spectrum of compound **6** resembled the spectrum of daucosterol in literature [10]. Compound **6** was then identified as daucosterol by comparison of its TLC characteristics under the same conditions with the authentic sample [11].

Cytotoxicity activity of the isolated compounds on RD-A cell line.

Compounds **1-3** and **5** were assessed the cytotoxic activity on RD-A cell line, using paclitaxel as the reference compound. Compounds **1-3** did not affect the cell proliferation at the concentration of 100 µM (the cell cultures showed 90 - 100% viability). Compound **5** showed activity with an IC_{50} value of 69.64 ± 1.01 µM; the IC_{50} value of paclitaxel was IC_{50} 7.59 ± 0.59 µM. Lupeol (**5**) was reported cytotoxic against several cancer cell lines [12] with its ability to inhibit topoisomerase II. Meanwhile, auraptene (**4**) was proven cytotoxic, antitumor, and anticancer activities in *in vivo* and *in vitro* studies [13]. Compound **4** was identified in dichloromethane fraction of *L. acidissima* and showed cytotoxic effect on RD-A cell line with IC_{50} of 25.97 ± 1.23 µM in previous study [5]. The presence of lupeol and auraptene in the petroleum ether fraction of *L. acidissima* could explain the cytotoxicity in RD-A cell line of this fractions.

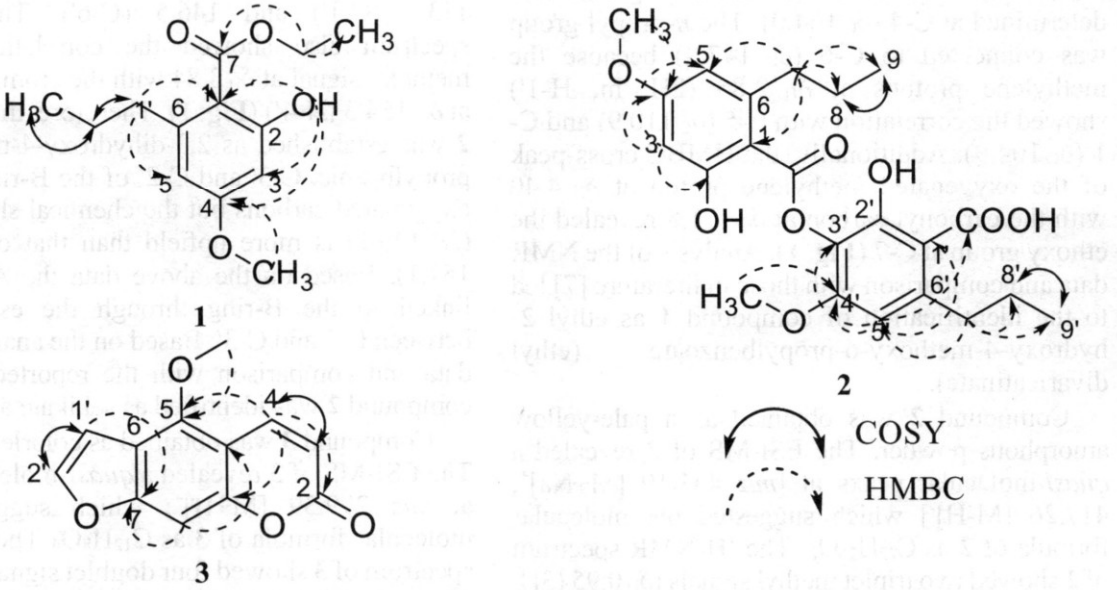

Fig 1. Structures of compounds **1**, **2** and **3** with COSY and HMBC correlation

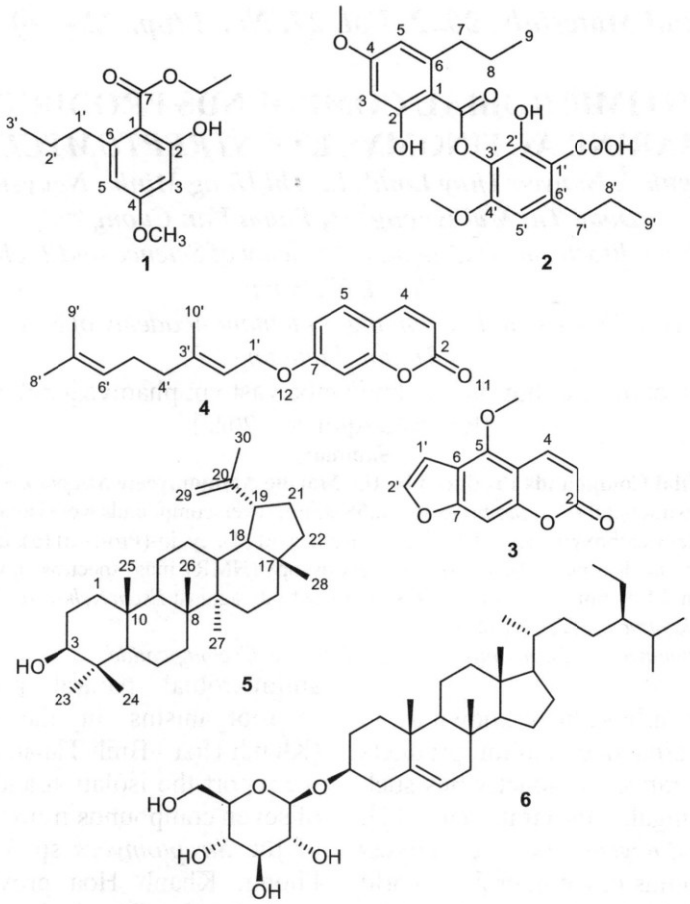

Fig 2. Chemical structures of compounds (**1-6**)

4. Conclusion

In this study, 6 compounds (**Fig.2**) were isolated from the petroleum ether fraction of *Limonia acidissima* which had cytotoxic activity on RD-A cell line. These compounds were identified as ethyl divaricatinate (**1**), sekikaic acid (**2**), bergapten (**3**), auraptene (**4**), lupeol (**5**), and daucosterol (**6**). Compounds **4** and **5** inhibited the RD-A cell viability (IC_{50} = 25.97 ± 1.23 µM and 69.64 ± 1.01 µM, respectively). Additionally, compounds **1** and **2** were reported in the *Limonia acidissima* for the first time.

References

1. Vo Van Chi (2012), *Dictionary of Vietnamese Medicinal Plants*, Medical Publishing House, Volume 1, 370-371. **2.** Vijayvargia P., Vijayvergia R. (2014), A review on *Limonia acidissima* L.: Multipotential medicinal plant, *International Journal of Pharmaceutical Sciences Review and Research*, 28(1), 191-195. **3.** Ngo Quoc Luan, Le Thi Hong Phuong, Dang Huynh Giup, Ngo Khac Khong Minh (2014), Preliminary chemical investigation on the fruit of *Limonia acidissima* L. in Tra Vinh Province, *Journal of Medicinal Materials*, 19(6), 358-362. **4.** Ngo Quoc Luan, Le Thi Hong Phuong, Dang Huynh Giup, Ngo Khac Khong Minh (2015), Contribution to investigation on chemical constituents and antimicrobial activity of the fruit of *Limonia acidissima* L. in Tra Vinh Province, *Journal of Medicinal Materials*, (6/2015), 358-362. **5.** Nguyen Minh Tu, Nguyen Le Thanh Tuyen, Do Thi Hong Tuoi, Tran Thi Van Anh (2020) Cytotoxicity of the isolated coumarines from the bark of *Limonia acidissima* Groff, Rutaceae, *Journal of Pharmaceutical Reearch,* 528 (4/2020), 72-75. **6.** Tran Thi Van Anh, Nguyen Minh Tu (2020), Chemical constituents of the stem bark of *Limonia acidissima* Groff, Rutaceae, *Journal of Medicinal Materials*, 25(3), 131-135. **7.** Tatipamula, V.B., Vedula, G.S. & Sastry, A.V.S (2019), Chemical and pharmacological evaluation of manglicolous lichen *Roccella montagnei* Bel em. D. D. Awasthi. *Future Journal Pharmaceutical Science*, 5, 8. **8.** Kumboonma, P., Senawong, T., Saenglee, S. Yenjai, C., Phaosiri, C. (2018), New histone deacetylase inhibitors from the twigs of *Melanorrhoea usitata*. *Medicinal Chemistry Research,* 27, 2004-2015. **9.** Liu R., Feng L., Sun A., Kong L. (2004), Preparative isolation and purification of coumarins from *Cnidium monnieri* (L.) Cusson by high-speed counter-current chromatography. *Journal of Chromatography A*, 1005, 71-76. **10.** Tatipamula V. B., Vedula G. S., Sastry A. V. S. (2019), Chemical and pharmacological evaluation of manglicolous lichen *Roccella montagnei* Bel em. D. D. Awasthi, *Future Journal of Pharmaceutical Sciences*, 5(8). DOI: 10.1186/s43094-019-0009-6. **11.** Park, J. H., Kwon S. J. (2009), Isolation of daucosterol and naphthalene glucoside from seeds of *Cassia mimosoides* var. *nomame* Makino. *Korean Journal of Plant Research*, 22(1), 26-30. **12.** Liu K., Zhang X., Xie L., Mao D., Chen H., Song, J., Long J., Li X., Luo J (2021). Lupeol and its derivatives as anticancer and anti-inflammatory agents: Molecular mechanisms and therapeutic efficacy, *Pharmacological Research*, 164, 105373. **13.** Zahra T. N., Nilufar T. N., Samira E. (2021). A review of auraptene as an anticancer agent. *Frontiers in Pharmacology*, 12, 698352.