Vietnam Journal of Science and Technology ***56*** *(4A) (2018) 199-204*

TRITERPENE ACIDS FROM *DOCYNIA INDICA* FRUITS AND THEIR CYTOTOXIC ACTIVITY

Le Xuan Duy[1, 2], Tran Quoc Toan[1], Do Huu Nghi[1, 2], Le Tat Thanh[1], Vu Dinh Hoang[2, 3], Young Ho Kim[4], Nguyen Manh Cuong[1, 2, *]

[1]*Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology (VAST), 18 Hoang Quoc Viet, Cau Giay, Ha Noi, Viet Nam*

[2]*Graduate University of Science and Technology, VAST, 18 Hoang Quoc Viet, Cau Giay, Ha Noi, Viet Nam*

[3]*School of Chemical Engineering, Hanoi University of Science and Technology, 1 Dai Co Viet road, Ha Noi, Viet Nam*

[4]*College of Pharmacy, Chungnam National University, Daejeon, Korea*

[*]Email: *nmcuong_inpc@yahoo.com.vn*

Received: 22 August 2018; Accepted for publication: 6 October 2018

ABSTRACT

Five triterpene acids (**1**–**5**) were isolated from ethyl acetate extract of *Docynia indica* fruits. Their structures were determined to be pomolic acid (**1**), maslinic acid (**2**), ursolic acid (**3**), 23-hydroxyursolic acid (**4**) and euscaphic acid (**5**) by comparison of physicochemical, NMR and mass spectral data with those reported in the literatures. All triterpenes (**1**–**5**) were isolated for the first time from *Docynia indica*. Compounds **1**–**5** were evaluated for their cytotoxic activity using MTT assay, of which compounds **3** (ursolic acid) and **4** (23-hydroxyursolic acid) showed cytotoxic activity against HeLa and Hep-G2 cancer cell lines.

Keywords: Docinya indica, triterpene acids, cancer cell lines, cytotoxic activity.

1. INTRODUCTION

Docynia indica (commonly called "Táo mèo" in Viet Nam), belonging to the Rosaceae family, is widely distributed in Viet Nam, India, Myanmar and some Southern provinces of China [1, 2]. In Viet Nam, *D. indica* grows mainly in the northern mountainous provinces such as Son La, Lai Chau, Ha Giang, Lao Cai and Yen Bai [1]. The fruits of *D. indica* have been used in traditional remedies for the treatment of infectious diseases, digestive disorders, dyhypeslipidemia, and hypertension [1, 2]. It was also found to possess biological activities, including anti-inflammatory [3], antioxidant and antimicrobial [4], The study results showed that the fruits of *D. indica* contain flavonoids, triterpenoids and megastigmane glycosides [3, 5]. Catechin and ferulic acid were the major phenolics present in *D. indica* fruits [4]. In this paper, we report the isolation, structural elucidation and cytotoxic activity against HeLa and HepG2 cancer cell lines of compounds **1**–**5** isolated from the ethyl acetate extract of *D. indica* fruits.

2. MATERIALS AND METHODS

2.1. Plant materials

The fruits of *Docynia indica* (Wall.) Decne. were collected in Bac Yen district, Son La province, Viet Nam in September, 2017. The plant was identified by Assoc. Prof. Tran The Bach, Institute of Ecology and Biological Resources (VAST), Viet Nam. A voucher specimen was deposited in Institute of Natural Products Chemistry (VAST), Ha Noi, Viet Nam.

2.2. General experimental procedures

^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were measured on a Bruker Avance 500 MHz spectrometer, Institute of Chemistry. ESI-MS were obtained from an Agilent 1100 Series LC/MSD Trap SL, Institute of Chemistry. Chromatographic separation was carried out on silica gel (Si 60 F254, 40-60 mesh, Merck) and RP-18 column. All solvents were redistilled before use. Pre-coated TLC plates (Si 60 F254) were used for analytical purposes.

2.3. Extraction and isolation

Dried powdered fruits of *D. indica* (3.0 kg) were extracted with methanol (3 × 8.0 L) at room temperature and then concentrated under reduced pressure to yield a black crude methanol extract (350 g). The crude metanol extract was suspended in hot methanol-water (1/1, v/v) and successively partitioned with hexane, dichloromethane and ethyl acetate. The resulting fractions were concentrated under reduced pressure to give the corresponding hexane (22 g), dichloromethane (36 g) and ethyl acetate extracts (114 g).

The ethyl acetate fraction (114 g) was subjected to a silica gel chromatography column (CC), eluting with dichloromethane:methanol (100:0 to 0:100, v/v) to afford 9 fractions (E1 – E9). Fraction E3 (10.5 g) was chromatographed on a silica gel CC, eluting with dichloromethane/aceton (20:1, v/v) to afford 5 fractions (E3A – E3E). Fraction E3B (1.12 g) was further chromatographed on a silica gel CC, eluting with dichloromethane/methanol (30:1, v/v) to afford 4 subfractions (E3B1 – E3B4). Compounds **1** (12 mg) and **2** (14.5 mg) were obtained from subfraction E3B2 (162 mg) by using RP-18 column, eluting with methanol/water (5:1, v/v). The subfraction E3B3 (89 mg) was rechromatographed over a RP-18 column eluting with methanol/water (6:1, v/v) to yield compound **3** (11 mg). Fraction E3C (0.98 g) was further separated on a normal silica gel CC, eluting with dichloromethane/methanol (35:1, v/v) to afford 5 subfractions (E3C1 – E3C5). Compounds **4** (13 mg) and **5** (20 mg) were obtained from subfraction E3C3 (92 mg) by using RP-18 column, eluting with methanol/water (4:1, v/v).

Pomolic acid (**1**): white powder; EI-MS *m/z*: 473 $[M + H]^+$, ($C_{30}H_{48}O_4$); ^{1}H-NMR (500 MHz, Pyridine-d_5) δ_H (ppm): 0.90 (3H, s, H-25), 1.01 (3H, s, H-24), 1.10 (3H, s, H-26), 1.09 (3H, d, J = 6.4 Hz, H-30), 1.22 (3H, s, H-23), 1.44 (3H, s, H-29), 1.72 (3H, s, H-27), 3.06 (1H, s, H-18), 3.12 (1H, td, J = 12.8, 4.4 Hz, H-16), 3.42 (1H, dd, J = 10.8, 5.2 Hz, H-3), 5.09, (1H, s, OH-19), 5.60 (1H, t-like, H-12); ^{13}C-NMR (125 MHz, Pyridine-d_5) δ_C (ppm): 39.4 (C-1), 28.1 (C-2), 78.2 (C-3), 39.4 (C-4), 55.9 (C-5), 18.9 (C-6), 33.6 (C-7), 40.3 (C-8), 47.8 (C-9), 37.3 (C-10), 24.0 (C-11), 128.0 (C-12), 140.0 (C-13), 42.1 (C-14), 29.3 (C-15), 26.4 (C-16), 48.4 (C-17), 54.6 (C-18), 72.7 (C-19), 42.4 (C-20), 26.9 (C-21), 37.3 (C-22), 28.8 (C-23), 16.8 (C-24), 16.5 (C-25), 17.4 (C-26), 24.7 (C-27), 180.0 (C-28), 27.1 (C-29), 16.5 (C-30).

Maslinic acid (**2**): white powder; EI-MS *m/z*: 473 $[M + H]^+$, ($C_{30}H_{48}O_4$); ^{1}H-NMR (500 MHz, Pyridine-d_5) δ_H (ppm): 5.27 (1H, t, *J* = 3.5 Hz, H-12), 3.62 (1H, m, H-2), 2.91 (1H, d, *J* = 10.0 Hz, H-3), 1.17 (3H, s, H-23), 1.02 (3H, s, H-27), 1.01 (3H, s, H-24), 0.95 (3H, s, H-30), 0.91 (3H, s, H-25), 0.82 (3H, s, H-26), 0.81 (3H, s, H-29); ^{13}C-NMR (125 MHz, Pyridine-d_5) δ_C (ppm): 47.3 (C-1), 68.0 (C-2), 83.2 (C-3), 39.4 (C-4), 55.5 (C-5), 18.5 (C-6), 33.2 (C-7), 39.6 (C-8), 47.9 (C-9), 38.2 (C-10), 23.1 (C-11), 121.7 (C-12), 144.5 (C-13), 41.7 (C-14), 27.9 (C-15), 23.8 (C-16), 45.9 (C-17), 41.3 (C-18), 46.2 (C-19), 30.7 (C-20), 33.9 (C-21), 32.7 (C-22), 28.3 (C-23), 16.8 (C-24), 16.5 (C-25), 17.1 (C-26), 25.3 (C-27), 179.9 (C-28), 32.8 (C-29), 24.3 (C-30).

Ursolic acid (**3**): white powder; EI-MS *m/z*: 457 $[M + H]^+$, ($C_{30}H_{48}O_3$); ^{1}H-NMR (500 MHz, Pyridine-d_5) δ_H (ppm): 5.47 (1H, br s, H-12), 3,44 (1H, dd, *J* = 10.0, 5.5 Hz, H-3), 2.62 (1H, d, *J* = 11.0 Hz, H-18), 2.31 (1H, td, *J* = 13.0, 3.5 Hz, H-15), 2.10 (1H, td, *J* = 13.5, 4.0 Hz, H-16), 1.23 (3H, s, H-23), 1.21 (3H, s, H-27), 1.03 (3H, s, H-26), 1.01 (3H, s, H-24), 0.98 (3H, d, *J* = 6.0 Hz, H-29), 0.95 (3H, d, *J* = 6.0 Hz, H-30), 0.87 (3H, s, H-25); ^{13}C-NMR (125 MHz, Pyridine-d_5) δ_C (ppm): 38.6 (C-1), 28.2 (C-2), 72.9 (C-3), 39.0 (C-4), 55.2 (C-5), 18.4 (C-6), 33.1 (C-7), 39.5 (C-8), 47.6 (C-9), 37.0 (C-10), 23.3 (C-11), 125.3 (C-12), 138.9 (C-13), 42.0 (C-14), 29.2 (C-15), 24.5 (C-16), 47.6 (C-17), 53.0 (C-18), 39.0 (C-19), 38.9 (C-20), 30.5 (C-21), 36.8 (C-22), 28.3 (C-23), 16.1 (C-24), 15.1 (C-25), 17.0 (C-26), 23.5 (C-27), 179.8 (C-28), 17.5 (C-29), 21.0 (C-30).

23-Hydroxyursolic acid (**4**): white powder; EI-MS *m/z*: 473 $[M + H]^+$, ($C_{30}H_{48}O_4$); ^{1}H-NMR (500 MHz, Pyridine-d_5) δ_H (ppm): 5.48 (1H, br s, H-12), 4.22 (1H, dd, *J* = 9.6, 6.0 Hz, H-3), 4.18 (1H, d, *J* = 10.4 Hz, H-23a), 3.72 (1H, d, *J* =10.4 Hz, H-23b), 1.17 (3H, s, H-27), 1.06 (3H, s, H-26), 1.04 (3H, s, H-24), 0.98 (3H, d, *J* = 6.4 Hz, H-29), 0.96 (3H, s, H-25), 0.92 (3H, d, *J* = 6.4 Hz, H-30); ^{13}C-NMR (125 MHz, Pyridine-d_5) δ_C (ppm): 38.4 (C-1), 27.2 (C-2), 72.8 (C-3), 42.4 (C-4), 48.0 (C-5), 18.1 (C-6), 30.6 (C-7), 39.5 (C-8), 47.5 (C-9), 36.6 (C-10), 23.2 (C-11), 125.0 (C-12), 138.7 (C-13), 42.0 (C-14), 28.2 (C-15), 24.5 (C-16), 47.5 (C-17), 53.1 (C-18), 39.0 (C-19), 38.9 (C-20), 37.0 (C-21), 32.8 (C-22), 67.3 (C-23), 12.7 (C-24), 15.7 (C-25), 17.1 (C-26), 23.4 (C-27), 178.7 (C-28), 17.1 (C-29), 20.9 (C-30).

Euscaphic acid (**5**): white powder; EI-MS *m/z*: 489 $[M + H]^+$, ($C_{30}H_{48}O_5$); ^{1}H-NMR (500 MHz, Pyridine-d_5) δ_H (ppm): 5.55 (1H, br s, H-12), 4.27 (1H, dt, *J* = 10.0, 3.5 Hz, H-2), 3.72 (1H, d, *J* = 2.5 Hz, H-3), 3.11 (1H, ddd, *J* = 13.5, 13.0, 4.5 Hz, H-16), 3.01 (1H, s, H-18), 2.29 (1H, ddd, *J* = 13.5, 13.0, 4.0 Hz, H-15), 1.59 (3H, s, H-27), 1.38 (3H, s, H-29), 1.22 (3H, s, H-23), 1.07 (3H, d, *J* = 6.0 Hz, H-30), 1.05 (3H, s, H-26), 0.94 (3H, s, H-25), 0.85 (3H, s, H-24); ^{13}C-NMR (125 MHz, Pyridine-d_5) δ_C (ppm): 42.0 (C-1), 66.7 (C-2), 79.6 (C-3), 39.0 (C-4), 48.8 (C-5), 18.8 (C-6), 33.6 (C-7), 40.8 (C-8), 47.8 (C-9), 39.4 (C-10), 24.2 (C-11), 128.6 (C-12), 138.9 (C-13), 42.2 (C-14), 29.2 (C-15), 26.9 (C-16), 48.7 (C-17), 54.8 (C-18), 73.3 (C-19), 42.6 (C-20), 26.3 (C-21), 38.7 (C-22), 28.7 (C-23), 16.4 (C-24), 16.4 (C-25), 17.2 (C-26), 24.5 (C-27), 183.8 (C-28), 26.7 (C-29), 16.2 (C-30).

2.4. Cytotoxicity assay

Cell viability was assessed through MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay [6, 7]. Dilute cells in 96-well microplates to a density of 5×10^4 cells/well including 200 µL mixtures. The samples at different concentrations ranging from 1 to 50 µg/mL and Paclitaxel used as positive control were added to the cells and incubated at 37 °C and 5 % CO_2 for 48 h. At the end of incubations, 20 µL of MTT (Sigma-Aldrich, Singapore) was added to the wells and incubated for at 37 °C for 4 h. Absorbance was recorded

at 540/720 nm using Infinite F50 ELISA plate reader (Tecan, Männedorf, Switzerland). All the experiments were repeated at least thrice independently. The growth inhibition was assessed using the following formula: Inhibition rate (%) = (1 - OD_{saml}/OD_{con}) × 100 %, where OD_{sampl} and OD_{con} are the optical densities of the experimental sample groups and control, respectively. Cytotoxicity is expressed as IC_{50} value (the concentration of test agents that cause 50 % inhibition or cell death), which was obtained by nonlinear regression by using TableCurve 2D program (SPSS Statistical Software, Chicago, IL).

3. RESULTS AND DISCUSSION

The methanol extract of *D. indica* fruits was partitioned into hexane-, dichloromethane-, ethyl acetate and water extracts. Chromatographic purification of the ethyl acetate extract led to the isolation of five compounds (**1**–**5**) (Figure 1).

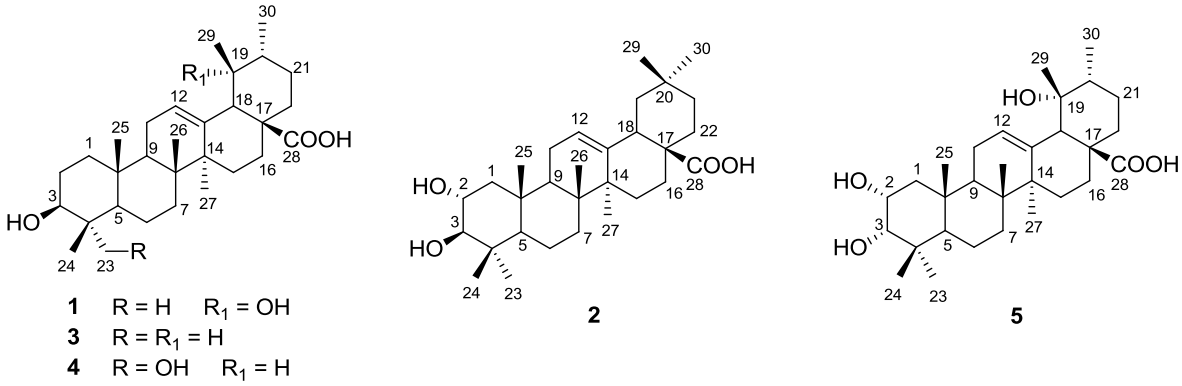

Figure 1. Structures of triperpene acids (**1**-**5**) from *Docynia indica* fruits.

Compound **1** was obtained as a white powder. The molecular formula of **1** was $C_{30}H_{48}O_4$ due to the presence of an ion peak at *m*/*z* 473 $[M + H]^+$ in the electron ionization mass spectrometry (ESI-MS). In the ^{1}H-NMR spectrum, an olefinic methine (δ_H 5.60, H-12), a proton of a hydroxyl group (δ_H 5.09, OH-19) and an oxymethine proton at δ_H 3.42 (H-3) were observed. The ^{1}H-NMR spectrum of **1** also showed signals for seven methyl groups [δ_H 0.90 (H-25), 1.01 (H-24), 1.10 (H-26, H-30), 1.22 (H-23), 1.44 (H-29), and 1.72 (H-27)] (Figure 1). The ^{13}C-NMR spectrum of **1** showed signals corresponding to 30 carbons, including a carboxylic carbon at δ_C 180.0 (C-28), a quaternary oxygenated carbon at δ_C 72.7 (C-19), two olefinic carbons at δ_C 128.0 (C-12), 140.0 (C-13) and seven methyl carbons at δ_C 28.8 (C-23), 16.8 (C-24), 16.5 (C-25), 17.4 (C-26), 24.7 (C-27), 27.1 (C-29) and 16.5 (C-30) (Figure 1). These above ^{1}H-NMR and ^{13}C-NMR data suggested that **1** was assignable to a dammarane-type triterpene [8]. By comparison with the published data, compound **1** was determined to be pomolic acid [8].

Compound **2** was obtained as a white powder. The molecular formula of **2** was $C_{30}H_{48}O_4$ due to the presence of an ion peak at *m*/*z* 473 $[M + H]^+$ in the electron ionization mass spectrometry (ESI-MS). The ^{1}H-NMR spectrum of **2** also showed signals for an olefinic methine (δ_H 5.27, H-12), two oxymethine protons at δ_H 3.62 (H-2), 2.91 (H-3) and seven methyl groups [δ_H 1.17 (H-23), 1.02 (H-27), 1.01 (H-24), 0.95 (H-30), 0.91 (H-25), 0.82 (H-26) and 0.81 (H-29) (Figure 1). The ^{13}C-NMR spectrum of **2** showed signals corresponding to 30 carbons, including a carboxylic carbon at δ_C 179.9 (C-28), two oxygenated methine carbon at δ_C 68.0 (C-

2), 83.2 (C-3), two olefinic carbons at δ_C 121.7 (C-12), 144.5 (C-13) and seven methyl carbons at δ_C 28.3 (C-23), 16.3 (C-24), 17.1 (C-25), 17.1 (C-26), 24.3 (C-27), 28.8 (C-29), 24.3 (C-30) (Figure 1). These above ^{1}H- and ^{13}C-NMR data suggested that **2** were assignable to an oleane-type triterpene [9]. Thus, the structure of **2** was determined to be maslinic acid by comparison with the published data [9].

Compound **3** was obtained as a white powder. The molecular formula of **3** was clarified as $C_{30}H_{48}O_3$ based on the ion at *m/z* 457 $[M + H]^+$ in the ESI-MS spectrum. The ^{1}H-NMR and ^{13}C-NMR spectra of **3** were similar to those of **1** except for the lack of hydroxyl group at C-19 (δ_C 39.0) in **3** (Figure 1). Therefore, compound **3** was identified as ursolic acid when compared with literature data [10].

Compound **4** was obtained as a white powder. Its molecular formula was $C_{30}H_{48}O_4$ due to the observation of an ion at *m/z* 473 $[M + H]^+$ in the ESI-MS spectrum. The ^{1}H-NMR and ^{13}C-NMR spectra of **4** were similar to ursolic acid (**3**). However, the methyl group located at C-23 of compound **3** was replaced by a methylene hydroxy group [δ_H 4.18 (H-23a) and 3.72 (H-23b), δ_C 67.3 (C-23)] of **4** (Figure 1). Based on the above analysis, the structure of compound **4** was determined to be 23-hydroxyursolic acid by comparison with the published data [9, 11].

Compound **5** was obtained as a white powder. The molecular formula of **5** was $C_{30}H_{48}O_5$ due to the presence of an ion peak at *m/z* 489 $[M + H]^+$ in the ESI-MS. The ^{1}H-NMR and ^{13}C-NMR spectra of **5** were similar to those of **1** except for the presence of hydroxyl group at C-2 [δ_H 5.55 (H-2) and δ_C 66.7 (C-2)] and a hydroxyl at C-3 was α-orientation [δ_H 3.72 (1H, d, J = 2.5 Hz, H-3)] in **5** (Figure 1). Therefore, compound **5** was identified as euscaphic acid when compared with literature data [12].

Table 1. Cytotoxic activity of isolated compounds (**1**–**5**) against HeLa and Hep-G2 cancer cell lines.

Compounds	IC_{50} (μM)	
	HeLa	Hep-G2
1	> 50	> 50
2	> 50	> 50
3	29.8	33.2
4	31.7	30.7
5	> 50	> 50
Paclitaxel[a]	0.027	0.038

[a] Used as positive control.

Compounds **1**–**5** were evaluated for their *in vitro* cytotoxic activity against HeLa and Hep-G2 cancer cell lines using MTT assay method. As a result, ursolic acid (**3**) and 23-hydroxyursolic acid (**4**) showed weak cytotoxic activity against HeLa cancer cell line with IC_{50} values of 29.8 and 31.7 μM, respectively (Table 1). In the case of Hep-G2 cancer cell line, ursolic acid (**3**) and 23-hydroxyursolic acid (**4**) also showed cytotoxic activity with IC_{50} values of 33.2 and 30.7 μM, respectively. Neverthless, compounds **1**, **2** and **5** did not show cytotoxic activity against two cancer cell lines in this test.

4. CONCLUSION

Five known compounds, pomolic acid (**1**), maslinic acid (**2**), ursolic acid (**3**), 23-hydroxyursolic acid (**4**) and euscaphic acid (**5**) were isolated from the ethyl acetate extract of *D. indica* fruits. Their chemical structures were determined by the interpretation of NMR spectral data and comparison with published data. Compounds **3** and **4** showed cytotoxic activity against HeLa and Hep-G2 cancer cell lines.

REFERENCES

1. Do T. L. - The medicinal plants and traditional medicines in Viet Nam, Medical Publishing, Ha Noi, 2004, pp. 355-357 (in Vietnamese).
2. Vo V. C. - Dictionary of Medicinal Plants in Viet Nam, Medical Publishing House, Ha Noi, 2012, pp. 1097-1098 (in Vietnamese).
3. Dung H. V., Bach N. V., Trung T. N., Nhiem N. X., Tai B. H., Kiem P. V., Kim S. H. - Megastigmane glycosides from *Docynia indica* and their anti-inflammatory activities, Helvetica Chimica Acta **99** (2016) 681-686.
4. Shende K. M., Singh N. I., Negi P. S. - Phytochemical characterization and biological activities of *Docynia indica* (wall) fruit extracts, Journal of Molecular Genetics and Medicine **10** (2016) 2041-2045.
5. Thu N. T. B., Tuan V. V., Thuong P. T. - Flavonoids and chlorogenic acid from ethyl acetate fraction of the fruits of *Docynia indica*, Journal of Medicinal Materials **20** (2015) 283-285 (in Vietnamese).
6. Mosmann T. - Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, Journal of Immunological Methods **65** (1983) 55-63.
7. Yang L., Tu D., Zhao Z., Cui J. - Cytotoxicity and apoptosis induced by mixed mycotoxins (T-2 and HT-2 toxin) on primary hepatocytes of broilers *in vitro*, Toxicon **129** (2017) 1-10.
8. Cheng J. J., Zhang L. J., Cheng H. L. - Cytotoxic hexacyclic triterpene acids from *Euscaphis japonica*, Journal of Natural Products **73** (2010) 1655-1658.
9. Kim D. H., Han K. M., Chung I. S., Kim D. K., Kim S. H., Kwon B. M., Baek N. I. - Triterpenoids from the flower of *Campsis grandiflora* K. Schum. as human acyl-CoA: cholesterol acyltransferase inhibitors, Archives of Pharmacal Research **28** (2005) 550-556.
10. Alves J. S., de Castro J. C., Freire M. O., da-Cunha E. V. L., Barbosa-Filho J. M., de Silva M. S. - Complete assignment of the ^{1}H and ^{13}C NMR spectra of four triterpenes of the ursane, artane, lupane and friedelane groups, Magnetic Resonance in Chemistry **38** (2000) 201-206.
11. Furuya T., Orihara Y., Hayashi C. - Triterpenoids from *Eucalyptus perriniana* cultured cells, Phytochemistry **26** (1987) 715-719.
12. Woo K. W., Han J. Y., Choi S. U., Kim K. H., Lee K. R. - Triterpenes from *Perilla frutescens* var. *acuta* and their cytotoxic activity, Natural Product Science **20** (2014) 71-75.