Malaysian Journal of Chemistry, 2014, Vol. 16, 32–37

Chemical Constituents of the Leaves of *Eberhardtia Aurata* (Sapotaceae)

V. T. Nguyen[1,2], V. C. Pham[1], T. M. H. Doan[1*], B. N. Truong[1], T. H. Tran[2], M. Litaudon[3] and V. M. Chau[1]

[1]Institute of Marine Biochemistry, Vietnam Academy of Science and Technology,
18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam
[2]Hanoi University of Science and Technology
[3]Institut de Chimie des Substances Naturelles, Institute of Natural Product Chemistry (CNRS), France

*Corresponding author (e-mail: doanhuong7@yahoo.com)

In the framework of scientific co-operation between the Institute of Marine Biochemistry, (Vietnam Academy of Science and Technology) and the Institute of Natural Product Chemistry (Natural Product Chemistry, France) on phytochemistry of the Vietnamese flora, the plant *Eberhardtia aurata* (Pierre ex Dubard) Lecomte was selected for its cytotoxic activity on KB cell lines [14.6% inhibition at 1 μg/ml for the ethyl acetate (EtOAc) extract of the leaves]. From the EtOAc extract of the leaves of this plant, 4 oleanane triterpenes, taraxerol (**1**), taraxerone (**2**), taraxeryl acetate (**3**), 3 β-octacosanoyloxy-12-oleanen-28-ol (**4**) and 1 sterol, spinasterol (**5**) were isolated. Their structures were elucidated by using MS and NMR spectroscopic methods, including 2D NMR spectroscopy (COSY, HMQC, HMBC).

Key words: *Eberhardtia aurata*; Sapotaceae; triterpenes; ethyl acetate; cytotoxic activity

Received: November 2014; Accepted: March 2015

Eberhardtia aurata (Pierre ex Dubard) Lecomte is a plant in the Sapotaceae family. *Eberhardtia aurata* are used in Vietnam as folk medicines to treat whooping cough [1]. In the framework of scientific co-operation between the Institute of Marine Biochemistry (Vietnam Academy of Science and Technology) and the Institute of Natural Product Chemistry (CNRS, France) on phytochemistry of the Vietnamese flora, the plant *Eberhardtia aurata* (Pierre ex Dubard) Lecomte was selected for its cytotoxic activity on KB cell lines (14.6% inhibition at 1 μg/mL for the EtOAc extract of the leaves). We have previously studied the stem bark of this plant and several terpenoids were isolated and characterized [2, 3]. In this paper, we report the isolation and structural characterization of five compounds, 4 oleanane triterpenes, taraxerol (**1**); taraxerone (**2**); taraxeryl acetate (**3**); 3β-octacosanoyloxy-12-oleanen-28-ol (**4**) and 1 sterol, spinasterol (**5**) from the leaves of *Eberhardtia aurata*.

MATERIALS AND METHODS

General Experimental Procedures

Optical rotations were recorded on a Polax-2 L polarimeter in $CHCl_3$. Melting points were recorded on a Buchi B-545 instrument, and IR spectra were measured on a Nicolet Impact-410 FT-IR spectrometer. ESIMS were recorded on an Agilent 1100 LC-MSD Trap spectrometer. The ^{13}C NMR spectra were recorded on a Bruker 500.13 MHz spectrometer operating at 125.76 MHz, and ^{1}H and 2D NMR spectra were recorded on a Bruker 500.13 MHz spectrometer operating at 500.13 MHz. ^{1}H chemical shifts were referenced to $CDCl_3$ and CD_3OD at 7.27 and 3.33 ppm, respectively, while the ^{13}C chemical shifts were referenced to the central peak of $CDCl_3$ at 77.0 and 49.0 ppm for CD_3OD. For HMBC experiments the delay (1/2J) was 70 ms, and for the NOESY experiments the mixing time was 150 ms.

Plant Material

The leaves *Eberhardtia aurata* was collected in Thuan Chau, Son La, Vietnam, in October 2005, and a specimen (VN 1575F) was deposited at the Institute of Ecology and Natural Resources, Vietnam Academy of Science and Technology.

Extraction and Isolation

The dried and ground mixture of the leaves (1.0 kg) of *Eberhardtia aurata* were extracted with EtOAc (5 × 3 l), and then with MeOH (3 × 2 l) at room temperature. The resulting EtOAc and MeOH extracts were evaporated under reduced pressure to give 53.29 g and 60 g of crude extracts, respectively.

The EtOAc residue (53 g) was fractionated over a column chromatography on silica gel (63–200 µm, 0–100% EtOAc in *n*-hexan), to afford 20 fractions (F1–F20). Fraction 7 was subjected to silica gel CC, eluted with a solvent gradient of *n*-hexane/EtOAc giving 7 fractions F7.1–F7.17. Subfraction F7.1 was purified on silica gel CC, eluting with a solvent gradient of *n*-hexan/EtOAc yielded **1** (26 mg). Fraction 6 was subjected to silica gel CC, eluted with a solvent gradient of *n*-hexane/EtOAc giving 6 fractions F6.1–F6.6. Subfraction F6.2 was separated on silica gel CC (solvent gradient of *n*-hexane/EtOAc), followed by recrystallization from a mixture of *n*-hexan/EtOAc (9/1) to afford **2** (9 mg). Fraction F7.4 on silica gel CC, eluting with a solvent gradient of *n*-hexan/EtOAc, followed by recrystallization from from a mixture of *n*-hexan/EtOAc (9/1) to afford **3** (20 mg). Purification of subfraction F7.8 on silica gel CC, eluting with a solvent gradient of *n*-hexan/acetone yielded **4** (27 mg). Purification of subfraction F7.6 was crystallized from a mixture of n-hexan/EtOAc (9/1) to afford **5** (25 mg).

Taraxerol (1)

It was isolated as white amorphous solid; mp 283 – 284°C; $[\alpha]_D^{25}$ + 7 (c 0.11, $CHCl_3$) [lit: mp 282 – 283°C, $[\alpha]_D^{25}$ + 7 (c 0.52, $CHCl_3$)]; ESI-MS *m/z*: 427 $[M+H]^+$, ^{1}H-NMR ($CDCl_3$ 500 MHz) δ (ppm): 0.77 (1H, *d*, *J*= 2.0 Hz, H-5), 0.80 (3H, *s*, CH_3), 0.82 (3H, *s*, CH_3), 0.91 (6H, *s*, 2x CH_3), 0.93 (3H, *s*, CH_3), 0.95 (3H, *s*, CH_3), 0.98 (3H, *s*, CH_3), 1.09 (3H, *s*, CH_3), 1.92 (1H, *dd*, *J*=14.5, 2.0 Hz, H-16), 2.04 (1H, dt, *J*=13.0, 3.0 Hz, H-7), 3.19 (1H, *dd*, *J*=11.0, 4.0 Hz, H-3), 5.53 (1H, *dd*, *J*=8.0, 3.0 Hz, H-15). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ (ppm)**:** 15.1 (C-25). 15.2 (C-24), 17.3 (C-11), 18.6 (C-6), 21.0 (C-30), 25.6 (C-27), 26.6 (C-2), 27.7 (C-23), 28.6 (C-20), 29.5 (C-28), 29.6 (C-26), 32.9 (C-22), 33.1 (C-29), 33.5 (C-21), 34.9 (C-7), 35.6 (C-13), 36.5 (C-12), 37.4 (C-10), 37.5 (C-16), 37.6 (C-1), 37.8 (C-13), 38.5 (C-8), 38.8 (C-4), 41.1 (C-19), 48.6 (C-9), 49.0 (C-18), 55.4 (C-5), 78.7 (C-3), 116.6 (C-15), 157.9 (C-14).

Taraxerone (2)

It was isolated as white solid, mp 240 – 241 °C; $[\alpha]_D^{25}$ + 10 (c 0.1, $CHCl_3$) [lit: mp 241 – 243 °C, $[\alpha]_D^{25}$ + 14.8 (c 0.16, $CHCl_3$)]; ESI-MS *m/z*: 425 $[M+H]^+$, ^{1}H-NMR ($CDCl_3$ 500 MHz) δ (ppm): 0.83 (3H, *s*, CH_3), 0.89 (3H, *s*, CH_3), 0.91 (3H, *s*, CH_3), 0.95 (3H, *s*, CH_3), 1.06 (3H, *s*, CH_3), 1.08 (3H, *s*, CH_3), 1.09 (3H, *s*, CH_3), 1.14 (3H, *s*, CH_3), 2.34 (1H, *m*, H-1), 2.56 (1H, *m*, H-2), 5.55 (1H, *dd*, *J*=8.0; 3.0 Hz, H-15). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ (ppm): 14.8 (C-25), 17.4 (C-11), 19.9 (C-6), 21.3 (C-30), 21.4 (C-24), 25.5 (C-27), 26.1 (C-23), 28.8 (C-20), 29.8 (C-26), 29.9 (C-28), 33.1 (C-22), 33.3 (C-29), 33.6 (C-21), 34.1 (C-2), 35.1 (C-7), 35.7 (C-12), 36.7 (C-16), 37.5 (C-10), 37.7 (C-17), 37.7 (C-13), 38.3 (C-1), 38.9 (C-8), 40.6 (C-19), 47.5 (C-4), 48.7 (C-9), 48.8 (C-18), 55.8 (C-5), 117.2 (C-15), 157.6 (C-14), 217.5 (C-3).

Taraxeryl Acetate (3)

It was isolated as white solid, mp 240 – 241°C; $[\alpha]_D^{25}$ + 10 (c 0.1, $CHCl_3$) [lit: mp 241 – 243°C, $[\alpha]_D^{25}$ + 14.8 (c 0.16, $CHCl_3$)]; ESI-MS *m/z*: 469 $[M+H]^+$, ^{1}H-NMR ($CDCl_3$ 500 MHz) δ (ppm): 0.82 (3H, *s*, CH_3), 0.86 (3H, *s*, CH_3), 0.86 (3H, *s*, CH_3), 0.87 (6H, *s*, 2 x CH_3), 0.96 (3H, *s*, CH_3), 0.96 (3H, *s*, CH_3), 1.12 (3H, *s*, CH_3), 2.04 (3H, *s*, $OCOCH_3$), 4.45 (1H, dd, *J*=10.0; 6,0 Hz, H-3), 5.53 (1H, *dd*, *J*=8,0; 3,0 Hz, H-15).

3β-Octacosanoyloxy-12-oleanen-28-ol (4)

It was isolated as white solid, mp 285 – 286°C; $[\alpha]_D^{25}$ + 85 (c 0.1, $CHCl_3$); ESI-MS *m/z*: 849 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$ 500 MHz) δ (ppm): 0.83–0.89 (15H, *m*, 5 × CH_3); 0,94 (3H, *s*, CH_3), 0,95 (3H, *s*, CH_3), 1,16 (3H, *s*, CH_3), 2,89 (2H, *t*, *J*=7,0 Hz, **-CH_2**COO-), 3,21 (1H, *d*, *J*=10.5 Hz, H-28a), 3,55 (1H, *d*, *J*=10.5 Hz, H-28b), 4,49 (1H, *dd*, *J*=10,5; 5,5 Hz, H-3), 5,19 (1H, *t*, *J*=3,5 Hz, H-12). ^{13}C-NMR ($CDCl_3$, 125 MHz) δ (ppm): 14,1 (CH_3-28'); 15,5 (C-24); 16,7 (C-25); 16,7 (C-26); 18,2 (C-6); 22,0 (C-16); 22,7 (C-27'); 23,5 (C-11); 23,6 (C-30); 25,1 (C-3'); 25,5 (C-15); 25,9 (C-27); 28,0 (C-23); 28,0-29.7 $(CH_2)_n$; 30,9 (C-22); 31,0 (C-26'); 31,9 (C-20); 32,5 (C-7); 33,1 (C-29); 34,1 (C-21); 34,8 (C-2') ; 36,8 (C-17); 36,9 (C-10); 37,3 (C-4); 38,2 (C-1); 39,8 (C-8); 41,7 (C-14); 42,3 (C-18); 46,4 (C-19); 47,5 (C-9); 55,2 (C-5); 69,7 (C-28); 80,5 (C-3); 122,3 (C-12); 144,2 (C-13); 173,6 (C-1').

Spinasterol (5)

The compound was obtained as colourless needles; mp 193 – 195°C; $[\alpha]_D^{25}$ – 2.2 (c 0.18, $CHCl_3$) [lit: mp 167 – 168°C, $[\alpha]_D^{25}$ – 3 (c 1.5, $CHCl_3$)]; ^{1}H-NMR (500 MHz, $CDCl_3$) δ(ppm): 0,55 (3H, *s*, CH_3-18), 0,79 (3H, *s*, CH_3-19), 0,81 (3H, *t*, *J*= 7,0 Hz, CH_3-29), 0,83 (3H, *d*, *J*= 6,0 Hz, CH_3-27), 0,85 (3H, *d*, *J*= 6,0 Hz, CH_3-26), 1,03 (3H, *d*, *J*= 6,0 Hz, CH_3-21), 1.09 (1H, *m*, H-1a), 1.18 (3H, *m*, H-28a), 1.23 (1H, *m*, H-12a), 1,26 (3H, *m*, H-4a, H-16a, H-17), 1.39 (3H, H-2a, H-5, H-15a), 1.42 (1H, *m*, H-28b), 1.48 (1H, *m*, 11a), 1.52 (1H, *m*, H-15b), 1.55 (2H, *m*, H-24, H-25), 1.58 (1H, *m*, H-11b), 1.65 (1H, *m*, H-9), 1.72 (1H, *m*, H-4), 1.75 (2H, *m*, H-6b, H-16b), 1.78 (1H, *m*, H-2b), 1.82 (2H, *m*, H-1b, H-14), 2.03 (2H, *m*, H-12b, H-20), 3,59 (1H, *m*, H-3), 5,03 (1H, *dd*, *J*= 9,0; 15,5 Hz; H-23), 5,16 (1H, br *s*, H-7), 5,16 (1H, *dd*, *J*= 9,0; 15,5 Hz; H-22); ^{13}C-NMR (125 MHz, $CDCl_3$) δ(ppm): 12,0 (C-18), 12,2 (C-29), 13,0 (C-19), 19,0 (C-27), 21,1 (C-26), 21,4 (C-21), 21,6 (C-11), 23,0 (C-15), 25,4 (C-28), 28,5 (C-16), 29,6 (C-6), 31,5 (C-2), 31,9 (C-25), 34,2 (C-10), 37,2 (C-1), 38,0 (C-4), 39,5 (C-12), 40,3 (C-5), 40,8 (C-20), 43,3 (C-13), 49,5 (C-9), 51,2 (C-24), 55,1 (C-14), 55,9 (C-17), 70,1 (C-3), 117,5 (C-7), 129,5 (C-23), 138,1 (C-22), 139,6 (C-8).

Cytotoxic Activity Assay

The cancer cell lines (KB, LU-1, HepG2 and MCF-7) were obtained from ATCC (Manassas, VA) and were maintained in Dulbecco's D-MEM medium, supplemented with 10% fetal calf serum, L-glutamine (2 mM), penicillin G (100 UI/ml), streptomycin (100 μg/ml) and gentamicin (10 μg/ml). Stock solutions of compounds (**1** – **5**) were prepared in DMSO/H_2O (1/9), and the cytotoxicity assays were carried out in 96-well microtiter plates against different cancer cells (3×10^3 cells/ml) using a modification of the published method [4]. After 72 h incubation at 37°C in air/CO_2 (95:5) with or without test compounds, cell growth was estimated by colorimetric measurement of stained living cells by neutral red. Optical density was determined at 540 nm with a Titertek Multiscan photometer. The IC_{50} value was defined as the concentration of sample necessary to inhibit the cell growth to 50% of the control. Ellipticine was used as a reference compound.

RESULTS AND DISCUSSION

Compound **1** was isolated as white amorphous solid mp 240 – 241°C; $[\alpha]_D^{25}$ + 10 (c 0.1, $CHCl_3$) (lit: mp 241 – 243°C, $[\alpha]_D^{25}$ + 14.8 (c 0.16, $CHCl_3$) [5]. The ^{1}H NMR and ^{13}C NMR spectral data of **1** revealed the resonance of a triterpenoid compound including eight methyl groups and a vinylic proton. The ^{1}H NMR spectrum of **1** showed eight three proton singlets at δ_H 0.80 (3H, *s*, CH_3), 0.82 (3H, *s*, CH_3), 0.91 (6H, *s*, 2x CH_3), 0.93 (3H, *s*, CH_3), 0.95 (3H, *s*, CH_3), 0.98 (3H, *s*, CH_3), 1.09 (3H, *s*, CH_3). The resonance of vinylic proton (H-15) was shown as a doublet of doublet at δ_H 5.53 (1H, *dd*, *J*=8.0, 3.0 Hz, H-15). A doublet of doublet at δ_H 3.19 was assigned for oxymethine proton (H-3) with a coupling constants of 11.0 and 4.0 Hz. The large coupling of this proton with the vicinal methylene protons suggested a *β* orientation of hydroxyl group at C-3. The ^{13}C-NMR and DEPT spectra of **1** exhibited the signal of 30 carbons, including 8 methyl groups, ten methylenes, 1 sp^2 methine, 1 hydroxymethin, 3 sp^3 methine and seven quaternary carbons. Complete analysis of NMR-spectra and comparison of the NMR data indicated this compound was β-taraxerol [5–7].

Compound **2** was isolated as white solid mp 240 – 241 °C; $[\alpha]_D^{25}$ + 10 (c 0.1, $CHCl_3$) (lit: mp 241 – 243 °C, $[\alpha]_D^{25}$ + 14.8 (c 0.16, $CHCl_3$) [8]. The 1D-NMR spectra (^1H and ^{13}C) of compound **2** were very close to those of **1**, except the resonance at δ_H 3.19 signal for the oxymethine proton at C-3 in the spectrum of **1**, was absent in **2**. This suggested that the hydroxyl group at C-3 in compound **1** was replaced by a carbonyl function. The ^{13}C-NMR and DEPT spectra of **1** exhibited the signal of 30 carbons, including 1 carbonyl at δ_H 217.5 (C-3), 8 methyl groups, ten methylenes, 1 sp^2 methine, 3 sp^3 methine, and seven quaternary carbons. Complete analysis of NMR-spectra and comparison of the NMR data indicated this compound was Taraxerone [6, 8].

Compound **3** was obtained as white solid, mp 240 – 241 °C; $[\alpha]_D^{25}$ + 10 (c 0.1, $CHCl_3$) (lit: mp 241 – 243 °C, $[\alpha]_D^{25}$ + 14.8 (c 0.16, $CHCl_3$) [5]. Its ESI-MS showed the protonated molecular ion $[M + H]^+$ at *m/z* 469. The 1D-NMR spectra (^1H and ^{13}C) of compound **2** were very closed to those of **1**, except the additional signal of an acetyl group at δ_H 2.04 (3H, *s*, OAc); δ_C 21.3 (OAc). NMR-data comparison with those reported in the literature permitted establishing the structure of **3** as β-taraxeryl acetate [9].

Compound **4** was isolated as white solid, mp 285 – 286 °C; $[\alpha]_D^{25}$ + 85 (c 0.1, $CHCl_3$). Its ESI-MS showed the protonated molecular ion $[M+H]^+$ at *m/z* 849. The ^{13}C-NMR spectrum of 4 exhibited resonance signals at δ_C 122.3 and 144.2, characteristic of the 12-oleanen triterpene type, in agreement with literature. The ^1H NMR spectrum of **4** exhibited two doublet of AB system at δ_H 3.21 (1H, *d*, *J*=10.5 Hz, H-28a), 3.55 (1H, *d*, *J*=10.5 Hz, H-28b) ascribed to one hydroxymethylene group in structure. A doublet of doublet at δ_H 4.49, was assigned for oxymethine proton (H-3) with a coupling constants of 10.5 and 5.5 Hz which implied that H-3 was in the axial α position. The presence of one olefinic proton was confirmed by the triplet at δ_H 5.19 (1H, *t*, *J*=3.5 Hz) assigned to H-12. ^{13}C-NMR and DEPT spectra of **1** displayed seven methyl groups in the triterpene pattern, one oxymethine group at δ_C 80.5 (C-3), and one hydroxymethylene groups at δ_C 69.7 (C-28). The 1D- NMR (^1H and ^{13}C) spectra also indicated the presence of the long chain ester with the resonance peaks at δ_C 173.6 (C-1'), 14.1 (CH_3-28'), 28.0–29.7 $(CH_2)_n$; δ_H 2.89 (2H, *t*, *J*=7.0 Hz, -CH_2COO-), 1.25 $(CH_2)_n$. Complete analysis of NMR, MS spectra and comparison of the NMR data indicated this compound was 3β-Octacosanoyloxy-12-oleanen-28-ol [10].

Compound **5** was obtained as colourless needles mp 193 –195 °C; $[\alpha]_D^{25}$ – 2.2 (c 0.18, $CHCl_3$) (lit: mp 167 – 168 °C, $[\alpha]_D^{25}$ – 3 (c 1.5, $CHCl_3$) [11]. The ^1H-NMR spectrum of **5** indicated resonances for three olefinic protons at δ_H 5.03 (1H, *dd*, *J*= 9.0, 15.5 Hz, H-23), 5.16 (1H, br *s*, H-7), 5.16 (1H, *dd*, *J*= 9.0, 15.5 Hz, H-22); a carbinyl proton at δ_H 3.59 (1H, *m*, H-3); and six methyl protons at δ_H 00,55 (3H, *s*, CH_3-18), 0.79 (3H, *s*, CH_3-19), 0.81 (3H, *t*, *J*= 7.0 Hz, CH_3-29), 0.83 (3H, *d*, *J*= 6.0 Hz, CH_3-27), 0.85 (3H, *d*, *J*= 6.0 Hz, CH_3-26), 1.03 (3H, *d*, *J*= 6.0 Hz, CH_3-21). The ^{13}C-NMR spectral data of **5** indicated resonances for twenty-nine carbons with the following functionalities: six methyl carbons, four olefinic carbons, a carbinyl carbon, seven methine carbons, nine methylene carbons, and two quaternary carbons. These are characteristic resonances of a sterol with an alcohol and two olefinic bonds. The HMBC correlation of CH_3-19 (δ_H 0.79) and H-7 (δ_H 5.16) with C-5 (δ_C 40.3) confirmed the presence of 7,8-double bond. The another double bond could be assigned to C-22 and C-23 in view of the HMBC correlation derived from H-22 (δ_H 5.16) and H-21 (δ_H 1.03) with C-17 (δ_C 55.9). Complete analysis of 1D and 2 D-NMR spectrum and comparison of the NMR data indicated this compound was spinasterol [11–13]. Spinasterol is a phytosterol found in a variety of plant sources. Recently, the anti-tumor activity of spinasterol was demonstrated *in vivo* in studies that showed that it greatly decreased the incidence of skin tumors without co-carcinogen or co-tumor promoter activities. Several published studies have shown that spinasterol also has anti-inflammatory effects [14, 15].

Compound 1-5 were evaluated for their cytotoxicity against four cancer cell lines, KB (mouth epidermal carcinoma cells), MCF7 (human

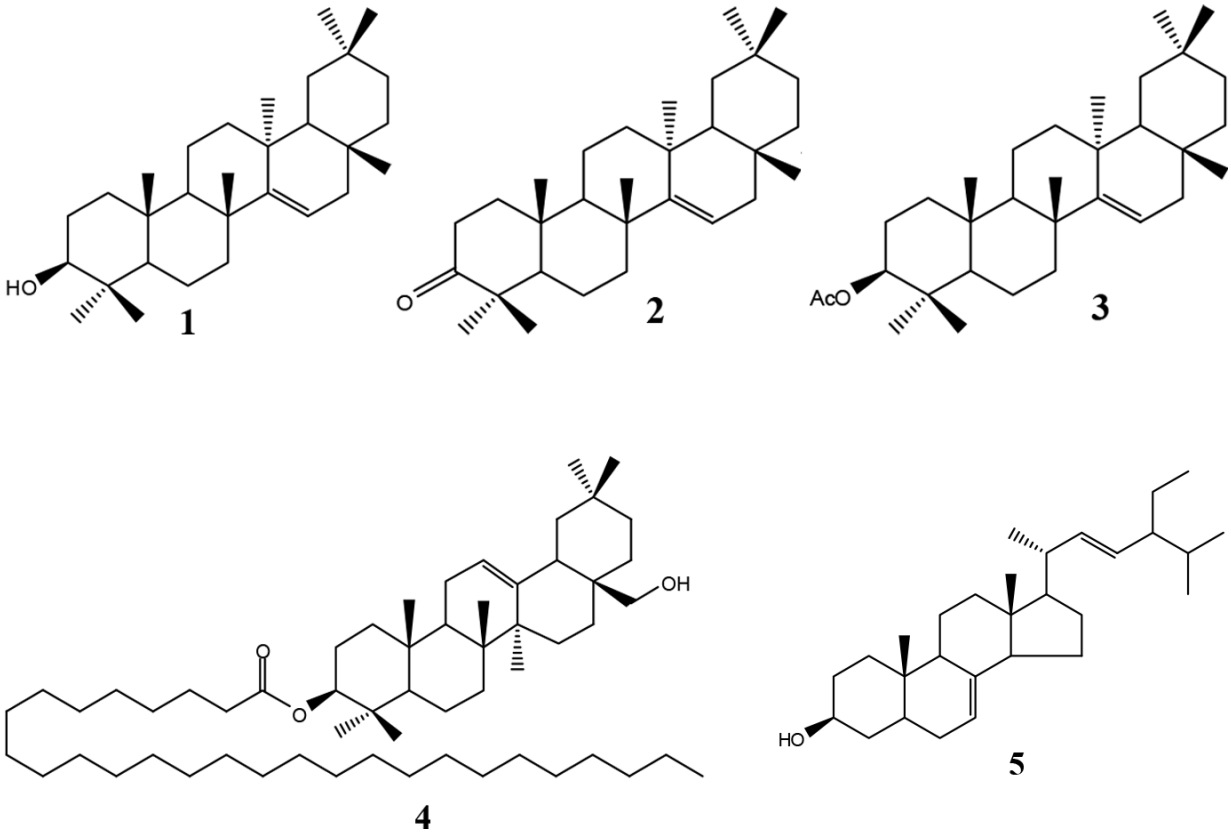

Figure 1. Form of chemical compound isolation from stem bark *Eberhartia aurata.*

breast cancer cells), LU1 (human lung cancer cells) and HepG2 (human hepatocellular liver carcinoma cell line). However, all compounds (1-5) were non-cytotoxic (IC_{50} >100 μM). Ellipticine was used as positive control (IC_{50} 0.39–0.52 μM).

CONCLUSION

In conclusion, 4 oleanane triterpenes, taraxerol (**1**), taraxerone (**2**), taraxeryl acetate (**3**), 3β-octacosanoyloxy-12-oleanen-28-ol (**4**) and 1 sterol, spinasterol (**5**) were isolated from the EtOAc extract of the leaves of *Eberhardtia aurata*. Compounds **1**, **2** and **4** were previously isolated from the stem bark of this plant. *In vitro* cytotoxic activity evaluation indicated that all the isolated compound were not cytotoxic (IC_{50} >100 μM) against four tested cancer cell lines.

ACKNOWLEDGEMENT

The authors thank Mr Dao Dinh Cuong and Mr Nguyen Quoc Binh (VAST – Vietnam) for plant collection and botanical determination. This work was supported by the Franco-Vietnamese Co-operation programme (Study of the flora of Vietnam).

REFERENCES

1. VO, V.C. (1997) *Vietnamese Medicinal Plants Dictionary*, NXB khoa học và Kỹ thuật, vol. **1**, pp. 1035–1036.
2. Doan, T.M.H., Pham, V.C., Litaudon, M. and Nguyen, V.H. (2009) Oleanan and ursan Triterpens from the stem bark *of Eberhardtia aurata* (Sapotaceae), *Journal of Science and Technology* (Vietnamese), **47**(1), 95–99.

3. Doan, T.M.H., Pham, V.C., Litaudon, M. and Nguyen, V.H. (2009) Chemical constituents of the stem bark of *Eberhardtia aurata* (Sapotaceae), *Journal of Science and Technology* (Vietnamese), **47**(6), 35–39.
4. Mosmann, T. (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays, *Journal of Immunological Methods,* **65**, 55–63.
5. Phan, V.K., Phan, T.S., Cha, V.M., Kim, Y.H., Phan, M.G. and Phạm, H.D. (2003) Study on chemical constituent of *Acanthopanax trifoliatus* (L.) Merr., Araliaceae), *Journal of Chemistry* (Vietnamese), **41**(4), 39–44.
6. Fakir, S.T., Md. Hossain, S., Chowhdury, A.M. S.U., Farhana, A., Al-Mansur, M. and Choudhury, M. (2009) HasanDhaka University phytochemical studies on the leaves of *Xylia dolabriformis*, *J. Pharm. Sci.,* **8**(2), 171–172.
7. Hernández-Chávez, I., Torres-Tapia, L.W., Simá-Polanco, P., Cedillo-Rivera, R., Moo-Puc, R. and Peraza-Sánchez, S.R. (2012) Antigiardial activity of *Cupania dentata* bark and its constituents, *J. Mex. Chem. Soc.,* **56**(2), 105–108.
8. Trinh, T.T., Tran, V.S., Frank, K. and Wessjohann, L. (2008) Triterpens from the roots of *Codonopsis pilosula*, *Journal of Chemistry,* **46**(4), 515–520.
9. Chatterji, N., Rastogi, R.P. and Dhar, M.L. (1965) Chemical examination of *Bacopa monniera.* II. The constitution of *bacoside A*, *Indian Journal of Chemistry,* **3**(1), 24–29.
10. Wandji, J., Tillequin, F., Mulholland, D.A., Wansi, J.D. , Fomum, T.Z., Fuendjiep, V. and Libot, F., and Tsabang, N. (2002) Fatty acid esters of triterpenoids and steroid glycosides from *Gambeya africana, Planta Med*., **68**(9), 822–826.
11. Ragasa, C.Y., Espineli, D.L., Mandia, E.H., Don, M-J. and Shen, C.C. (2012) A new triterpene from *Glinus oppositifolius, Chinese Journal of Natural Medicines,* **10**(4), 0284−0286.
12. Ragasa, C.Y. and Lim, K. (2005) Sterols from *Cucurbita maxima*[J], *Philipp. J. Sc.,* **134**(2), 83–87.
13. Barton, D.H.R. and Cox, J.D. (1948) Application of the method of molecular rotation differences to steroid. V. Olefinic unsaturation at the 7,8-position, *Journal of the Chemical Society*, **67,** 1354–1357.
14. Villaseñor, I.M. and Domingo, A.P. (2000) Anticarcinogenicity potential of spinasterol isolated from squash flowers, *Teratog Carcinog Mutagen*, **20**, 99–105.
15. Zhou, C.C., Sun, X.B., Liu, J.Y., Luo, S.Q. and Lu, C.Y. (1985) Anti-inflammatory effect of alpha-spinasterol, *Yao Xue Xue Bao*, **20**, 257–261.