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Isolation and characterization of triterpenes from the stem bark of *Platanus kerrii* Gagnep. and anticancer activity

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Abstract *Platanus kerrii* Gagnep. belong to the family of Platanaceae, endemic to Southeast Asia, distributed on the mountains along the borders of countries Vietnam - Laos. From the stem bark of this plant, two substances were isolated in *n*-hexane extract. The structures of these compounds have been identified as betulinal (1) and oleanolic acid (2) by modern spectroscopic analysis. The cytotoxicity of these compounds was evaluated against hepatocellular carcinoma (Hep-G2) and human breast carcinoma cells (MCF-7). Betulinal exhibited medium cytotoxicity with Hep-G2 and MCF-7 with IC_{50} 50.07, 85.63 (μg/ml) respectively while oleanolic acid showed no potiential anticancer with all cancer cell lines.

Keywords *Platanus kerrii*, stem bark, betulinal, oleanolic acid.

Introduction

The *Platanus* genus is the only living member of the Platanaceae family and consists of nine accepted species widely distributed throughout different regions of the world [1]. It is a small genus of trees known in English as the plane tree. These plants are widely planted along roads, parks, both in cities and in rural areas to improve the climate [2]. Previous studies on the *Platanus* genus indicate that this genus has interesting properties, such as *Platanus orientalis* in folk medicine used for the treatment of diseases including dermatological, gastrointestinal, rheumatic and inflammatory diseases [3], toothache, skin disease, fever, and body pain [4]. Especially, *Platanus orientalis* possesses anti-hepatotoxic, anti-oxidant and cytotoxic activities and elicits anti-inflammatory and antinoceptive effects [5-6]. Regarding chemical compositions, tocopherols, esters of phytols with fatty acids and several polyphenols were isolated from the leaves [7-8], triterpenoids, proanthocyanidins and proanthocyanidin glycosides were also isolated from the barks [9-10] and kaempferol derivatives and caffeic acid were isolated from the buds of the *Platanus* plants [11].

Platanus kerrii is woody perennial evergreen tree, endemic to Southeast Asia, distributed on the mountains along the borders of countries Vietnam - Laos and Vietnam - Cambodia [12]. The chemical composition and pharmacological activity of this plant have not been studied. In this paper, we reported the isolation and characterization of 2 steroids including betulinal (1) and oleanolic acid (2) from the bark of *Platanus kerrii*.

Materials and methods

Plant material

The barks of *Platanus kerrii* were collected from the forest in Huong Son district, Ha Tinh province, Vietnam in June 2017. The plant was identified by Dr. Tran The Bach, Institute of Ecology and Biological Resources, VietNam

Academy Of Science And Technology. A voucher specimen (PK-01) was deposited at the Faculty of Natural Sciences, Hong Duc University. The barks after collection was dried under shade and crushed into powder.

Used equipment and chemicals

The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded on a Bruker AM500 FT-NMR spectrometer using TMS as an internal standard. The electrospray ionization mass spectra (ESI-MS) were obtained on an Agilent 1260 series single quadrupole LC/MS system. Column chromatography (CC) was performed on silica gel (Merck, 230-400 mesh) or Sephadex LH-20. Thin layer chromatography used precoated silica gel plates (Merck 60 F_{254}). Compounds were visualized by spraying with Ce-Mo stain.

Isolation and characterization

The material powder (2.25 kg) was extracted with *n*-hexane (3 L x 3 times, 24 hours/time) at room temperature. The combined extracts were evaporated *in vacuo* to obtain *n*-hexane residue (3 g). The *n*-hexane residue was chromatographed on silica gel column and eluted using gradient solvents with *n*-hexane-ethyl acetate (100:1 to 0:1, v/v) to afford 12 fractions (H1-H12**)**. The H6 fraction (100 mg) was subjected to the chromatography column and eluted with n-hexane-dichloromethane (1:1, v/v) to give 6 sub-fractions H6.1-H6.6. The H6.6 fraction (45 mg) was purified by column chromatography on silica gel with dichloromethane-methanol (8:2, v/v) as eluent to give compound **1** (8 mg). The H12 fraction (0.45 g) was purified on Sephadex LH-20 column chromatography, eluted with MeOH-dichloromethane (8:2, v/v) to afford 2 sub-fractions H12.1-H12.2. The sub-fraction H12.2 (140 mg) was separated with silica gel column chromatography with the eluent *n*-hexane - ethyl acetate (7:3, v/v), followed by purification with Sephadex column to give compound **2** (3 mg).

Betulinal (**1**): White solid, mp: 192-193 ^{o}C. ESI-MS *m/z* 441.1 $[M+H]^{+}$, molecular fomular $C_{30}H_{48}O_2$ (M= 440). ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 9.68 (1H, s, H-28), 4.75 (1H, d, *J* = 1.5, Ha-29), 4.63 (1H, d, *J* = 1.5, Hb-29), 3.18 (1H, dd, *J* =11.5, 4.5 Hz, H-3), 2.86 (1H, m, H-19), 2.09-1.99 (2H, m, Ha-16, H*b*-13), 1.69 (3H, s, H-30), 0.97 (3H, s, H-27), 0.96 (3H, s, H-23), 0.91 (3H, s, H-25), 0.82 (3H, s, H-26), 0.75 (3H, s, H-24). ^{13}C NMR (125-MHz, $CDCl_3$): 206.7 (C-28), 149.6 (C-20), 110.1 (C-29), 79.0 (C-3), 59.3 (C-17), 55.3 (C-5), 50.5 (C-9), 48.1 (C-18), 47.5 (C-19), 42.6 (C-14), 40.8 (C-8), 38.8 (C-1), 38.7 (C-4), 38.7 (C-13), 37.2 (C-10), 34.3 (C-7), 33.2 (C-22), 29.9 (C-16), 29.3 (C-21), 28.8 (C-15), 28.0 (C-23), 27.4 (C-2), 25.5 (C-12), 20.7 (C-11), 19.0 (C-30), 18.3 (C-6), 16.1 (C-26), 15.9 (C-25), 15.3 (C-24), 14.3 (C-27).

Oleanolic acid (**2**): White solid, mp: 303-304 ^{o}C. ESI-MS *m/z* 429.1 $[M+H]^{+}$, molecular formula $C_{30}H_{48}O_3$ (M = 456). ^{1}H-NMR (500 MHz, $CDCl_3$, δ, ppm, *J*/Hz): 5.29 (1H, br s, H-12), 3.21 (1H, dd, J= 11.0, 3.5 Hz, H-3), 2,83 (1H, dd, J= 5.0, 10 Hz, H-18), 1.25 (3H, s, H-24); 0.98 (3H, s, H-23), 0.92 (3H, s, H-30), 0.91 (3H, s, H-25), 0.90 (3H, s, H-29), 0.77 (3H, s, H-24); 0.75 (3H, s, H-26). ^{13}C-NMR (125 MHz, $CDCl_3$, δ, ppm): 182.3 (C-28), 143.6 (C-13); 122.6 (C-12); 79.0 (C-3); 55.2 (C-5); 47.6 (C-4); 46.5 (C-17); 45.9 (C-19); 41.6 (C-14); 41.0 (C-18); 39.3 (C-8); 38.7 (C-4); 38.4 (C-1); 37.1 (C-10); 33.8 (C-21); 33.0 (C-29); 32.6 (C-7); 32.4 (C-22); 30.6 (C-20); 28.1 (C-23); 27.7 (C-15); 27.2 (C-2); 25.9 (C-27); 23.5 (C-30), 23.4 (C-16); 22.9 (C-11); 18.3 (C-6); 17.1 (C-26); 15.5 (C-25); 15.3 (C-24).

Cytotoxicity Assay

Two human cancer cell lines including hepatocellular carcinoma (Hep-G2) and human breast carcinoma (MCF-7) were obtained from the American Type Culture Collection (Manassas, VA) and cultured in DMEM medium containing 2μ M L-glutamine, 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 10 mM HEPES, 1.0 mM sodium pyruvate and Fetal Bovine Serum (FBS) 10%, under standard conditions (5% CO_2, 37 °C, 98% humidity). The culturing time depended on the cell line types. The inhibitory effects of compounds on the viability of human cancer cells were determined by the procedures established by the National Cancer Institute as described previously [13]. Culture wells were inoculated with 200μL of cell suspensions with 3.10^4 cells/mL and the test samples of compound (1) at 128, 25.6, 5.12, 1.02 and for compound (2) at 128, 21.33 and 3.55 μg/mL concentrations in DMSO. Ellipticine purchased from Sigma was also dissolved in DMSO, and tested as a positive control at several concentrations of

0.08, 0.4, 2.0, and 10.0 µg/mL. The plates were incubated for 72 h under the standard conditions (5% CO_2, 37 °C, 98% humidity). 1 mg/mL solution of MTT (50 µL) was added to each well with cell suspensions and incubated for 4 h at 37 °C in dark under humid atmosphere with 5% CO_2. At the end of incubation, the supernatant was carefully removed, and DMSO (100 µL) was added to each well. The precipitate was resuspended and incubated in dark at room temperature for 15 min. The optical density was measured on a Tecan Genios microplate reader at 450 nm. The results are expressed as the percentage of cell viability and survival in the presence of compounds. A dose-response curve was generated and the half maximal cytotoxic concentration (IC_{50}) was determined for each compound as well as each cell line.

Results and Discussion

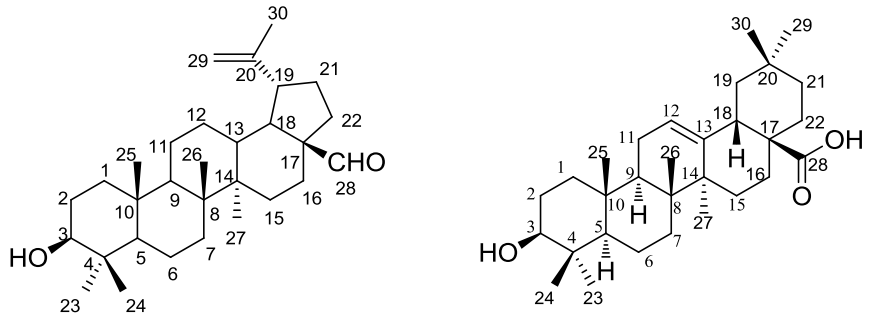

Figure 1: Chemical structures of isolated compounds from Platanus kerrii

Betulinal (1)

Compound **1** was obtained from *n*-hexane extract as a white solid, mp 192-193 ^oC. The ESI-MS spectrum showed a molecular ion peak *m/z* 441.1 $[M+H]^+$, suggested for molecular formula of **1** is $C_{30}H_{48}O_2$ (M= 440). The ^1H-NMR spectrum showed characterize a lupan-ring triterpene compound with the signal of two olefinic protons of CH_2=C at δ_H 4.75 (1H, d, *J* = 1.5, Ha-29), 4.63 (1H, d, *J* = 1.5, Hb-29), and 6 methyl singlet groups at δ_H 1.69 (3H, s, H-30), 0.97 (3H, s, H-27), 0.96 (3H, s, H-23), 0.91 (3H, s, H-25), 0.82 (3H, s, H-26), and 0.75 (3H, s, H-24). In addition, there are an aldehyde group signal at δ_H 9.68 (1H, s, H-28) and a oxymethine group signal at δ_H 3.18 (1H, dd, *J* =11.5, 4.5 Hz, H-3). The ^{13}C-NMR and DEPT spectra of **1** showed 20 carbon signals including 6 CH_3 groups at δ_C 28.0 (C-23), 19.0 (C-30), 16.1 (C-26), 15.9 (C-25), 15.3 (C-24), 14.3 (C-27), twelve methylene groups which have a CH_2=C group at δ_C 149.6 (C-20), 110.1 (C-30), six methine groups which have a CHOH group at δ_C 79.0 (C-3), 1 aldehyde group at δ_C 206.7 (C-28) and six quaternary carbon.

Analytical NMR and MS data suggest that the chemical structure of compound **1** is a lupan-ring triterpene compound with an aldehyde group and identified as betulinal. The NMR data is in good agreement with values in the reported literature (table 1) [14].

Oleanolic acid (3*β*-Hydroxy-olean-12-en-28-oic acid) (2)

Compound **2** was obtained as a white solid, mp 303-304 ^oC. The ESI-MS showed a molecular ion peak *m/z* 457 $[M+H]^+$, suggested for molecular formula of **2** is $C_{30}H_{48}O_3$ (M= 456). The ^1H NMR spectrum revealed typical signals of oleanan triterpene with seven singlet signals of methyl groups at δ_H 1.25 (3H, s, H-24); 0.98 (3H, s, H-23), 0.92 (3H, s, H-30), 0.91 (3H, s, H-25), 0.90 (3H, s, H-29), 0.77 (3H, s, H-24); 0.75 (3H, s, H-26). In addition, signals of an olefinic proton and methine hydroxyl proton are found at δ_H 5.29 (1H, br s, H-12) and 3.21 (1H, dd, *J* = 11.0, 3.5 Hz, H-3), respectively. The ^{13}C-NMR and DEPT spectra of **2** showed the signals of 30 carbons including a signal of carboxylic acid at 182.3 (C-28), signals of 2 olefinic carbon at δ_C 143.6 (C-13) and 122.6 (C-12), and signals of 7 methyl groups at 33.0 (C-29); 28.1 (C-23); 25.9 (C-27); 23.5 (C-30); 17.1 (C-26); 15.5 (C-25) and 15.3 (C-24). On the basis of the above spectral evidences, compound **2** is determined as oleanolic acid. The analytical NMR data of **2** are in accordance with those published [15].

Table 1: ^{1}H and ^{13}C-NMR data of compound **1-2** and reference compounds

C	Compound 1				Compound 2			
	[a,b]δ_H	[@]δ_H	[a,c]δ_C	[@]δ_C	[a,b]δ_H	[#]δ_H	[a,c]δ_C	[#]δ_C
1			38.8	39.0			38.4	38,3
2			27.4	27.6			27.2	28,0
3			38.8	39.0	3.21 (1H, dd, J= 11.0, 3.5 Hz)	3.21 (1H, dd, J= 11.0, 3.5 Hz)	79.0	79.9
4	3.18 (1H, dd, *J* =11.5, 4.5 Hz)	3.16 (1H, dd, *J* =11.5, 4.5 Hz)	79.0	79.0			38.7	39,9
5			38.7	38.9			55,2	56.9
6			55.3	55.5			18.3	19.6
7			18.3	18.5			32.6	33.9
8			34.3	34.5			39,3	40.7
9			40.8	41.0			47,6	49.6
10			50.5	50.6			37.1	37.9
11			37.2	37.4			22.9	24.2
12			25.5	25.7	5.29 (1H, br s)	5.29 (1H, br s)	122,6	123.5
13			38.7	38.8			143,6	145.4
14			42.6	42.7			41,6	43.0
15			28.8	29.0			27.7	28.9
16			29.9	30.1			23.4	24.3
17			59.3	59.4			45,9	47.3
18			48.1	48.3	2.83 (1H, dd, J= 5.0, 10 Hz, H-18)	2.83 (1H, dd, J= 5.0, 10 Hz, H-18)	41.0	42.8
19	2.86 (1H, m, H-19)	2.84 (1H, m, H-19)	47.5	47.6			46.5	47.5
20			149.6	149.6			30.6	31.8
21			29.3	29.4			33.8	34.9
22			33.2	33.4			32.4	33.9
23	0.96 (3H, s)	0.95 (3H, s)	28.0	28.1	0.98 (3H, s)	0.98 (3H, s)	28.1	28.0
24	0.75 (3H, s)	0.74 (3H, s)	15.3	15.5	1.25 (3H, s)	1.25 (3H, s)	15.3	16.0
25	0.91 (3H, s)	0.90 (3H, s)	15.9	16.1	0.91 (3H, s)	0.91 (3H, s)	15.5	16.4
26	0.82 (3H, s)	0.80 (3H, s)	16.3	16.3	0.75 (3H, s)	0.75 (3H, s)	17.1	17.8
27	0.97 (3H, s)	0.96 (3H, s)	14.3	14.5	0.97 (s)	0.97 (s)	25.9	26.4
28	9.68 (1H, s)	9.65 (1H, s)	206.7	206.8			181.9	181.9
29	4.75 (1H, d, *J* = 1.5), 4.63 (1H, d, *J* = 1.5)	4.74 (1H, d, *J* = 1.5), 4.61 (1H, d, *J* = 1.5)	110.1	110.1	0.90 (3H, s)	0.90 (3H, s)	33.0	33.6
30	1.69 (3H, s)	1.68 (3H, s)	19.0	19.2	0.92 (3H, s)	0.92 (3H, s)	23.5	27.6

[a]125 MHz, [b] $CDCl_3$, [c]500 MHz, @: betulinal [14], # oleanolic acid [15].

The isolated compounds were evaluated for the cytotoxicity against two human tumor cell lines including hepatocellular carcinoma (Hep-G2) and human breast carcinoma (MCF-7) by colorimetric MTT assay [13]. The cytotoxicity test results are summarized in Table 2.

Betulinal showed moderate activity against 2 cancer cell line HepG2 and MCF-7 with IC_{50} values of **50.07** and **85.63** µg/ml, respectively while oleanolic acid showed no activity in the tested cell lines.

Table 2: Cytotoxicity (IC_{50}) of isolated compounds

No	Compound	IC_{50}(µg/ml)	
		HepG2	**MCF7**
1	Betulinal (**1**)	50:07	85:63
2	Oleanolic acid (**2**)	>128	>128
3	Ellipticine	0.35	0.38

Conclusion

An investigation of the *n*-hexane extract of the bark of *Platanus kerrii* led to the isolation of two triterpene compounds including betulinal (**1**) and olenanolic acid (**2**). Their chemical structures were elucidated by spectroscopic NMR and MS data and comparison with the reported literatures. These compounds were isolated from *P. kerrii* and *Platanus* genus for the first time. The isolated compounds were tested for cytotoxicity and betulinal (1) showed moderate anticancer activity against hepatocellular carcinoma (Hep-G2) and human breast carcinoma (MCF-7) cancer cell lines.

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