Indian Journal of Natural Products and Resources
Vol. 13(1), March 2022, pp. 32-35

Cytotoxic constituents from Vietnamese *Pterospermum truncatolobatum* Gagnep.

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Received 22 October 2020; Revised 16 February 2022

Pterospermum truncatolobatum Gagnep. has long been used as a traditional medicine in Vietnam. Its crude extract showed cytotoxicity against human epidermal carcinoma (KB) cell lines. However, its chemical constituent and biological activity remains unknown. In the course of our investigation on the Vietnamese medicinal plants, four compounds, taraxerol (**1**), betulonic acid (**2**), *β*-sitosterol (**3**) and eicosanoic acid (**4**) were purified from methanolic extract of *P. truncatolobatum* by silica gel column chromatography. Their structures were determined by spectral (Mass spectrometry and nuclear magnetic resonance) analysis and by comparison with the literature reports. Of which, betulonic acid (**2**) showed moderate cytotoxicity against all four cancer cell lines, KB, MCF7 (human breast carcinoma), LU (human lung carcinoma), and HepG2 (hepatocellular carcinoma).

Keywords: Betulonic acid, Cancer, Cytotoxic, Medicinal plant, *Pterospermum truncatolobatum* Gagnep., Taraxerol.

IPC code; Int. cl. (2021.01)- A61K 36/00, A61K 127/00, A61K 135/00, A61P

Introduction

The Sterculiaceae is a big plant family in Vietnam, currently comprises 22 genus and 86 species[1]. They have been used as food, medicine, fiber and wood. Phytochemical investigation on the plants of this family revealed that they contained various secondary metabolites such as alkaloids, phenyl propanoids, flavonoids and terpenoids. Many of them have been found to be effective in treatment of common diseases such as cold, sore throats, cancer, inflammation[2-5]. The *Pterospermum* genus has 40 species, mostly distribute in tropical and subtropical Asia[6]. Chemical investigation of the plants of this genus indicated that some biologically active compounds were characterized such as cytotoxic naphthol from *P. yunnanense*[7], cytotoxic triterpenoids from *P. heterophyllum*[8], antioxidant phenolic and osteogenic compounds from *P. acerifolium*[4,9], and cytotoxic phenolic compounds from *P. lanceifolium*[10]. So far, there was no report on the chemical constituents and biological activity of the plant *Pterospermum truncatolobatum* Gagnep. When screening the biological activity of the Vietnamese medicinal plant, we found that the *n*-hexane, dichloromethane, ethyl acetate and *n*-butanol extracts of a rare plant, *P. truncatolobatum* showed moderate cytotoxic activity against KB cell lines, with the IC_{50} values of 14.57, 54.09, 7.6, and 182.5 μg/mL, respectively. Thus, phytochemical study of the extracts of the leaves and stems of this plant was carried out. This paper describes the isolation, structural elucidation and cytotoxicity of four compounds from the *n*-hexane and ethyl acetate extracts of *P. truncatolobatum*.

Materials and Methods

Plant sample collection and identification

The *P. truncatolobatum* was collected in March 2018 in Langson province, north of Vietnam and identified by Nghiem Duc Trong, Department of Botany, Hanoi Pharmacy University. Voucher specimen (LKL-1801) has been deposited at the Faculty of Chemistry, Hanoi University of Education, Vietnam.

General

Nuclear magnetic resonance spectroscopy (NMR) was recorded on a Bruker AMX-500 (500 MHz for ^{1}H-NMR spectrum and 125 MHz for ^{13}C-NMR spectrum) with an internal standard TMS. The chemical shifts (δ) are expressed in parts per million (ppm). HR-MS was recorded on a SCIEX X500 QTOF system. TLC was performed on silica gel plates (Kiesegel 60 F254, Merck). The spots of TLC were detected under UV lights (254, 302, and 366 nm) and by spraying with 10% H_2SO_4 in methanol, followed by heating at 120 °C. Column chromatography was carried out on silica gel 60 (0.2-0.5 mm, 0.04-0.063 mm, Merck).

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Extraction and Isolation

The fresh leaves and stems of *P. truncatolobatum* (4.0 kg) were dried and extracted with methanol. All solvent was removed to give the crude extract (131 g), which was partitioned between *n*-hexane, CH_2Cl_2, EtOAc, *n*-butanol and water. The *n*-hexane extract (10.8 g) was subjected to a silica gel column chromatography, using *n*-hexane/EtOAc gradient to give 11 sub-fractions. Sub-fraction 7 (0.71 g) was recrystallized to give compound **3** (17 mg). Compounds **1** (37 mg) and compound **4** (32 mg) were isolated from sub-fraction 8 (0.87 g) by silica gel column chromatography, eluting with *n*-hexane/EtOAc (6/1) and followed by recrystallization. The EtOAc extract (10 g) was chromatographed on a silica gel column, using *n*-hexane/EtOAc from 6/1 to 1/1 to give 8 sub-fractions. Sub-fraction 2 (225 mg) was purified by silica gel column, eluting with *n*-hexane/EtOAc (4/1) to afford compound **2** (18 mg) as white crystals.

Spectral data of isolated compounds

Taraxerol (**1**): ^{1}H NMR: δ_H 5.53 (dd, J = 3.5, 8.5 Hz, H-15), 3.19 (dd, J = 4.5, 11.0 Hz, H-3), 2.04 (m, H-19), 1.93 (m, H-16), 1.66 (H-2, H-7), 1.64 (m, H-2, H-16), 1.63 (m, H-1, H-11), 1.61 (m, H-6a), 1.51 (m, H-6b), 1.49 (m, H-11), 1.46 (m, H-18), 1.45 (m, H-7), 1.38 (m, H-22a), 1.36 (m, H-19), 1.31 (m, H-12), 1.26 (m, H-21), 1.09 (s, H-27), 1.03 (m, H-12), 0.99 (m, H-22b), 0.98 (m, H-1, H-9), 0.96 (s, H-23), 0.95 (s, H-29), 0.93 (s, H-24), 0.91 (s, H-26, H-30), 0.87 (m, H-5), 0.80 (s, H-28), 0.77 (s, H-25). ^{13}C NMR: δ_C 158.1 (C-14), 116.9 (C-15), 79.0 (C-3), 55.6 (C-5), 49.3 (C-18), 48.8 (C-9), 41.3 (C-19), 39.0 (C-8), 38.8 (C-4), 38.0 (C-1), 37.8 (C-10, C-13, C-17), 36.7 (C-16), 35.8 (C-12), 35.1 (C-7), 33.7 (C-21), 33.4 (C-29), 33.1 (C-22), 29.9 (C-26), 29.8 (C-28), 28.8 (C-20), 28.0 (C-23), 27.2 (C-2), 25.9 (C-27), 21.3 (C-30), 18.8 (C-6), 17.5 (C-11), 15.5 (C-25), 15.4 (C-24).

Crystal data for **1**: Program used to solve structure ShelXT[11]. Refinement: on Least Squares minimization. Crystal size: 0.4 × 0.4 × 0.3 mm^3. $C_{30}H_{50}O$, MW 426, monoclinic, $P2_12_12_1$, a = 13.5489(7) Å, b = 6.2054(3) Å, c = 30.2721(15) Å, α = 90.00°, β = 94.620(3)°, γ = 90.00°, V = 2536.9(2) Å^3, Z = 4, MoKα radiation, λ = 0.71073 Å, μ = 0.064 mm^{-1}, 6087 reflections, 298 parameters; R = 0.0833, R_w = 0.1911, S = 1.307.

Betulonic acid (**2**): ^{1}H NMR: δ_H 4.74 (d, J = 1.5 Hz, H-29a), 4.62 (d, J = 1.5 Hz, H-29b), 3.01 (m, H-19), 2.50 (m, H-2), 2.42 (m, H-2), 1.99 (m, H-15, H-22), 1.70 (s, H-30), 1.7 (m, H-12), 1.64 (m, H-18), 1.52 (m, H-6), 1.50 (m, H-21), 1.48 (m, H-22), 1.46 (m, H-16), 1.45 (m, H-11a), 1.44 (m, H-7), 1.42 (m, H-15), 1.38 (m, H-1, H-9), 1.34 (m, H-5, H-11b), 1.07 (s, H-23), 1.06 (m, H-12), 1.02 (s, H-24), 1.00 (s, H-27), 0.99 (s, H-26), 0.93 (s, H-25). ^{13}C NMR: δ_C 218.1 (C-3), 181.2 (C-28), 150.3 (C-20), 109.7 (C-29), 56.3 (C-17), 55.0 (C-5), 49.9 (C-9), 49.2 (C-18), 47.3 (C-4), 46.9 (C-19), 42.5 (C-14), 40.7 (C-8), 39.6 (C-1), 38.5 (C-13), 37.0 (C-22), 36.3 (C-10), 34.1 (C-2), 33.6 (C-7), 32.1 (C-16), 30.6 (C-15), 29.7 (C-21), 26.7 (C-23), 25.5 (C-12), 21.4 (C-11), 21.0 (C-24), 19.6 (C-6), 19.4 (C-30), 15.9 (C-25), 15.8 (C-26), 14.6 (C-27). +IDA-TOF-MS: *m/z* 477.3332 $[M+Na]^+$, calcd. for $C_{30}H_{46}O_3Na$: 477.3345.

***β*-sitosterol** (**3**): ^{1}H NMR: δ_H 5.36 (s, H-6), 3.52 (m, H-3), 1.01 (s, H-19), 0.92 (d, J = 6.5 Hz, H-21), 0.85 (t, J = 7.2 Hz, H-29), 0.84 (d, J = 6.5 Hz, H-26), 0.82 (d, J = 6.0 Hz, H-27), 0.68 (s, H-18). ^{13}C NMR: δ_C 140.8 (C-5), 121.7 (C-6), 71.8 (C-3), 56.8 (C-14), 56.1 (C-17), 50.2 (C- 9), 45.9 (C-24), 42.4 (C-4), 42.3 (C-13), 39.8 (C-12), 37.3 (C-1), 36.5 (C-10), 36.2 (C-20), 34.0 (C-22), 31.9 (C-2), 31.9 (C-8), 31.9 (C-7), 29.2 (C-25), 28.3 (C-16), 26.2 (C-15, C-23), 23.1 (C-28), 21.1 (C-11), 19.8 (C-26), 19.4 (C-27), 19.1 (C-21), 19.1 (C-18), 12.0 (C-29), 11.9 (C-19).

Eicosanoic acid (**4**): ^{1}H NMR: δ_H 2.34 (t, H-2), 1.63 (m, H-3), 1.26 (m, H-4 → H-19), 0.87 (m, H-20). ^{13}C NMR: δ_C 179.9 (C-1), 34.0 (C-2), 31.9 (C-18), 29.6 (C-6 → C16), 29.4 (C-17), 29.1 (C-4), 24.7 (C-3), 22.7 (C-19), 14.1 (C-20).

Bioassay

Cytotoxic assay was carried out as described in the literature[12].

Results and Discussion

The ^{1}H NMR spectrum of compound **1** showed the presence of one olefinic proton at 5.53 ppm, one carbinol at 3.19 ppm together with eight singlet methyls at 1.09 (3H), 0.96 (3H), 0.95 (3H), 0.93 (3H), 0.91 (6H), 0.80 (3H) and 0.77 (3H) ppm. In addition, its ^{13}C NMR spectrum displayed 30 carbon signals, including two olefinic carbons (158.1 and 116.9 ppm), one oxygen-bearing carbon (79.0 ppm) and eight singlet methyls as written in section Spectral Data of Isolated Compounds. This spectral data suggested that this compound is a triterpenoid with a hydroxyl group at C-3 and a double bond[13]. The relative configuration of 3-OH was determined as *β*-equatorial by the proton splitting pattern of H-3 in its ^{1}H NMR spectrum with big coupling constants

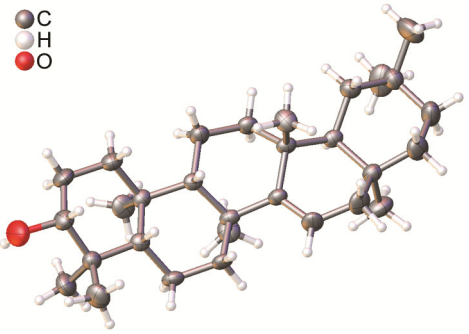

Fig. 1 — The ORTEP drawing of compound **1**

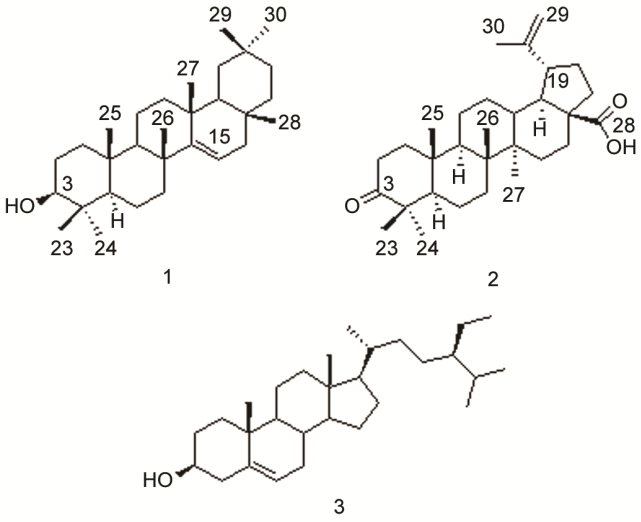

Fig. 2 — Structures of compounds **1-3**

(J = 4.5, 11.0 Hz). In addition, the NMR spectral data of compound **1** were identical with those of taraxerol[13]. Furthermore, a suitable crystal of compound **1** was obtained and its ORTEP drawing (Fig. 1) was described, confirming the structure of taraxerol as shown in Fig. 2.

The HR-MS of compound **2** has a *quasi*-molecular ion peak at m/z 477.3332 $[M+Na]^+$, corresponding to the molecular formula of $C_{30}H_{46}O_3Na$ with 7 degrees of unsaturation. Analysis of its ^1H NMR spectrum revealed the presence of one *exo*-methylene at 4.74 and 4.62 ppm, six singlet methyls at 1.70, 1.07, 1.02, 1.00, 0.99, 0.93 ppm. The ^{13}C NMR spectrum of compound **2** displayed 30 carbon signals, including one conjugated ketone (218.1 ppm), one carboxylic acid (181.2 ppm), one *exo*-methylene (109.7 and 150.3 ppm). Then, the structure of compound **2** was deduced by the interpretation of its 2D NMR spectra (HSQC, HMBC, ROESY). The ketone group was located at C-3 based on the HMBC correlations between H-1, H-2, H-23, H-24 and C-3. In addition, the carboxylic group was at C-28 since H-18 was coupled to C-28 in its HMBC spectrum. Furthermore, *exo*-methylene was bonded at C-20 since H-29 was correlated with C-19 and C-30 in its HMBC spectrum. Finally, H-5 and H-18 were α-oriented since H-5 was coupled to H-24; H-18 was coupled to H-27 in its ROESY spectrum. Consequently, compound **2** was found to be betulonic acid, which was previously isolated from *Hopea odorata* Roxb.[14].

Table 1 — Cytotoxic activity of compound **2** (IC_{50}, μg/mL)

Cancer cells	Compound **2**	Ellipticine
Hep-G2	20.33±1.67	0.36±0.03
Lu	67.15±3.65	0.32±0.03
KB	16.0±0.95	0.24±0.02
MCF7	37.47±1.52	0.51±0.03

Compounds **3** and **4** were characterized as β-sitosterol (**3**)[15] and eicosanoic acid (**4**)[16] by comparing their spectral data with those of published papers.

Previously, betulonic acid (**2**) has shown anti-inflammatory, anti-melanoma, anti-protozoal, and anti-viral activities[14,17,18]. In this study, all four extracts of this plant showed cytotoxicity against KB cell lines, and then we tested the activity of compound **2** toward four cancer cells, using ellipticine as a standard. The result was illustrated in Table 1. Accordingly, compound **2** had non-selective activity against all four cancer cells, especially it could inhibit moderately Hep-G2 and KB cell lines with its IC_{50} values of 20.33 and 16.0 μg/mL, respectively.

Conclusion

Four secondary metabolites from the extracts of Vietnamese medicinal plant, *Pterospermum truncatolobatum* were successfully purified and structural determined as taraxerol (**1**), betulonic acid (**2**), β-sitosterol (**3**) and eicosanoic acid (**4**). The moderate cytotoxic activity of betulonic acid (**2**) against all four cancer cell lines suggested the possible application of *P. truncatolobatum* for cancer treatment.

Conflict of interest

The authors declare there is no conflict of interest.

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