Journal of Medicinal Materials, 2023, Vol. 28, No. 2 (pp. 67 - 72)

DITERPENES FROM THE STEM BARK OF *TAXUS WALLICHIANA* ZUCC. AND THEIR CYTOTOXIC ACTIVITY

Ninh Thi Ngoc[1], Nguyen Viet Phong[1], Tran Hong Quang[1], Dang Viet Cuong[1], Tran Thi Hong Hanh[1], Nguyen Xuan Cuong[1], Chu Hoang Ha[2], Nguyen Hoai Nam[1,*]

[1]*Institute of Marine Biochemistry, Vietnam Academy of Science and Technology (VAST);*

[2]*Institute of Biotechnology, VAST, Hanoi, Vietnam*

*Corresponding author: namnguyenhoai@imbc.vast.vn

(Received December 12th, 2022)

Summary

Diterpenes from the Stem Bark of *Taxus wallichiana* Zucc. and their Cytotoxic Activity

By various chromatographic methods, seven known diterpenes, including two tricyclic-diterpenes containing a tropone ring (**1** and **2**) and five 11(15→1)-*abeo*-taxane diterpenes (**3**–**7**), were isolated from the methanol extract of the stem bark of *Taxus wallichiana* Zucc. The chemical structures of the isolated compounds were confirmed by ESI–MS, 1D, and 2D NMR as well as by comparison with the literatures. All isolated compounds were tested *in vitro* for their cytotoxic activity against the HepG2, SK-LU-1, MCF-7, LNCaP, and SK-Mel-2 human cancer cell lines. Among them, compounds **1** and **2** showed cytotoxicity against all tested cancer cell lines, with IC_{50} values ranging from 17.8 to 61.5 μM.

Keywords: *Taxus wallichiana* Zucc., Taxaceae, *Diterpene, Cytotoxic activity.*

1. Introduction

Taxus wallichiana Zucc., also known as "thông đỏ" in Vietnam, is a member in the Taxaceae family, which distributes in many countries in Asia, including India, Nepal, China, Indonesia, Malaysia, and Vietnam [1]. In India, this plant has been used in folk medicine for the treatment of bronchitis, asthma, epilepsy, snakebites, and scorpion stings [2]. Whereas, the wood powder of this plant has been commonly used to treat diabetes in traditional Vietnamese medicine [3]. Previous studies have reported that the stem bark of *T. wallichiana* exhibits various pharmacological activities, such as antibacterial, anticonvulsant, anti-inflammatory, and immunomodulatory activities [1],[4]. The previously phytochemical investigations of *T. wallichiana* have revealed that taxane-type diterpenoids are the main secondary metabolites in this plant, along with lignans, biflavonoids, and phytosterols [3],[5]. As part of our ongoing investigations of the secondary metabolites and respective biological effects from herbal and medicinal plants in Vietnam, in the present study, we isolated and structurally elucidated seven diterpenes, including two tricyclic-diterpenes containing a tropone ring (**1** and **2**) and five 11(15→1)-*abeo*-taxane diterpenes (**3–7**), from the stem bark of *T. wallichiana*. In addition, *in vitro* assays were performed to determine the cytotoxic activity of all isolated compounds.

2. Material and methods

2.1. General experimental procedures

All NMR spectra (^1H, ^{13}C, HSQC, and HMBC) were carried out on an AVANCE NEO 600 FT-NMR spectrometer (Bruker, Karlsruhe, Germany) at ppm rel. to tetramethylsilane (TMS) as internal standard, *J* in Hz at 294 K. Column chromatography (CC) was performed on *silica gel* (Kieselgel 60, 70-230 mesh and 230-400 mesh; Merck, Darmstadt, Germany) and reversed phased (RP) ODS-A gel (12 nm, S-150 μm; YMC, Tokyo, Japan). Preparative high-performance liquid chromatography (HPLC) was conducted on an Agilent 1200 HPLC system (Agilent Technologies, USA) equipped with a G1361A preparative pump, a G1315D diode array detector, a G2260A preparative autosampler, and a COSMOSIL 5C18-MS-II Packed column (250 × 10 mm, 5 μm). Thin layer chromatography (TLC) was performed using glass plates pre-coated with *silica gel* $60F_{254}$ and RP-18 F_{254s} (Merck, Darmstadt, Germany). Compounds were detected under UV light and then visualized by spraying the plates with 10% sulfuric acid reagent followed by heating at 110°C for 1 min.

2.2. Plant materials

The stem bark of *T. wallichiana* was collected in Lam Dong province, Vietnam in June 2020. The plant material was identified by one of the

authors, Dang Viet Cuong. A voucher specimen (IMBC.TWV.6.2020) was deposited at the Institute of Marine Biochemistry, VAST.

2.3. Extraction and isolation

The dried stem bark of *T. wallichiana* (3 kg) was cut into small pieces and extracted three times with MeOH (10 L × 3 times) at room temperature. Evaporation of the solvent under reduced pressure gave a MeOH residue (320 g). The MeOH residue was suspended in H_2O and successively partitioned with CH_2Cl_2 to yield a CH_2Cl_2 extract and water layer. The CH_2Cl_2 extract was chromatographed with CC on RP-18 gel using a stepwise eluent of 50-100% MeOH in H_2O to obtain ten fractions, D1-D10. Fraction D7 was separated by RP-18 CC, eluting with acetone-H_2O (1:1, v/v) and further purified by preparative HPLC, using an isocratic elution of 56% ACN in H_2O over 60 min to furnish **1** (10.0 mg) and **2** (2.0 mg). Fractions D8 and D9 were combined and separated on *silica gel* by CC and eluted with CH_2Cl_2-EtOAc (8:1, v/v) to obtain three fractions, D8A–D8C. Fraction D8B was chromatographed by preparative HPLC, using an isocratic eluent of 82% MeOH in H_2O at a flow rate of 3.5 mL/min over 60 min to obtain compound **3** (7.0 mg). From fraction D5, compounds **5** (3.0 mg), **6** (7.0 mg), and **7** (8.0 mg) were purified by *silica gel* CC using CH_2Cl_2-EtOAc (2:1, v/v) as eluent, followed by preparative HPLC with an isocratic mixture solvent of 70% MeOH in H_2O at a flow rate of 3.5 mL/min over 60 min. Compound **4** (6.5 mg) was isolated from fraction D3 by *silica gel* CC, eluted with CH_2Cl_2-EtOAc (1:1, v/v), and subsequently purified by preparative HPLC with an isocratic mixture solvent of 45% ACN in H_2O at a flow rate of 3.5 mL/min over 60 min.

Taxamairin A (1): yellow amorphous powder; $C_{21}H_{22}O_4$; ESI-MS: *m/z* 339 $[M+H]^+$; ^1H NMR (600 MHz, $CDCl_3$): δ_H (ppm) 7.95 (1H, br s, H-4), 7.32 (1H, d, *J* = 9.6 Hz, H-6), 6.11 (1H, d, *J* = 10.2 Hz, H-7), 6.95 (1H, br s, H-11), 1.46 (6H, s, H-12, H-13), 7.78 (1H, br s, H-17), 3.36 (1H, m, H-18), 1.33 (6H, d, *J* = 6.6 Hz, H-19, H-20), 3.89 (3H, s, 15-OCH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ_C (ppm) 188.2 (C-1), 120.9 (C-2), 146.2 (C-3), 133.8 (C-4), 130.2 (C-5), 147.9 (C-6), 123.8 (C-7), 200.9 (C-8), 50.5 (C-9), 151.4 (C-10), 131.3 (C-11), 26.9 (C-12), 26.9 (C-13), 146.9 (C-14), 148.3 (C-15), 136.6 (C-16), 119.4 (C-17), 27.5 (C-18), 23.4 (C-19), 23.4 (C-20), 62.1 (15-OCH_3).

Taxamairin D (2): yellow amorphous powder; $C_{21}H_{24}O_4$; ESI-MS: *m/z* 341 $[M+H]^+$; ^1H NMR (600 MHz, $CDCl_3$): δ_H (ppm) 7.54 (1H, br s, H-4), 6.45 (1H, d, *J* = 11.4 Hz, H-6), 6.02 (1H, dd, *J* = 4.5, 9.0 Hz, H-7), 4.04 (1H, br s, H-8), 6.84 (1H, br s, H-11), 1.33 (3H, s, H-12), 1.13 (3H, s, H-13), 7.70 (1H, br s, H-17), 3.30 (1H, m, H-18), 1.31 (6H, d, *J* = 6.6 Hz, H-19, H-20), 3.87 (3H, s, 15-OCH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ_C (ppm) 189.6 (C-1), 121.8 (C-2), 136.2 (C-3), 127.9 (C-4), 132.1 (C-5), 133.1 (C-6), 129.3 (C-7), 74.3 (C-8), 42.9 (C-9), 150.9 (C-10), 131.1 (C-11), 20.0 (C-12), 26.7 (C-13), 147.3 (C-14), 146.6 (C-15), 144.3 (C-16), 119.0 (C-17), 27.4 (C-18), 23.5 (C-19), 23.5 (C-20), 61.9 (15-OCH_3).

Taxayuntin C (3): white amorphous powder; $C_{42}H_{48}O_{14}$; ESI-MS: *m/z* 777 $[M+H]^+$; ^1H NMR (600 MHz, $CDCl_3$): δ_H (ppm) 6.60 (1H, d, *J* = 12.5 Hz, H-2), 3.12 (1H, d, *J* = 9.0 Hz, H-3), 4.97 (1H, d, *J* = 9.6 Hz, H-5), 2.56 (1H, m, Ha-6), 1.90 (1H, m, Hb-6), 5.62 (1H, t, *J* = 10.2 Hz, H-7), 6.29 (1H, d, *J* = 12.6 Hz, H-9), 6.45 (1H, d, *J* = 9.0 Hz, H-10), 5.69 (1H, t, *J* = 9.0, 18.0 Hz, H-13), 2.42 (1H, m, Ha-14), 1.93 (1H, m, Hb-14), 1.18 (6H, s, H-16, H-17), 1.94 (3H, s, H-18), 1.76 (3H, s, H-19), 4.48 (1H, d, *J* = 9.0 Hz, Ha-20), 4.16 (1H, d, *J* = 9.0 Hz, Hb-20); 2-OBz: 8.00 (2H, dd, *J* = 1.2, 9.6 Hz), 7.87 (2H, d, *J* = 9.0 Hz), 7.56 (1H, t, *J* = 9.0 Hz); 10-OBz: 7.48 (2H, t, *J* = 9.6 Hz), 7.43 (2H, t, *J* = 9.0 Hz), 7.62 (1H, t, *J* = 9.0 Hz); OAc: 2.18 (3H, s), 2.17 (3H, s), 2.16 (3H, s), 1.76 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$): δ_C (ppm) 70.0 (C-1), 68.6 (C-2), 45.0 (C-3), 79.5 (C-4), 84.6 (C-5), 35.9 (C-6), 70.7 (C-7), 43.7 (C-8), 76.5 (C-9), 68.3 (C-10), 136.1 (C-11), 148.1 (C-12), 78.8 (C-13), 37.8 (C-14), 75.7 (C-15), 25.5 (C-16), 27.8 (C-17), 12.0 (C-18), 12.5 (C-19), 74.6 (C-20); 2-OBz: 164.1, 133.5, 130.0, 129.7, 129.7, 129.6, 129.1; 10-OBz: 165.9, 133.4, 128.9, 128.9, 128.8, 128.8, 128.6; OAc: 170.7, 169.9, 169.8, 169.0, 22.0, 21.4, 21.2, 20.7.

10,13-Deacetylabeobaccatin IV (4): white amorphous powder; $C_{28}H_{40}O_{12}$; ESI-MS: *m/z* 569 $[M+H]^+$; ^1H NMR (600 MHz, $CDCl_3$): δ_H (ppm) 5.45 (1H, t, *J* = 7.8 Hz, H-2), 3.10 (1H, d, *J* = 7.8 Hz, H-3), 4.91 (1H, dd, *J* = 1.2, 7.8 Hz, H-5), 2.52 (1H, m, Ha-6), 1.88 (1H, m, Hb-6), 6.23 (1H, d, *J* = 10.2 Hz, H-7), 5.96 (1H, d, *J* = 10.8 Hz, H-9), 6.03 (1H, d, *J* = 7.2 Hz, H-10), 4.47 (1H, t, *J* = 6.6 Hz, H-13), 2.23 (1H, m, Ha-14), 1.57 (1H, m, Hb-14), 1.12 (3H, s, H-16), 1.05 (3H, s, H-17), 1.93 (3H, s, H-18), 1.65 (3H, s, H-19), 4.50 (1H, d, *J* = 7.8 Hz, Ha-20), 4.39 (1H, d, *J* = 7.2 Hz, Hb-20); OAc: 2.14 (3H, s), 2.07 (3H, s), 2.02 (3H, s), 2.00 (3H, s); ^{13}C NMR (150

MHz, $CDCl_3$): δ_C (ppm) 67.6 (C-1), 70.3 (C-2), 43.8 (C-3), 80.1 (C-4), 85.4 (C-5), 34.8 (C-6), 68.8 (C-7), 43.9 (C-8), 76.8 (C-9), 68.0 (C-10), 134.5 (C-11), 149.2 (C-12), 77.5 (C-13), 39.7 (C-14), 75.1 (C-15), 25.1 (C-16), 27.4 (C-17), 11.7 (C-18), 12.8 (C-19), 75.3 (C-20); OAc: 171.3, 170.2, 169.8, 169.7, 22.4, 21.6, 21.4, 20.8.

7,9,10,13-Tetradeacetylabeobaccatin VI (5): white amorphous powder; $C_{29}H_{38}O_{10}$; ESI-MS: *m/z* 547 $[M+H]^+$; ^1H NMR (600 MHz, $CDCl_3$): δ_H (ppm) 6.07 (1H, d, *J* = 7.8 Hz, H-2), 3.03 (1H, d, *J* = 7.2 Hz, H-3), 4.89 (1H, d, *J* = 9.6 Hz, H-5), 2.57 (1H, m, Ha-6), 1.84 (1H, m, Hb-6), 4.23 (1H, d, *J* = 8.4 Hz, H-7), 4.34 (1H, d, *J* = 9.6 Hz, H-9), 4.54 (1H, d, *J* = 9.6 Hz, H-10), 4.59 (1H, d, *J* = 6.0 Hz, H-13), 2.26 (1H, m, Ha-14), 1.78 (1H, m, Hb-14), 1.12 (3H, s, H-16), 1.06 (3H, s, H-17), 1.95 (3H, s, H-18), 1.88 (3H, s, H-19), 4.46 (1H, d, *J* = 7.8 Hz, Ha-20), 4.10 (1H, d, *J* = 7.8 Hz, Hb-20); 2-OBz: 8.01 (2H, d, *J* = 7.8 Hz), 7.46 (2H, t, *J* = 7.8 Hz), 7.06 (1H, t, *J* = 7.2 Hz); 4-OAc: 2.20 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$): δ_C (ppm) 68.9 (C-1), 68.8 (C-2), 44.6 (C-3), 80.8 (C-4), 85.1 (C-5), 37.5 (C-6), 72.5 (C-7), 42.7 (C-8), 80.5 (C-9), 67.7 (C-10), 137.4 (C-11), 147.0 (C-12), 77.8 (C-13), 39.8 (C-14), 76.6 (C-15), 25.1 (C-16), 27.8 (C-17), 11.3 (C-18), 12.2 (C-19), 74.8 (C-20); 2-OBz: 166.1, 133.6, 130.0, 129.7, 129.7, 128.6, 128.6; 4-OAc: 171.1, 22.5.

Wallitaxane E (6): white amorphous powder; $C_{34}H_{42}O_{13}$; ESIMS: *m/z* 659 $[M+H]^+$; ^1H NMR (600 MHz, $CDCl_3$): δ_H (ppm) 4.48 (1H, dd, *J* = 3.6, 6.6 Hz, H-2), 3.32 (1H, d, *J* = 7.2 Hz, H-3), 5.00 (1H, d, *J* = 8.4 Hz, H-5), 2.72 (1H, m, Ha-6), 2.00 (1H, m, Hb-6), 3.98 (1H, dd, *J* = 7.8, 9.0 Hz, H-7), 2.75 (1H, m, Ha-13), 2.38 (1H, m, Hb-13), 2.33 (1H, m, Ha-14), 2.12 (1H, m, Hb-14), 1.58 (3H, s, H-16), 1.55 (3H, s, H-17), 2.30 (3H, s, H-18), 1.77 (3H, s, H-19), 4.56 (1H, d, *J* = 9.0 Hz, Ha-20), 4.63 (1H, d, *J* = 9.0 Hz, Hb-20); 15-OBz: 7.80 (2H, dd, *J* = 1.2, 8.4 Hz), 7.45 (2H, dd, *J* = 7.8, 15.6 Hz), 7.58 (1H, m); 7-Xyl: 4.17 (1H, d, *J* = 6.6 Hz, H-1′), 3.28 (1H, m), 3.53 (1H, t, *J* = 8.4 Hz), 3.65 (1H, m), 3.93 (1H, dd, *J* = 6.6, 12.0 Hz), 3.24 (1H, m); 4-OAc: 2.17 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$): δ_C (ppm) 68.4 (C-1), 69.8 (C-2), 44.8 (C-3), 79.6 (C-4), 84.6 (C-5), 35.9 (C-6), 77.8 (C-7), 55.4 (C-8), 205.6 (C-9), 192.3 (C-10), 134.9 (C-11), 168.5 (C-12), 39.6 (C-13), 26.8 (C-14), 91.4 (C-15), 22.3 (C-16), 21.9 (C-17), 18.3 (C-18), 8.8 (C-19), 75.7 (C-20); 15-OBz: 165.9, 134.9, 134.3, 133.1, 131.8, 129.5, 128.9; 7-Xyl: 103.8, 73.2, 75.6, 69.3, 65.2; 4-OAc: 170.0, 21.7.

Wallifoliol (7): white amorphous powder; $C_{29}H_{34}O_{10}$; ESIMS: *m/z* 543 $[M+H]^+$; ^1H NMR (600 MHz, $CDCl_3$): δ_H (ppm) 5.80 (1H, d, *J* = 12.0 Hz, H-2), 2.78 (1H, br s, H-3), 4.83 (1H, d, *J* = 7.8 Hz, H-5), 2.76 (1H, m, Ha-6), 1.85 (1H, dd, *J* = 7.8, 16.2 Hz, Hb-6), 4.32 (1H, t, *J* = 7.2 Hz, H-7), 4.55 (1H, d, *J* = 6.0 Hz, H-13), 2.27 (1H, dd, *J* = 7.2, 14.4 Hz, Ha-14), 2.13 (1H, dd, *J* = 6.0, 15.0 Hz, Hb-14), 1.23 (3H, s, H-16), 1.34 (3H, s, H-17), 2.09 (3H, s, H-18), 1.69 (3H, s, H-19), 4.67 (1H, d, *J* = 8.4 Hz, Ha-20), 4.25 (1H, d, *J* = 8.4 Hz, Hb-20); 2-OBz: 7.97 (2H, d, *J* = 7.2 Hz), 7.48 (2H, t, *J* = 7.8 Hz), 7.61 (1H, t, *J* = 7.2 Hz); 4-OAc: 1.74 (3H, s); ^{13}C NMR (150 MHz, $CDCl_3$): δ_C (ppm) 59.9 (C-1), 68.3 (C-2), 43.3 (C-3), 80.6 (C-4), 84.3 (C-5), 37.4 (C-6), 71.1 (C-7), 48.3 (C-8), 84.9 (C-9), 174.5 (C-10), 131.0 (C-11), 140.1 (C-12), 79.6 (C-13), 37.2 (C-14), 90.3 (C-15), 24.8 (C-16), 22.5 (C-17), 11.0 (C-18), 10.2 (C-19), 74.2 (C-20); 15-OBz: 164.8, 129.8, 129.5, 131.0, 128.7, 128.7, 133.7; 4-OAc: 170.4, 21.4.

2.4. Cytotoxic assay

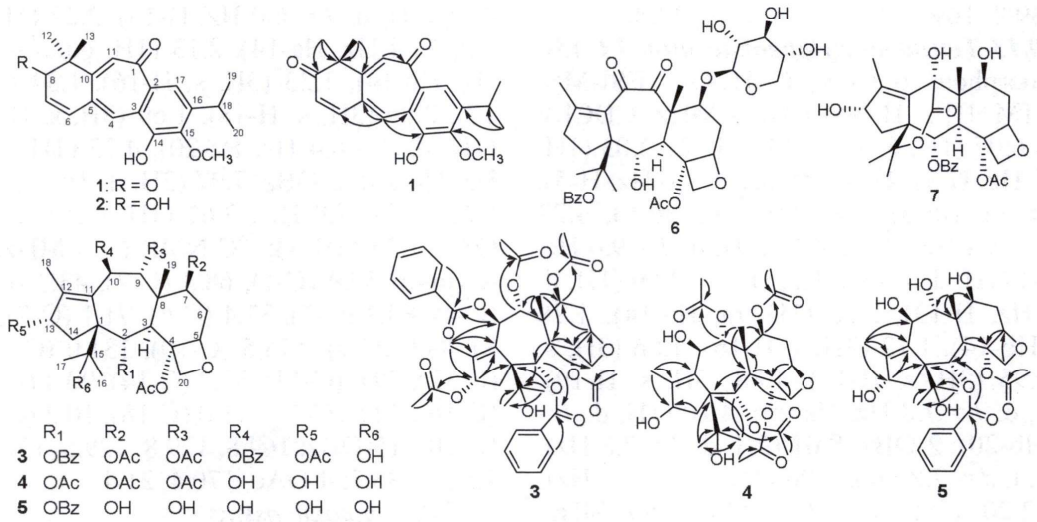
The cytotoxic effects of all isolated compounds (**1-7**) against five human cancer cell lines, including HepG2 (liver cancer), SK-LU-1 (lung cancer), MCF-7 (breast cancer), LNCaP (prostate cancer), and SK-Mel-2 (sarcoma cancer), were examined by SRB assay, followed our previously described protocol [6]. Statistical analysis was determined using GraphPad Prism 9.0 (GraphPad Software Inc., San Diego, CA, USA).

3. Results and discussion

Compound **1** was obtained as a yellow amorphous powder. The molecular formula of **1** was identified as $C_{15}H_{14}O_6$ based on an ESI-MS molecular ion peak at *m/z* 338 $[M]^+$. The ^1H NMR spectrum had the characteristics of an *abeo*-abietane skeleton with an isopropyl group [δ_H 3.36 (1H, m, H-18), 1.33 (6H, d, *J* = 6.6 Hz, H-19, H-20)], two methyl groups [δ_H 1.46 (6H, s, H-12, H-13)], and a carbonyl group [δ_C 188.2 (C-1)]. The assignment of all protonated carbons was established by the HSQC experiment. The position of an additional carbonyl group at C-8 was determined by the chemical shift [δ_C 200.9 (C-8)] and the HMBC correlations of H-7, H-12, and H-13 with C-8 (Fig. 1). Thus, compound **1** was determined to be taxamairin A [7].

Compound **2** was obtained as a yellow amorphous powder. The molecular formula of **2** was identified as $C_{21}H_{24}O_4$ based on an ESI-MS molecular ion peak at *m/z* 340 $[M]^+$. The ^1H and ^{13}C NMR spectra of compound **2**

showed the characteristics of an *abeo*-abietane skeleton, which was similar to those of taxamairin A (**1**), except for the linkage of a hydroxy group in **2** instead of a carbonyl group in **1**. The attachment of a hydroxy group at C-8 was confirmed by the connectivity in the HMBC spectrum between proton at δ_H 6.02 (1H, dd, J = 4.5, 9.0 Hz, H-7), 1.33 (3H, s, H-12), and 1.13 (3H, s, H-13), with the carbon at δ_C 74.3 (C-8) (Fig. 1). Thus, the structure of **2** was established as taxamairin D [8].



	R_1	R_2	R_3	R_4	R_5	R_6
3	OBz	OAc	OAc	OBz	OAc	OH
4	OAc	OAc	OAc	OH	OH	OH
5	OBz	OH	OH	OH	OH	OH

Fig. 1. Chemical structures and key HMBC correlations of isolated compounds (**1**–**7**).

Compound **3** was isolated as a white amorphous powder and its molecular formula $C_{42}H_{48}O_{14}$ was deduced by ESI-MS analysis, with a protonated ion peak at *m/z* 777 $[M+H]^+$. The ^1H NMR spectrum showed the presence of an 11(15→1)*abeo*-taxane skeleton with signals for four methyl singlets [δ_H 1.18 (6H, s, H-16, H-17), 1.94 (3H, s, H-18), 1.76 (3H, s, H-19)], an exomethylene [δ_H 4.48 (1H, d, J = 9.0 Hz, Ha-20), 4.16 (1H, d, J = 9.0 Hz, Hb-20)], four acetoxy groups [δ_H 2.18, 2.17, 2.16, 1.76 (each 3H, s)], and two benzoyloxy groups [2-OBz δ_H 8.00 (2H, dd, J = 1.2, 9.6 Hz), 7.87 (2H, d, J = 9.0 Hz), 7.56 (1H, t, J = 9.0 Hz) and 10-OBz δ_H 7.48 (2H, t, J = 9.6 Hz), 7.43 (2H, t, J = 9.0 Hz), 7.62 (1H, t, J = 9.0 Hz)]. Its ^{13}C NMR spectrum showed resonances for six carbonyls (δ_C 170.7, 169.9, 169.8, 169.0, 165.9, 164.1), eight methyls [δ_C 25.5 (C-16), 27.8 (C-17), 12.0 (C-18), 12.5 (C-19), 22.0, 21.4, 21.2, 20.7 (OAc)], a pair of olefinic carbons [δ_C 136.1 (C-11), 148.1 (C-12)], a methine [δ_C 45.0 (C-3)], two methylenes [δ_C 35.9 (C-6), 37.8 (C-14)], two quaternary carbons [δ_C 70.0 (C-1), 43.7 (C-8)], two oxygenated quaternary carbons [δ_C 79.5 (C-4), 75.7 (C-15)], and two aromatic rings (δ_C 128.6–133.5). The positions of acetoxy and benzoyloxy groups were determined by analyzing the HMBC correlations from H-2, H-7, H-9, H-10, and H-13 to carbonyl carbons 164.1 (2-OBz), 169.9 (7-OAc), 169.8 (9-OAc), 165.9 (10-OBz), and 170.7 (13-OAc), respectively (Fig. 1), as well as by comparison with those reported in the literature [9]. Based on the spectroscopic evidence, compound **3** was assigned as taxayuntin C.

Compound **4** was isolated as a white amorphous powder. The molecular formula $C_{28}H_{40}O_{12}$ was deduced by ESI-MS. The ^1H and ^{13}C NMR spectra showed the presence of an 11(15→1)*abeo*-taxane skeleton. In addition, analyzing the signals and correlations in ^{13}C NMR and HSQC spectra revealed signals of four acetoxy groups at (δ_C 171.3, 170.2, 169.8, 169.7, 22.4, 21.6, 21.4, 20.8). The locations of four acetoxy groups were determined by HMBC correlations from H-2, H-7, and H-9 to carbonyl carbon signals 2-OAc, 7-OAc, and 9-OAc, respectively (Fig. 1) as well as the down-field carbon chemical shift of C-4 (δ_C 80.1). Thus, the structure of **4** was established as 10,13-deacetylabeobaccatin IV [10].

Compound **5** was obtained as a white amorphous powder. The molecular formula $C_{29}H_{38}O_{10}$ was identified by ESI-MS analysis. The ^1H and ^{13}C NMR spectra revealed the presence of an 11(15→1)*abeo*-taxane skeleton. Furthermore, the ^{13}C NMR and HSQC spectra showed the signals of an acetoxy group (δ_C 171.1, 22.5) and a benzoyloxy group (δ_C 166.1, 133.6, 130.0, 129.7, 129.7, 128.6, 128.6). The locations of acetoxy and

benzoyloxy groups were determined by the down-field carbon chemical shift of C-4 (δ_C 80.8) and from H-2 to 166.1 (2-OBz), respectively (Fig. 1). Thus, the structure of **5** was elucidated as 7,9,10,13-tetradeacetylabeobaccatin VI [11].

Compound **6** was isolated as a white amorphous powder and its molecular formula $C_{34}H_{42}O_{13}$ was deduced by ESI-MS analysis, with a protonated ion peak at *m/z* 659 $[M+H]^+$. The ^1H and ^{13}C NMR spectra showed the presence of an 11(15→1)*abeo*-taxane skeleton, an acetoxy group (δ_C 170.0, 21.7), a benzoyloxy group (δ_C 165.9, 134.9, 134.3, 133.1, 131.8, 129.5, 128.9), and two carbonyl groups (δ_C 205.6, 192.3). The locations of a benzoyloxy, an acetoxy, and two carbonyl groups were determined by HMBC correlations between H-3 and C-2; H-3, H-5, and C-4; H_3-19 and C-9; and H_3-18 and C-10, respectively (Fig. 1). In addition, ^{13}C NMR and HSQC spectra showed the signals of a sugar unit, which was deduced to be a β-xylosyl moiety by comparison of its NMR data with those reported in the literature. The location of the xylosyl group was determined to be at C-7 based on the HMBC correlation between anomeric proton δ_H 4.17 (1H, d, J = 6.6 Hz, H-1′) and δ_C 77.8 (C-7). Thus, the structure of **6** was assigned as wallitaxane E [3].

Compound **7** was isolated as a white amorphous powder and exhibited a molecular peak at *m/z* 543 $[M+H]^+$, corresponding to the molecular formula $C_{29}H_{34}O_{10}$. The ^1H and ^{13}C NMR spectra of **7** showed strong variations compared to those of taxayuntin C (**3**). The carbon signals of C-9 (δ_C 84.9) and C-10 (δ_C 174.5) of **7** are typical of carbons bearing hydroxy and ester groups, respectively, indicating that the acetoxy group at C-9 of **3** (δ_C 76.5) was changed to a hydroxy group, whereas the benzoyloxy group at C-10 (δ_C 68.3) was oxidized to the carbonyl of the ester group. This evidence suggested the breaking of the C-10/C-11 bond in **3**. In addition, the carbon signals of C-1 (δ_C 59.9) and C-15 (δ_C 90.3) of **7** are strongly different with those of compound **3** [δ_C 70.0 (C-1), 75.7 (C-15)], suggesting that the hydroxy group linked to C-15 in **3** was changed by the linkage with a carbonyl group at C-10 in **7**. Comparison of the NMR data of **7** with those reported in previously published literature resulted in the confirmation of **7** as wallifoliol [12].

To investigate the cytotoxic effects of the compounds isolated from *T. wallichiana*, their cytotoxicity against five human cancer cell lines (HepG2, SK-LU-1, MCF-7, LNCaP, and SK-Mel-2) was evaluated using SRB assays [6]. As shown in Table 1, compound **1** exhibited cytotoxicity against all tested cancer cell lines, with IC_{50} values ranging from 18.3 ± 0.7 – 42.4 ± 0.8 μM. Compound **2** showed a little weaker activity against SK-LU-1, MCF-7, SK-Mel-2, and HepG2 cancer cell lines, with IC_{50} values of 53.8 ± 3.0, 29.7 ± 2.7, 21.7 ± 1.7, and 61.5 ± 2.7 μM, respectively. In contrast, the 11(15→1)-*abeo*-taxane diterpenes group (**3**–**7**) did not exert cytotoxic effects on any of the five cancer cell lines (IC_{50}>100 μM). These results suggest that the tricyclic nucleus containing a tropone ring in the diterpene structure could play an important role in the cytotoxic activity.

Table 1. Cytotoxic activity of isolated compounds **1**–**7**

Compound	IC_{50} values (μM)[a]				
	SK-LU-1	MCF-7	SK-Mel-2	HepG2	LNCaP
1	39.1 ± 1.0	25.7 ± 1.7	18.3 ± 0.7	42.4 ± 0.8	21.0 ± 1.1
2	53.8 ± 3.0	29.7 ± 2.7	21.7 ± 1.7	61.5 ± 2.7	17.8 ± 1.6
3	>100[c]	>100	>100	>100	>100
4	>100	>100	>100	>100	>100
5	>100	>100	>100	>100	>100
6	>100	>100	>100	>100	>100
7	>100	>100	>100	>100	>100
Ellipticine[b]	2.0 ± 0.3	1.7 ± 0.1	1.6 ± 0.1	2.0 ± 0.2	1.5 ± 0.2

[a] Mean ± SD (n = 3), [b] Positive control, [c] inactive (IC_{50} > 100 μM).

4. Conclusion

In conclusion, the chemical study on the stem bark of *T. wallichiana* led to the isolation and structural elucidation of seven known diterpenes. Among them, two tricyclic-diterpenes containing a tropone ring (**1**–**2**) showed cytotoxic activity against five human cancer cell lines (HepG2, SK-LU-1, MCF-7, LNCaP, and SK-Mel-2) with IC_{50} values ranging from 17.8 to 61.5 μM. Our study not only contributed to the diversity of isolated compounds but also provided insights into the cytotoxic activity of diterpenes isolated from *T. wallichiana.*

Acknowledgments: *This work was financially supported by Vietnam Academy of Science and Technology under grant number TDCNSH.06/20-22.*

References

1. Sharma H., Garg M. (2015), A review of traditional use, phytoconstituents and biological activities of Himalayan yew, *Taxus wallichiana. Journal of Integrative Medicine*, 13(2), 80-90. **2**. Rikhari H. C., Palni L. M. S., Sharma S., Nandi S. K. (1998), Himalayan yew: stand structure, canopy damage, regeneration and conservation strategy. *Environmental Conservation*, 25(4), 334-341. **3**. Dang P. H., Nguyen H. X., Duong T. T. T., Tran T. K. T., Nguyen P. T., Vu T. K. T., Vuong H. C., Phan N. H. T., Nguyen M. T. T., Nguyen N. T., Awale S. (2017), α-Glucosidase inhibitory and cytotoxic taxane diterpenoids from the stem bark of *Taxus wallichiana. Journal of Natural Products*, 80(4), 1087-1095. **4**. Qayum M., Nisar M., Rauf A., Khan I., Kaleem W. A., Raza M., Karim N., Saleem M. A., Bawazeer S., Uysal S., Zengin G., Jahan S., Ramadan M. F. (2019), In-vitro and in-silico anticancer potential of taxoids from *Taxus wallichiana* Zucc. *Biologia Futura*, 70(4), 295-300. **5**. Dang P. H., Nguyen H. X., Nguyen H. H. T., Vo T. D., Le T. H., Phan T. H. N., Nguyen M. T. T., Nguyen N. T. (2017), Lignans from the roots of *Taxus wallichiana* and their α-glucosidase inhibitory activities. *Journal of Natural Products*, 80(6), 1876-1882. **6**. Quang T. H., Phong N. V., Anh D. V., Hanh T. T. H., Cuong N. X., Ngan N. T. T., Trung N. Q., Oh H., Nam N. H., Minh C. V. (2021), Bioactive secondary metabolites from a soybean-derived fungus *Aspergillus versicolor* IMBC-NMTP02. *Phytochemistry Letters*, 45(93-99. **7**. Liang J. Y., Min Z. D., Tanaka T., Mizuno M., Iinuma M., Nakanishi T., Inada A. (1988), Studies on the diterpenes from *Taxus mairei*: I. Structures of taxamairins A, B and C. *Acta Chimica Sinica,* 6(1), 37-41. **8**. Yang S. J., Fang J. M., Cheng Y. S. (1998), Diterpenes from *Taxus mairei. Phytochemistry*, 49(7), 2037-2043. **9**. Wang F. S., Peng L. V., Zhao Y., Gu K., Zhao Q. S., Sun H. D. (2003), Taxoids from the leaves and stems of *Taxus chinensis. Acta Botanica Yunnanica*, 25(3), 369-376. **10**. Chattopadhyay S. K., Sharma R. P., Appendino G., Gariboldi P. (1995), A rearranged taxane from the Himalayan yew. *Phytochemistry*, 39(4), 869-870. **11**. Appendino G., Cravotto G., Enriù R., Jakupovic J., Gariboldi P., Gabetta B., Bombardell E. (1994), Rearranged taxanes from *Taxus baccata. Phytochemistry*, 36(2), 407-411. **12**. Arnone A., Bava A., Fronza G., Malpezzi L., Nasini G. (2010), Microbial transformations of the taxan ring of 10-DAB by some strains of the fungus *Curvularia lunata*: Formation of the bis-abeotaxanes wallifoliol and 4-deacylwallifoliol. *Journal of Natural Products*, 73(6), 1049-1052.

Journal of Medicinal Materials, 2023, Vol. 28, No. 2 (pp. 72 - 77)

FLAVONOIDS FROM ETHYL ACETATE FRACTION OF THE WHOLE PLANT OF *PYRROSIA LANCEOLATA* (L.) FARW.

Ly Tu Loan[1], Dao Thi Thanh Thuyen[2], Nguyen Bui Thanh Linh[2], Ma Chi Thanh[2,*]

[1]Faculty of Pharmacy, Ton Duc Thang University, Ho Chi Minh City, Vietnam;

[2]Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City, Vietnam

*Corresponding author: mcthanh@ump.edu.vn

(Received February 03st, 2023)

Summary

Flavonoids from Ethyl Acetate Fraction of the Whole Plant of *Pyrrosia lanceolata* (L.) Farw.

The medicinal fern, *Pyrrosia lanceolata* (L.) Farw. (Polypodiaceae), has been used in folk medicine in Vietnam and Southeast Asia for the treatment of swelling, urocystitis, urinary calculus, bloody urine, coughs, and bronchitis. Five compounds were isolated and identified as vitexin (**1**), naringenin 7-*O*-(2-*β*-D-apiofuranosyl)-*β*-D-glucopyranoside (**2**), naringin (**3**), neoeriocitrin (**4**), and hypolaetin-7-O-*β*-D-glucopyranoside (**5**) from the ethyl acetate fraction of *Pyrrosia lanceolata*. Their structures were determined by means of spectroscopic methods (MS, 1D and 2D NMR).

Keywords: *Pyrrosia lanceolata*, Polypodiaceae, *Fern, Flavonoid.*

1. Introduction

Ferns (Polypodiophyta class) are a large group of vascular plants, including sixty-one genera, distributed worldwide from tropical to temperate regions, which are the most diverse plants in tropical countries in Asia [1]. Ferns showed remarkable activities such as anti-oxidative, anti-bacterial, anti-inflammatory effects... The genus *Pyrrosia* (Polypodiaceae family) has more than 100 species, mostly grow on wet tree trunks, rocks, and rocky crevices in forests [2]. Some species in the genus *Pyrrosia* are commonly used in the traditional medicine for the treatment of inflammatory urinary tract disease. The *P. lanceolata* species has been used as a diuretic in folk medicine in Vietnam [3].

The previous studies on the genus *Pyrrosia* (such as *P. lingua*, *P. petiolosa*, *P. serpens* and *P. linearifolia*) revealed the presence of phenolic compounds, flavonoids [4],[5],[6], triterpenoids [7],[8], xanthones [9], and other less-polar compounds in the essential oil [10]. To the best of our knowledge, until now phytochemical and pharmacological studies of *P. lanceolata* were very limited. Our present study on phytochemical constituents from the ethyl acetate fraction of the whole plants of *P. lanceolata* led to the isolation of five flavonoids. Their structures were identified by spectroscopic data and compared to those reported in the literature. In the present paper, five compounds vitexin (**1**), naringenin 7-*O*-(2-*β*-D-apiofuranosyl)-*β*-D-glucopyranoside