Compounds from the ethyl acetate extract of *Pteris multifida* Poir. collected in Nam Dinh province, Vietnam

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Abstract:

Pteris multifida Poir. is an evergreen herbaceous fern that is distributed in temperate and tropical regions of East Asia and cultivated in many other countries. It is an ethnomedicinal plant used for detoxicating, cooling, reducing pain, and as a treatment for many diseases like dysentery, typhoid, cholecystitis, diarrhoea, enteritis, jaundice, and eczema among others. Several compounds including flavonoids, sesquiterpenoids, diterpenoids, coumarins, lignans, and sterols with anti-spasmodic, anti-bacterial, anti-hyperlipidemic, anti-oxidative, and cytotoxic activities have been isolated from this plant. In the present study, three sesquiterpenoids, (2S,3S)-pretosin Q (1), (2R,3S)-pterosin C (2), (2S,3S)-pterosin C (3); two lignans, pinoresinol (4); lariciresinol 9-O- β -glucopyranoside (5), three flavonoids, naringenin (6), apigenin (7), and luteolin (8) were obtained from the ethyl acetate extract of the whole of *P. multifida* collected in Nam Dinh province, Vietnam. The chemical structures were elucidated by various spectroscopic means including one- and two-dimensional (1D, 2D)-NMR, mass spectrometry, and comparison with published data. Compounds 4-6 were isolated from *P. multifida* for the first time.

Keywords: flavonoid, lignan, Pteris multifida Poir., sesquiterpenoid.

Classification numbers: 2.2, 3.4

1. Introduction

The genus Pteris is a large member of the family Pteridaceae. It contains about 250 species that mostly occur in the tropical and subtropical regions of the world, with about 28 species found in Vietnam [1]. Some Pteris species have been utilised in folk medicines, as food sources, and as phytoremediations [2]. P. multifida, an evergreen herbaceous fern, is native to temperate and tropical regions of East Asia and cultivated in many other countries. This plant grows extensively throughout the north and middle regions of Vietnam and is most commonly found in damp and shady locations. P. multifida is traditionally used for detoxicating, cooling, reducing pain, and as a treatment for many diseases like dysentery, typhoid, cholecystitis, diarrhoea, enteritis, jaundice, and eczema among others [3]. Pharmacological properties of P. multifida include anti-tumour, anti-inflammatory, anti-hyperlipidaemic, antimutagenic, and free radical-scavenging activities [4-7]. Previously, the presence of flavonoids, sesquiterpenoids, diterpenoids, coumarins, lignans, and sterols has been reported in this plant [8-14]. We report herein the isolation and structural elucidation of eight compounds from the ethyl acetate extract of the entire the whole of *P. multifida* fern collected in the Nam Dinh province, Vietnam. Their structures were identified as (2S,3S)-pretosin Q (1), (2R,3S)-pterosin C (2), (2S,3S)-pterosin C (3), pinoresinol (4), lariciresinol 9-O- β -glucopyranoside (5), naringenin (6), apigenin (7), and luteolin (8) by spectral analysis and comparison with spectroscopic data reported in previous literature.

2. Materials and methods

2.1. General experimental procedures

NMR spectra were recorded with a Bruker Avance II spectrometer at 600 MHz (¹H-NMR) and 150 MHz (¹³C-NMR) using TMS as an internal reference. Mass spectra were carried out on an Agilent LC-MSD-Trap SL system mass spectrometer. Thin layer chromatography was performed on silica gel 60F254 and Rp-18 F254s, and spots were detected with UV light (254 and 365 nm) and then sprayed with a solution of vanillin/sulfuric acid followed by heating to 110°C. Silica gel 60 (0.063-0.2 mm), Sephadex LH-20 (25-100 μ m), and silica gel Rp-18 (40-63 μ m) were used for normal pressure column chromatography.

2.2. Plant material

The whole *P. multifida* plant was collected in the Nam Truc district, Nam Dinh province, Vietnam, in May 2021 and identified

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by Prof. Do Huu Thu, Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology (VAST). The specimens were kept at the Laboratory of Natural Products Research, Institute of Chemistry, VAST, Hanoi, Vietnam.

2.3. Extraction and isolation

The dried whole plant (1600 g) of P. multifida was ground and extracted with methanol-water (95:5, v/v) three times at room temperature. After removing the solvent under vacuum, the concentrated extract was successively partitioned with n-hexane, ethyl acetate, and n-butanol, respectively. The EtOAc-soluble fraction (22 g) was subjected to silica gel chromatography eluted with gradient mixtures of CH₂Cl₂-MeOH (from 10:0 to 4:1, v/v) to give ten fractions (PE1-PE10). Fraction PE3 was re-chromatographed on a silica gel column (n-hexane-acetone, 3.5:2) to afford compound 6 (7 mg). Fraction PE4 was further purified on a silica gel column (n-hexane-acetone, 3:2, v/v) and then on a Sephadex LH-20 column (CH₂Cl₂-MeOH, 1:9, v/v) to give compounds 4 (10 mg) and 7 (3 mg). Fraction PE6 was rechromatographed on a silica gel column (CH₂Cl₂-MeOH, 10:1, v/v) to give four sub-fractions (PE6.1-PE6.4). Sub-fraction PE6.2 was purified on a silica gel column (n-hexane-acetone, 2.5:2, v/v) to yield mixture compounds 2 and 3 (12 mg). Sub-fraction PE6.3 was purified on a silica gel column (n-hexane-acetone, 2.5:2, v/v) to afford compound 1 (10 mg). Fraction PE7 was purified on a silica gel column (CH₂Cl₂-MeOH, 15:1, v/v) to give compound 8 (6 mg). Compound 5 (6 mg) was obtained from fraction PE8 through column chromatography on silica gel (CH₂Cl₂-MeOH- H_2O , 9:1:0.1, v/v), then silica gel Rp-18 (MeOH-H₂O, 1:1).

(2*S*,3*S*)-*Pretosin Q* (1): White powder. ESI-MS *m*/z 251.2 [M+H]⁺. ¹H-NMR (600 MHz, CD₃OD): $\delta_{\rm H}$ 7.36 (1H, s, H-4), 5.34 (1H, dd, *J*=8.4, 5.4 Hz, H-12), 4.70 (1H, d, *J*=4.2 Hz H-3), 3.94 (1H, dd, *J*=11.4, 8.4 Hz, H-13a), 3.65 (1H, dd, *J*=11.4, 5.4 Hz, H-13b), 2.77 (3H, s, H-14), 2.59 (3H, s, H-11), 2.49-2.45 (1H, m, H-2), 1.32 (3H, d, *J*=7.2 Hz, H-10). ¹³C-NMR (150 MHz, CD₃OD): $\delta_{\rm c}$ 207.52 (C-1), 155.34 (C-9), 146.43 (C-5), 140.33 (C-7), 138.66 (C-6), 132.81 (C-8), 126.69 (C-4), 75.74 (C-3), 72.73 (C-12), 65.56 (C-13), 54.79 (C-2), 22.35 (C-11), 15.06 (C-14), 13.26 (C-10).

(2*R*,3*S*)-*Pterosin C* (2): ESI-MS *m*/z 235.3 [M+H]⁺. ¹H-NMR (600 MHz, CD₃OD): $\delta_{\rm H}$ 7.38 (1H, s, H-4), 5.16 (1H, d, *J*=6.6 Hz, H-3), 3.63 (2H, t, *J*=7.8 Hz, H-13), 3.03 (2H, t, *J*=7.8 Hz, H-12), 2.79-2.74 (1H, m, H-2), 2.66 (3H, s, H-14), 2.49 (3H, s, H-11), 1.19 (3H, d, *J*=7.2 Hz, H-10). ¹³C-NMR (150 MHz, CD₃OD): $\delta_{\rm C}$ 210.31 (C-1), 155.13 (C-9), 146.24 (C-5), 138.54 (C-7), 138.07 (C-6), 132.22 (C-8), 126.68 (C-4), 70.23 (C-3), 61.61 (C-13), 49.66 (C-2), 33.04 (C-12), 21.37 (C-11), 14.00 (C-14), 10.70 (C-10).

(2*S*, 3*S*)-*Pterosin C* (3): ESI-MS m/z 235.3 [M+H]⁺. ¹H-NMR (600 MHz, CD₃OD): $\delta_{\rm H}$ 7.37 (1H, s, H-4), 4.70 (1H, d, *J*=4.2 Hz, H-3), 3.62 (2H, t, *J*=7.8 Hz, H-13), 3.02 (2H, t, *J*=7.8 Hz, H-12), 2.66 (3H, s, H-14), 2.49 (3H, s, H-11), 2.48-2.43 (1H, m, H-2), 1.32 (3H, d, *J*=7.2 Hz, H-10). ¹³C-NMR (150 MHz, CD₃OD): $\delta_{\rm C}$ 207.59 (C-1), 154.53 (C-9), 146.24 (C-5), 138.21 (C-7), 137.85 (C-6), 132.39 (C-8), 125.63 (C-4), 75.90 (C-3), 61.61 (C-13), 54.68 (C-2), 33.04 (C-12), 21.41 (C-11), 14.07 (C-14), 13.28 (C-10).

Pinoresinol (4): White powder. ESI-MS *m/z* 359.3. ¹H-NMR (600 MHz, DMSO-*d*₆): $\delta_{\rm H}$ 6.88 (2H, d, *J*=1.8 Hz, H-2, H-2'), 6.75 (2H, dd, *J*=8.4, 1.8 Hz, H-6, H-6'), 6.72 (2H, d, *J*=8.4 Hz, H-5, H-5'), 4.60 (2H, d, *J*=4.2 Hz, H-7, H-7'), 4.11 (2H, dd, *J*=9.0, 6.6 Hz, H-9a, H-9'a), 3.76 (6H, s, 2xOCH₃), 3.72 (2H, dd, *J*=9.0, 4.2 Hz, H-9b, H-9'b), 3.04-3.02 (2H, m, H-8, H-8'). ¹³C-NMR (150 MHz, DMSO-*d*₆): $\delta_{\rm C}$ 147.50 (C-3, C-3'), 145.88 (C-4, C-4'), 132.21 (C-1, C-1'), 118.61 (C-6, C-6'), 115.11 (C-5, C-5'), 110.39 (C-2, C-2'), 85.13 (C-7, C-7'), 70.87 (C-9, C-9'), 55.58 (2xOCH₃), 53.56 (C-8, C-8').

Lariciresinol 9-O-β-glucopyranoside (5): White powder. ESI-MS m/z 545.3 [M+Na]⁺. ¹H-NMR (600 MHz, CD₂OD): δ_{u} 6.95 (1H, d, J=1.8 Hz, H-2), 6.82 (1H, dd, J=7.8, 1.8 Hz, H-6), 6.81 (1H, d, J=1.8 Hz, H-2'), 6.78 (1H, d, J=7.8 Hz, H-5), 6.73 (1H, d, J=8.4 Hz, H-5'), 6.67 (1H, dd, J=8.4, 1.8 Hz, H-6'), 4.32 (1H, d, J=7.8 Hz, H-1"), 4.23 (1H, dd, J=9.6, 7.2 Hz, H-9a), 3.99 (1H, dd, J=8.4, 6.6 Hz, H-9'a), 3.89 (1H, dd, J=11.4, 1.8 Hz, H-6"a), 3.87 (3H, s, OCH,), 3.86 (3H, s, OCH,), 3.76 (1H, dd, J=8.4, 6.6 Hz, H-9'b), 3.69 (1H, dd, J=11.4, 5.4 Hz, H-6"b), 3.62 (1H, dd, J=9.6, 6.0 Hz, H-9b), 3.38-3.36 (1H, m, H-3"), 3.32-3.30 (2H, m, H-4", H-5"), 3.27-3.24 (1H, m, H-2"), 3.02 (1H, dd, J=13.8, 4.8 Hz, H-7'a), 2.78-2.75 (1H, m, H-8'), 2.57-2.51 (2H, m, H-7'b, H-8). ¹³C-NMR (150 MHz, CD₂OD): δ_c 149.00 (C-3, C-3'), 147.07 (C-4), 145.76 (C-4'), 135.65 (C-1), 133.82 (C-1'), 122.19 (C-6'), 119.89 (C-5), 116.19 (C-5'), 115.99 (C-6), 113.52 (C-2'), 110.80 (C-2), 104.60 (C-1"), 84.15 (C-7), 78.29 (C-3"), 78.04 (C-5"), 75.18 (C-2"), 73.69 (C-9'), 71.71 (C-4"), 68.47 (C-9), 62.84 (C-6"), 56.45, 56.44 (2xOCH₃), 51.70 (C-8), 44.04 (C-8'), 33.72 (C-7').

Naringenin (6): White powder. ¹H-NMR (600 MHz, DMSO- d_6): $\delta_{\rm H}$ 12.15 (1H, s, 5-OH), 7.31 (2H, d, J=9.0 Hz, H-2', H-6'), 6.79 (2H, d, J=9.0 Hz, H-3', H-5'), 5.88 (1H, d, J=2.4 Hz, H-8), 5.87 (1H, d, J=2.4 Hz, H-6), 5.44 (1H, dd, J=12.6, 3.0 Hz, H-2), 3.26 (1H, dd, J=16.8, 12.6 Hz, H-3a), 2.67 (1H, dd, J=16.8, 3.0 Hz, H-3b). ¹³C-NMR (150 MHz, DMSO- d_6): $\delta_{\rm C}$ 196.40 (C-4), 166.63 (C-7), 163.47 (C-5), 162.93 (C-9), 157.71 (C-4'), 128.84 (C-1'), 128.33 (C-2', C-6'), 115.14 (C-3', C-5'), 101.75 (C-10), 95.76 (C-6), 94.94 (C-8), 78.41 (C-2), 41.95 (C-3).

Apigenin (7): Yellow powder. ¹H-NMR (600 MHz, CD₃OD): $\delta_{\rm H}$ 7.87 (2H, d, *J*=8.4 Hz, H-2', H-6'), 6.95 (2H, d, *J*=8.4 Hz, H-3', H-5'), 6.61 (1H, s, H-3), 6.48 (1H, d, *J*=1.8 Hz, H-8), 6.23 (1H, d, *J*=1.8 Hz, H-6). ¹³C-NMR (150 MHz, CD₃OD): $\delta_{\rm C}$ 184.05 (C-4), 166.14 (C-7), 166.27 (C-2), 163.23 (C-5), 162.67 (C-4'), 159.53 (C-9), 129.53 (C-2', C-6'), 123.28 (C-1'), 117.11 (C-3', C-5'), 105.32 (C-10), 102.87 (C-3), 100.17 (C-6), 94.97 (C-8).

Luteolin (8): Yellow powder. ¹H-NMR (600 MHz, CD₃OD): $\delta_{\rm H}$ 7.40 (1H, dd, *J*=7.8, 2.4 Hz, H-6'), 7.39 (1H, d, *J*=2.4 Hz, H-2'), 6.92 (1H, d, *J*=7.8 Hz, H-5'), 6.55 (1H, s, H-3), 6.45 (1H, d, *J*=1.8

Hz, H-8), 6.22 (1H, d, *J*=1.8 Hz, H-6). ¹³C-NMR (150 MHz, CD₃OD): $\delta_{\rm C}$ 183.93 (C-4), 166.39 (C-7), 166.10 (C-2), 163.18 (C-5), 159.45 (C-9), 150.98 (C-4'), 147.03 (C-3'), 123.75 (C-1'), 120.38 (C-6'), 116.78 (C-5'), 114.15 (C-2'), 105.32 (C-10), 103.88 (C-3), 100.19 (C-6), 95.06 (C-8).

Chemical structures of compounds **1-8** obtained from *P. multifida* are presented in Fig. 1.

Compounds 2 and 3 were obtained as a mixture with a ratio of 1:3 (based on the peak intensities in their ¹H- and ¹³C-NMR spectra). The ESI-MS spectrum showed only one *pseudo*-molecular ion peak at m/z 235.3 [M+H]⁺ suggesting that 2 and 3 are isomers. The ¹H- and ¹³C-NMR spectral data of compounds 2 and 3 were similar to those of 1 except for the presence of an additional methylene group and the absence of one oxymethine group at C-12. The

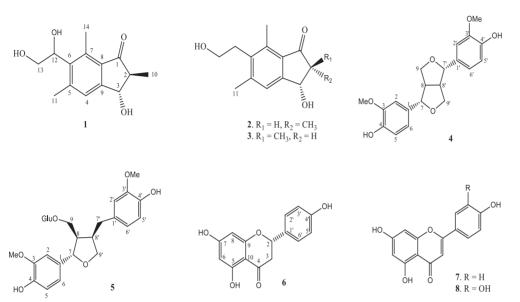


Fig. 1. Chemical structures of compounds 1-8 obtained from P. multifida.

3. Results and discussion

Compound 1 was isolated as a white powder and its molecular formula was established as C14H18O4 based on a pseudo-molecular ion peak at 251.2 [M+H]+ and NMR data. The carbon-13 nuclear magnetic resonance (13C-NMR) and heteronuclear single quantum coherence (HSQC) of 1 displayed 14 carbon signals including one ketone group at δ_{C} 207.52 (C-1), 5 quaternary aromatic carbons, one aromatic methine carbon, 3 aliphatic methine carbons in which two oxygenated methines, 1 oxygenated methylene carbon, and three methyl groups. The 1H-NMR spectrum showed signals of one aromatic proton [δ_{H} 7.36 (1H, s, H-4)], one oxygenated methylene [δ_{H} 3.94 (1H, dd, *J*=11.4, 8.4 Hz, H-13a), 3.65 (1H, dd, J=11.4, 5.4 Hz, H-13b)], three methine protons [$\delta_{\rm H}$ 5.34 (1H, dd, J=8.4, 5.4 Hz, H-12), 4.70 (1H, d, J=4.2 Hz, H-3), and 2.49-2.45 (1H, m, H-2)] and three methyl groups $[\delta_{H} 2.77 (3H, s, H-14), 2.59$ (3H, s, H-11), and 1.32 (3H, d, J=7.2 Hz, H-10)]. These indicated compound 1 was a pterosin sesquiterpene. The small coupling constant $J_{2,3}$ =4.2 Hz suggested the *trans*-configuration for the protons at C-2 and C-3 in 1 [9]. The HMBC spectrum showed correlations of H-10 to C-1, C-2, and C-3; of H-12 to C-6, C-7, C-13; of H-11 to C-4 and C-5; and of H-4 to C-3 and C-8. Based on the above results, the structure of compound 1 was identified as (2S,3S)-pretosin Q. This compound was previously obtained from P. multifida [3].

coupling constants $(J_{23}=6.6 \text{ Hz})$ and $J_{2,3}$ =4.2 Hz) confirmed the cis-, trans-configurations for the protons at C-2 and C-3 in 2 and 3. respectively. Additionally, the ¹³C-NMR spectral data of 2 and 3 were similar to those of the (2R.3S)-acetylpterosin C and (2S,3S)-acetylpterosin C, except for the absence of signals of the acetyl groups at C-13 and the chemical shifts of C-13 and C-12 significantly changed by -2.2 and +4.1 ppm, respectively [11]. As a result, the chemical structures of 2 and 3 were established as (2R,3S)-pterosin C and (2S,3S)pterosin C, respectively.

The ESI-MS spectrum of 4 showed a *pseudo*-molecular ion peak at m/z 359.3 [M+H]⁺. The ¹³C NMR and HSQC spectra

indicated the presence of only ten carbon peaks, including three aromatic quaternary carbons, three methine carbons, one oxymethylene, one oxymethine, one methine, and one methoxy group. These suggested that compound **4** was a symmetrical molecule and its molecular formula as $C_{20}H_{22}O_6$. The ¹H-NMR revealed six aromatic protons in a pair of ABX systems at $\delta_{\rm H}$ 6.88 (2H, d, *J*=1.8 Hz, H-2, H-2'), 6.75 (2H, dd, *J*=8.4, 1.8 Hz, H-6, H-6'), 6.72 (2H, d, *J*=8.4 Hz, H-5, H-5'), two methoxy groups at $\delta_{\rm H}$ 3.76 (6H, s), and aliphatic protons at $\delta_{\rm H}$ 4.60 (2H, d, *J*=4.2 Hz, H-7, H-7'), 4.11 (2H, dd, *J*=9.0, 6.6 Hz, H-9a, H-9'a), 3.72 (2H, dd, *J*=9.0, 4.2 Hz, H-9b, H-9'b), 3.04-3.02 (2H, m, H-8, H-8'). Therefore, the structure of **4** was determined to be pinoresinol. The ¹³C-NMR data (in DMSO-*d6*) of **4** were in good agreement with those of pinoresinol in [15].

The molecular formula of **5** was determined as $C_{26}H_{34}O_{11}$ from an ESI-MS pseudo-molecular ion peak at 545.3 [M+Na]⁺ and NMR spectral data. The ¹³C-NMR and HSQC spectra showed a total 26 carbon signals, which included three methines (δ_{C} 84.15, 51.70 and 44.04), three methylenes (δ_{C} 73.69, 68.47 and 33.72), two methoxy groups (δ_{C} 56.45, 56.44), twelve signals for two aromatic rings (δ_{C} 149.00- 110.80), and six signals for one glucose unit with an anomeric carbon at δ_{C} 104.60. The ¹H-NMR spectrum of **5** revealed the presence of two 1,3,4-trisubstitued benzenes [$\delta_{\rm H}$ 6.95 (1H, d, *J*=1.8 Hz, H-2), 6.82 (1H, dd, *J*=7.8, 1.8 Hz, H-6), 6.78 (1H, d, *J*=7.8 Hz, H-5); 6.81 (1H, d, *J*=1.8 Hz, H-2'), 6.73 (1H, d, *J*=8.4 Hz, H-5'), 6.67 (1H, dd, *J*=8.4, 1.8 Hz, H-6')], two methoxy groups [$\delta_{\rm H}$ 3.87 and 3.86 (each 3H, s)]. In addition, the ¹H-NMR spectrum also supported the presence of one β-glucose moiety with an anomeric proton signal at $\delta_{\rm H}$ 4.32 (1H, d, *J*=7.8 Hz, H-1"). The HMBC correlations between H-1" ($\delta_{\rm H}$ 4.32) with C-9 ($\delta_{\rm C}$ 68.47) indicated that the sugar unit was attached at the C-9 position. The NOE correlations between H-8 and H-8' and between H-7 and H-9 confirmed the benzyl group and the glucosyl group were on the same side, while the phenyl group was oriented to the other side of tetrahydrofuran ring. The ¹³C-NMR data of **5** were similar with those of lariciresinol [16] except for the presence of an additional β-glucose unit. Based on above evidence, the structure of **5** was elucidated to be lariciresinol 9-O-β-glucopyranoside.

Compound **6** was obtained as white powder. The ¹³C-NMR and HSQC spectra showed 15 carbon signals comprising one carbonyl carbon (δ_c 196.40), six quaternary carbons, seven methine carbons, and one methylene carbon. These suggested **6** was a flavonoid. The ¹H-NMR spectrum exhibited a one-proton singlet [δ_H 12.15] for a chelated hydroxyl group at C-5, two *meta*-coupled proton signals [δ_H 5.88 (1H, d, *J*=2.4 Hz) and 5.87 (1H, d, *J*=2.4 Hz)]

corresponding to H-8 and H-6 of ring A, one AA'BB' spin systems of 1',4'-disubstituted aromatic ring B [$\delta_{\rm H}$ 7.31 and 6.79 (both 2H, d, *J*=7.5 Hz)], and other proton signals of ring C [$\delta_{\rm H}$ 5.44 (1H, dd, *J*=12.6, 3.0 Hz, H-2), 3.26 (1H, dd, *J*=16.8, 12.6 Hz, H-3a), 2.67 (1H, dd, *J*=16.8, 3.0 Hz, H-3b)]. ¹H- and ¹³C-NMR data of **6** were in good agreement with those reported for naringenin [17]. Therefore, **6** was determined to be naringenin.

The structures of the remaining two compounds, 7 and 8, were determined to be apigenin [18] and luteolin [19], respectively, based on the analysis NMR data and by comparison with the literature. These flavonoids have been found in several plant species and have been previously obtained from *P. multifida*.

Figure 2 shows in simplified form the pathways involved in the biosynthesis of all isolated compounds starting primary metabolism. Farnesyl pyrophosphate is the starting material for all other sesquiterpenes including compounds 1, 2, and 3. The lignan biosynthesis of 4 and 5 were developed through a long sequence of reactions involving the shikimic acid pathway from cinnamic acid to feruloyl-CoA. Flavonoids 6, 7, and 8 are synthesised via shikimic acid and HAc-malonic acid pathways, respectively [20-22].

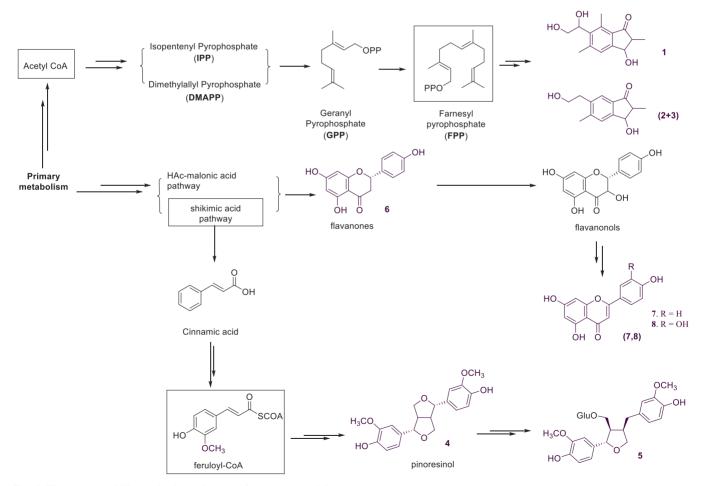


Fig. 2. The proposed biosynthetic pathways of compounds 1-8.

4. Conclusions

The chromatographic investigation of the ethyl acetate extract of the entire whole of *P. multifida* plant collected in the Nam Dinh province, Vietnam, led to the isolation of eight compounds. Their structures were identified as (2S,3S)-pretosin Q (1), (2R,3S)-pterosin C (2), (2S,3S)-pterosin C (3), pinoresinol (4), lariciresinol 9-O- β -glucopyranoside (5), naringenin (6), apigenin (7), and luteolin (8) by analysis of their 1D-NMR (¹H-, ¹³C-NMR), 2D-NMR (HSQC, HMBC, NOESY), ESI-MS spectra, and comparison with published data. This is the first report of the isolation of compounds **4-6** from *P. multifida*.

CRediT author statement

Duc Thien Dao: Methodology, Data curation, Writing -Original draft preparation; Hoang Sa Nguyen: Data curation, Writing - Original draft preparation; Huu Thu Do: Investigation; Thanh Tam Nguyen: Writing - Reviewing and Editing.

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COMPETING INTERESTS

The authors declare that there is no conflict of interest regarding the publication of this article.

REFERENCES

[1] P.H. Ho (2000), *An Illustrated Flora of Vietnam*, Youth Publishing House, **1**, pp.61-68 (in Vietnamese).

[2] W. Hu, M. Hou, K. Hao, et al. (2021), "Phytochemical and chemotaxonomic studies on *Pteris wallichiana* J. Agardh", *Biochemical Systematics and Ecology*, **95**, DOI: 10.1016/j.bse.2021.104242.

[3] M. Hou, Y. Chen, Y. Wang, et al. (2021), "Sesquiterpenoids and flavonoids from *Pteris multifida* Poir.", *Biochemical Systematics and Ecology*, 98, DOI: 10.1016/j.bse.2021.104320.

[4] J.W. Kim, J.Y. Seo, W.K. Oh, et al. (2017), "Anti-neuroinflammatory ent-kaurane diterpenoids from *Pteris multifida* roots", *Molecules*, 22(1), pp.27-36, DOI: 10.3390/molecules22010027.

[5] T.C. Wang, C.C. Lin, H.I. Lee, et al. (2010), "Anti-hyperlipidemic activity of spider brake (*Pteris multifida*) with rats fed a high cholesterol diet", *Pharmaceutical Biology*, **48(2)**, pp.221-226, DOI: 10.3109/13880200903085458.

[6] B.B. Hu, X.D. Zheng, H.S. Hu, et al. (2009), "Three antibacterial compounds from the roots of *Pteris multifida*", *Chemistry of Natural Compounds*, **45(1)**, pp.45-48, DOI: 10.1007/s10600-009-9258-5.

[7] T.C. Wang, M.C. Ti, S.C. Lo, et al. (2007), "Free radical-scavenging activity of aqueous extract of *Pteris multifida* Poiret", *Fitoterapia*, **78(3)**, pp.248-249, DOI: 10.1016/j.fitote.2006.11.004.

[8] H.B. Hu, X.D. Zheng, H. Cao, et al. (2006), "Xanthone *O*-glycosides from the roots of *Pteris multifida*", *Journal of the Chinese Chemical Society*, **53(2)**, pp.459-464, DOI: 10.1002/cbdv.202200499.

[9] X. Ge, G. Ye, P. Li, et al. (2008), "Cytotoxic diterpenoids and sesquiterpenoids from *Pteris multifida*", *Journal of Natural Product*, **71(2)**, pp.227-231, DOI: 10.1021/np0706421.

[10] J. Liu, J. Shu, R. Zhang, et al. (2011), "Two new pterosin dimers from *Pteris multifida* Poir.", *Fitoterapia*, **82(8)**, pp.1181-1184, DOI: 10.1016/j. fitote.2011.08.004.

[11] J. Shu, J. Liu, Y. Zhong, et al. (2012), "Two new pterosin sesquiterpenes from *Pteris multifida* Poir.", *Phytochemistry Letters*, **5**(2), pp.276-279, DOI: 10.1016/j.phytol.2012.01.011.

[12] G. Ni, G.J. Fu, D. Zhang, et al. (2015), "An unusual dihydrobenzofuroisocoumarin and *ent*-kauranediterpenoids from *Pteris multifida*", *Journal of Asian Natural Products Research*, **17(5)**, pp.423-429, DOI: 10.1080/10286020.2015.1040777.

[13] N.D. Chi, L.Q. Long, T.N.H. Thu, et al. (2014), "Some flavonoids were isolated from sedge grass (*Pteris multifida* Poir.) collected in Hanoi (in Vietnamese)", *Journal of Medicinal Materials*, **54**(9), pp.50-54.

[14] N.D. Chi, L.Q. Long, T.N.H. Thu, et al. (2015), "Some compounds isolated from water fractions of sedge grass (*Pteris multifida* Poir.) collected in Hanoi (in Vietnamese)", *Journal of Medicinal Materials*, **55(3)**, pp.25-29.

[15] S. Nishibe, H. Tsukamoto, S. Hisada, et al. (1984), "Effects of *O*-methylation and *O*-glucosylation on carbon-13 nuclear magnetic resonance chemical shifts of matairesinol, (+)-pinoresinol and (+)-epipinoresinol", *Chemical and Pharmaceutical Bulletin*, **32(11)**, pp.4653-4657, DOI: 10.1248/ cpb.32.2622.

[16] L.H. Xie, T. Akao, K. Hamasaki, et al. (2003), "Biotransformation of pinoresinol diglucoside to mammalian lignans by human intestinal microflora, and isolation of *Enterococcus faecalis* strain PDG-1 responsible for the transformation of (+)-pinoresinol to (+)-lariciresinol", *Chemical and Pharmaceutical Bulletin*, **51**(5), pp.508-515, DOI: 10.1248/cpb.51.508.

[17] C.C. Shen, Y.S. Chang, L.K. Hott, et al. (1993), "Nuclear magnetic resonance studies of 5,7-dihydroxyflavonoids", *Phytochemistry*, **34(3)**, pp.843-845.

[18] L.A.L. Silva, L.G. Faqueti, F.H. Reginatto, et al. (2015), "Phytochemical analysis of *Vernonanthura tweedieana* and a validated UPLC-PDA method for the quantification of eriodictyol", *Revista Brasileira de Farmacognosia*, **25(4)**, pp.375-381, DOI: 10.1016/0031-9422(93)85370-7.

[19] L.M. Ha, N.T. Phuong, D.T.T. Huyen, et al. (2019), "A contribution to study chemical constituents of *Desmodium gangeticum* of Vietnam", *Vietnam Journal of Science and Technology*, **57(1)**, pp.1-6, DOI: 10.15625/2525-2518/57/1/12758.

[20] G.A. Cordell (1976), "Biosynthesis of sesquiterpenes", *Chemical Reviews*, **76(4)**, pp.425-460, DOI: 10.1021/cr60302a002.

[21] N.G. Lewis, L.B. Davin, S. Sarkanen (1998), "Chapter 1: Lignin and lignan biosynthesis: Distinctions and reconciliations", *ACS Symposium Series*, **697**, pp.1-27, DOI: 10.1021/bk-1998-0697.ch001.

[22] M. Rehan (2021), "Biosynthesis of diverse class flavonoids via shikimate and phenylpropanoid pathway", *Bioactive Compounds - Biosynthesis, Characterization and Applications*, IntechOpen Publisher, 304pp, DOI: 10.5772/intechopen.96512.