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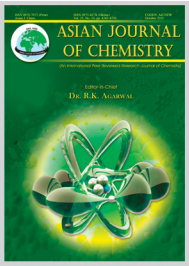

NOTE

Phytochemical Studies on *Pteris excelsa*

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Present study reports the chemical constituents of *Pteris excelsa* Gaud. The compounds were isolated by MCI gel chromatography and silica gel column chromatography. Their structures were identified by physicochemical properties and spectroscopic analysis. Three compounds were elucidated as luteolin 7-O-β-D-glucoside (**1**), β-sitosterol (**2**) and fructose (**3**). All the compounds were isolated from *Pteris excelsa* Gaud for the first time.

Key Words: *Pteris excelsa* Gaud, Chemical constituents, Luteolin 7-O-β-D-glucoside.

The group of ferns from the genus *Pteris* (Pteridaceae) contains 300 cosmopolitan species, with 66 species being endemic to China[1]. Several plants in the genus are widely used in traditional Chinese medicine for relieving internal heat or fever, swelling and diarrhea and removing toxicity. For example, *Pteris semipinnata* L. has been widely utilized as folk medicine to treat toothache, diarrhea, jaundices and viper bites.[2] *Pteris multifida* was mainly used as folk medicine for the treatment of cholera, dysentery, entero-haemorrhoids and as an antitumor and antiinflammatory agent.[3,4] *Pteris ensiformis* Burm has been one of the most popular constituents of herbal beverages in Taiwan for hundreds of years.[5] Previously, phytochemical investigations on the Pteris genus had led to the isolation of various phenolic compounds, flavonol glycosides, kauranes and pterosinsesquiterpenes.[6-11] Some extracts and compounds of the plants in the genus showed various bioactivities such as antitumor, antibacterial, cytotoxic and free radical-scavenging activities.[12-17] *Pteris excelsa* Gaud was distributed in North Korea and south-western China.[1] There was no report on the isolation of compounds from it. During the search for bioactive compounds from medicinal plants in Yunnan of China, we investigated the plant and isolated three compounds: luteolin 7-O-β-D-glucoside (**1**), β-sitosterol (**2**) and fructose (**3**).

Mass spectra were determined on an API Qstar Pulsa LC/TOF mass spectrometer. NMR spectra were measured on a Bruker DRX-500 spectrometer (500 MHz for ^{1}H and 125 MHz for ^{13}C) using the solvent DMSO-d_6, or $CDCl_3$ and TMS as internal standard. Silica gel (200-300 mesh, Qingdao Marine Chemical Co., China) and MCI gel CHP20P (75-150 mm; Mitsubishi Chemical Corporation, Tokyo, Japan) were used for column chromatography and silica gel GF_{254} for TLC (Qingdao Marine Chemical Co., China). Solvents were of industrial purity and distilled prior to use.

The whole plant of *Pteris excelsa* was collected from Kunming of Yunnan Province, China in April, 2006 and identified by Prof. Shugang LU, School of Life Science, Yunnan University, where a voucher specimen (0604011) is deposited.

Extraction and isolation: The air dried powdered whole plant of *Pteris excelsa* (50 g) was extracted with 95 % EtOH at room temperature. The solutions was concentrated under vacuum to yield the EtOH extract (5 g), which was added with H_2O (0.1 L) and the resulting solution was extracted with EtOAc to afford the EtOAc extract (1 g). The EtOAc extract was separated on a MCI gel column, eluting with H_2O, MeOH-H_2O (8:2, 9:1), MeOH and acetone successively to obtain 5 fractions. Fr. 1 (0.1 g) was crystallized to afford fructose (**3**) (12 mg). Fr. 3 (0.12 g) was isolated by column chromatography (silica gel, petroleum ether-EtOAc 20:1) to yield luteolin 7-O-β-D-glucoside (**1**) (3 mg). Fr. 5 (0.14 g) was purified by column chromatography (silica gel, petroleum ether-EtOAc 20:1 to 0:1) to obtain β-sitosterol (**2**) (17 mg).

Luteolin 7-O-β-D-glucoside (1): Compound **1** (3 mg) was obtained as light yellow amorphous powder; ESIMS m/z: 449 $[M+H]^+$; ^{1}H NMR (500 MHz, DMSO-d_6) δ: 12.99 (1H, s, 5-OH), 10.03 (1H, s, 4'-OH), 9.43 (1H, s, 3'-OH), 7.45 (1H, dd, J = 8.3, 2.2 Hz, H-6'), 7.42 (1H, d, J = 2.2, Hz, H-2'), 6.90

(1H, d, *J* = 8.3 Hz, H-5'), 6.78 (1H, d, *J* = 2.2 Hz, H-8), 6.76 (1H, s, H-3), 6.44 (1H, d, *J* = 2.2 Hz, H-6); ^{13}C NMR (125 MHz, DMSO-d_6) δ: 182.2 (C-4), 164.8 (C-2), 163.3 (C-7), 161.4 (C-5), 157.3 (C-9), 150.2 (C-4'), 146.1 (C-3'), 121.7 (C-1'), 119.5 (C-6'), 116.3 (C-5'), 113.9 (C-2'), 105.7 (C-10), 103.5 (C-3), 100.3 (C-1''), 99.9 (C-6), 95.1 (C-8), 77.5 (C-5''), 76.7 (C-3''), 73.5 (C-2''), 69.9 (C-4''), 61.0 (C-6''). The physical and spectral data showed complete agreement with the literature[18].

β-Sitosterol (2): Compound **2** (17 mg) was obtained as colourless needles; m.p. 137-138 ºC; ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 5.33 (1H, d, *J* = 5.0 Hz, H-6), 3.50 (1H, m, H-3), 0.66 (3H, s, Me-18), 0.80 (3H, d, *J* = 8.7, H-26), 0.82 (3H, d, *J* = 8.7, H-27), 0.84 (3H, t, *J* = 7.7, H-29), 0.92 (3H, d, *J* = 8.1, H-21), 0.99 (3H, s, H-19), ^{13}C NMR ($CDCl_3$, 125 MHz): δ140.8, 121.6, 71.7, 56.8, 56.1, 50.2, 49.8, 45.9, 42.3, 42.2, 39.8, 37.3, 36.5, 36.1, 34.0, 31.9, 31.5, 29.3, 28.2, 26.2, 24.3, 23.1, 21.1, 19.7, 19.3, 19.0, 18.8, 11.9, 11.8. Identified by mixed melting point and co-TLC with that of authentic samples[19].

Fructose (3): Compound **3** (12 mg) was obtained as colourless powder. ^{1}H NMR (DMSO-d_6, 500 MHz) δ: 4.04 (1H, d, *J* = 3.8 Hz), 3.92-3.64 (6H, m); ^{13}C NMR (DMSO-d_6, 125 MHz) δ: 109.7 (C-2), 85.2 (C-5), 83.7 (C-3), 79.5 (C-4), 63.3 (C-1), 61.1 (C-6); identified by co-chromatography with authentic standards.

The 95 % EtOH extract of the whole plant of *Pteris excelsa* was partitioned between water and ethyl acetate. The ethyl acetate fraction was subjected to MCI gel column chromatography eluted H_2O, MeOH-H_2O (8:2, 9:1), MeOH and acetone successively. In summary, **3** compounds were isolated from the acetyl acetate fractions of the 95 % EtOH extract of this plant, including luteolin 7-O-β-D-glucoside (**1**), β-sitosterol (**2**) and fructose (**3**). All the compounds were isolated from *Pteris excelsa* Gaud for the first time.

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