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.

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

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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

(**2**), naringin (**3**), neoeriocitrin (**4**), and hypolaetin-7-*O*-*β*-D-glucopyranoside (**5**) are firstly reported in *P. lanceolata*.

2. Experimental

2.1. Materials and reagents

The whole plant of *Pyrrosia lanceolata* (L.) Farw. was collected at Tao Dan Park (Ho Chi Minh City) in January 2019. The voucher specimen (PL012019.HCM) was deposited at the Faculty of Pharmacy, University of Medicine and Pharmacy at Ho Chi Minh City (UMP), Vietnam. The plant was identified by Dr. Vo Van Leo (Dept. of Pharmacognosy, UMP) and compared to the morphology characteristics as in botanical references [11]. The solvents were used for isolation and extraction: ethanol 96%, *n*-hexane, ethyl acetate, chloroform, methanol, and *n*-butanol (Scharlau, Spain). The solvents used were of analytical reagent (AR) grade.

2.2. General experimental procedures

Silica gel (40-63 μm, Merck) and Sephadex LH-20 (GE Healthcare, Sweden) were used for column chromatography (CC). The UV spectra were obtained on an UV-VIS SP8001 spectrophotometer (Axiom, Germany). TLC was performed using normal phase *silica gel* 60 F_{254} plates (Merck). Spots were detected by UV and 5% vanillin-sulfuric acid/absolute ethanol or 1% $FeCl_3$ solutions/95% ethanol. ESI-MS spectra were obtained from a Xevo TQ-S micro mass spectrometer (Waters, USA). APCI-MS spectra were obtained from an Agilent 6130-quadrupole mass spectrometer (Agilent Technologies, USA). NMR spectra were measured on a Bruker Avance Neo 400 MHz or 500 MHz spectrometer, using TMS as an internal standard. NMR solvents were purchased from Sigma-Aldrich (USA).

2.3. Extraction and isolation

The whole plant of *P. lanceolata* (2.4 kg) was crushed in a blender and percolated with 80% ethanol (40 L). The solvents were evaporated under reduced pressure to obtain the concentrated ethanol extract (800 mL). The ethanol extract was successively partitioned with *n*-hexane (2L x 5), ethyl acetate (2L x 4) and saturated *n*-BuOH (2L x 3), then the solvents were removed to obtain *n*-hexane (H, 66.3 g), ethyl acetate (E, 15.3 g), *n*-BuOH (B, 64.9 g), and water (W, 289.2 g) fractions, respectively. The ethyl acetate fraction (E, 12.0 g) was subjected to *silica gel* CC and eluted with $CHCl_3$-MeOH-H_2O (95:5:0, 90:10:10, 80:20:10, 70:30:10 and 65:35:10, v/v) to obtain 14 sub-fractions (E1-E14). Fraction E7 (600 mg) was subjected to Sephadex LH-20 CC (MeOH) to give 6 sub-fractions (E7.1-E7.6) and further purified by Sephadex LH-20 CC (MeOH) to obtain compound **1** (27.6 mg) from sub-fraction E7.4 (216.7 mg). Fraction E8 (250 mg) was subjected to Sephadex LH-20 CC (MeOH) to give 6 sub-fractions (E8.1-E8.6) and sub-fraction E8.2 (75.0 mg) was continued to be purified by Sephadex LH-20 CC (MeOH) to achieve compound **2** (17.0 mg). Fraction E9 (1.0 g) was loaded on Sephadex LH-20 CC (MeOH) CC to yield 4 sub-fractions (E9.1-E9.4). Compound **3** (24.8 mg) was isolated and further purified by Sephadex LH-20 CC (MeOH) from sub-fraction E9.4 (270 mg). After solvent removal, fractions E10 and E11 were precipitated in MeOH to obtain compounds **4** (222.2 mg) and **5** (10.4 mg), respectively.

Vitexin (1): pale-yellow amorphous powder. ESI-MS: *m/z* = 431.39 $[M-H]^-$, $C_{21}H_{20}O_{10}$. ^1H NMR (400 MHz, DMSO-d_6) δ_H: 13.17 (1H, s, 5-OH), 10.80 (1H, s, 7-OH), 10.35 (1H, s, 4′-OH), 8.03 (2H, d, *J* = 8.8 Hz, H-2′ and H-6′), 6.89 (2H, d, *J* = 8.8 Hz, H-3′ and H-5′), 6.79 (1H, s, H-3), 6.28 (1H, s, H-6), 4.69 (1H, d, *J* = 10.0 Hz, H-1″), 3.83 (1H, t, *J* = 9.2 Hz, H-2″), 3.38, (1H, m, H-4″) 3.27 (1H, m, H-3″), 3.22 (1H, m, H-5″), 3.76 (1H, m, H-6a″), 3.52 (1H, m, H-6b″). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 182.6 (C-4), 164.4 (C-2), 163.0 (C-7), 161.6 (C-4′), 160.8 (C-5), 156.4 (C-9), 129.5 (C-2′ and C-6′), 122.1 (C-1′), 116.3 (C-3′ and C-5′), 105.1 (C-8), 104.5 (C-10), 102.9 (C-3), 98.6 (C-6), 82.3 (C-5″), 79.1 (C-3″), 73.8 (C-1″), 71.3 (C-2″), 71.0 (C-4″), 61.8 (C-6″).

Naringenin 7-*O*-(2-*β*-D-apiofuranosyl)-*β*-D-glucopyranoside (2): colorless amorphous powder. APCI-MS: *m/z* = 565.00 $[M-H]^-$, $C_{26}H_{30}O_{14}$. ^1H NMR (400 MHz, CD_3OD) δ_H: 7.32 (2H, d, *J* = 8.4 Hz, H-2′ and H-6′), 6.81 (2H, d, *J* = 8.4 Hz, H-3′ and H-5′), 6.17 (1H, d, *J* = 2.0 Hz, H-8), 6.16 (1H, d, *J* = 2.0 Hz, H-6), 5.41 (1H, d, *J* = 1.6 Hz, H-1‴), 5.39 (1H, dd, *J* = 12.8, 3.2 Hz, H-2), 5.05 (1H, d, *J* = 7.2 Hz, H-1″), 3.98 (1H, dd, *J* = 9.6, 3.6 Hz, H-4a‴), 3.95 (1H, d *J* = 1.6 Hz, H-2‴), 3.86 (1H, d, *J* = 10.8 Hz, H-6a″), 3.78 (1H, d, *J* = 6.4 Hz, H-4b‴), 3.68 (1H, m, H-6b″), 3.52 (2H, s, H_2-5‴), 3.19 (1H, dd, *J* = 17.2, 12.8, H-3a), 2.76 (1H, dd, *J* = 17.2, 2.8 Hz, H-3b). ^{13}C NMR (100 MHz, CD_3OD) δ_C: 198.6 (C-4), 166.8 (C-7), 165.0 (C-9), 164.7 (C-5), 159.2 (C-4′), 130.8 (C-1′), 129.2 (C-2′ and C-6′), 116.4 (C-3′ and C-5′), 110.9 (C-1‴), 104.9 (C-10), 99.8 (C-1″), 97.9 (C-6), 96.8 (C-8), 80.7 (C-2 and C-3‴), 78.6 (C-2″), 78.5 (C-3″), 78.2 (C-5″), 78.1 (C-2‴), 75.5 (C-4‴), 71.2 (C-4″), 65.9 (C-5‴), 62.3 (C-6″), 44.0 (C-3).

Naringin (3): colorless needles, APCI-MS: m/z = 603.10 $[M+Na]^+$, $C_{27}H_{32}O_{14}$. ^1H NMR (400 MHz, CD_3OD) δ_H: 7.32 (2H, d, J = 8.4 Hz, H-2′ and H-6′), 6.83 (2H, d, J = 8.4 Hz, H-3′ and H-5′), 6.17 (1H, d, J = 2.0 Hz, H-8), 6.15 (1H, d, J = 2.0 Hz, H-6), 5.39 (1H, dd, J = 12.8, 2.8 Hz, H-2), 5.24 (1H, dd, J = 4.0, 1.6 Hz, H-1‴), 5.10 (1H, t, J = 7.2 Hz, H-1″), 3.92 (1H, m, H-2‴), 3.87 (1H, m, H-5‴), 3.85 (1H, m, H-6a″), 3.67 (1H, m, H-6b″), 3.62 (1H, m, H-2″), 3.60 (1H, m, H-5″), 3.58 (1H, m, H-3‴), 3.44 (1H, m, H-3″), 3.38 (2H, m, H-4″ and H-4‴), 3.15 (1H, dd, J = 16.8, 12.8 Hz, H-3a), 2.74 (1H, dd, J = 16.8, 2.8, Hz, H-3b), 1.28 (3H, d, J = 6.4 Hz, H-6‴). ^{13}C NMR (100 MHz, CD_3OD) δ_C: 198.5 (C-4), 166.6 (C-7), 165.0 (C-5), 164.7 (C-9), 159.1 (C-4′), 130.8 (C-1′), 129.1 (C-2′ and C-6′), 116.3 (C-3′ and C-5′), 104.9 (C-10), 102.6 (C-1″), 99.3 (C-1‴), 97.8 (C-6), 96.8 (C-8), 80.7 (C-2), 79.2 (C-2″), 79.0 (C-5″), 78.1 (C-3″), 73.9 (C-4‴), 72.19 (C-2‴), 72.17 (C-3‴), 71.2 (C-4″), 70.0 (C-5‴), 62.3 (C-6″), 44.0 (C-3), 18.2 (C-6‴).

Neoeriocitrin (4): colorless amorphous powder. ESI-MS: m/z = 595.07 $[M-H]^-$, $C_{27}H_{32}O_{15}$. ^1H NMR (400 MHz, CD_3OD) δ_H: 6.91 (1H, d, J = 1.6 Hz, H-2′), 6.78 (1H, dd, J = 8.0, 1.6 Hz, H-6′), 6.78 (1H, d, J = 8.0 Hz, H-5′), 6.17 (1H, d, J = 2.4 Hz, H-8), 6.15 (1H, d, J = 2.4 Hz, H-6), 5.32 (1H, dd, J = 12.8, 3.2 Hz, H-2), 5.24 (1H, s, H-1‴), 5.09 (1H, d, J = 7.2 Hz, H-1″), 3.92 (1H, m, H-2‴), 3.88 (1H, m, H-5‴), 3.85 (1H, m, H-6a‴), 3.67 (1H, m, H-6b‴), 3.63 (1H, m, H-2″), 3.60 (1H, m, H-3″), 3.57 (1H, m, H-3‴), 3.45 (1H, m, H-5″), 3.39 (1H, m, H-4″), 3.37 (1H, m, H-4‴), 3.12 (1H, dd, J = 17.2, 12.8 Hz, H-3a), 2.76 (1H, dd, J = 17.2, 3.2 Hz, H-3b), 1.28 (3H, d, J = 6.0 Hz, H-6‴). ^{13}C NMR (100 MHz, CD_3OD) δ_C: 197.1 (C-4), 165.2 (C-7), 163.6 (C-5), 163.2 (C-9), 145.6 (C-4′), 145.1 (C-3′), 130.1 (C-1′), 117.9 (C-6′), 114.8 (C-5′), 113.4 (C-2′), 103.5 (C-10), 101.1 (C-1‴), 98.0 (C-1″), 96.4 (C-6), 95.4 (C-8), 79.3 (C-2), 77.6 (C-2″), 77.6 (C-3″), 76.7 (C-5″), 72.5 (C-4‴), 70.8 (C-3‴), 70.8 (C-2‴), 69.8 (C-4″), 60.9 (C-6″), 42.8 (C-3), 16.8 (C-6‴).

Hypolaetin-7-*O*-*β*-D-glucopyranoside (5): pale-yellow amorphous powder. APCI-MS: m/z = 463.16 $[M-H]^-$, $C_{21}H_{20}O_{12}$; ^1H NMR (500 MHz, DMSO-d_6) δ_H: 12.37 (1H, s, 5-OH), 7.48 (1H, d, J = 2.0 Hz, H-2′), 7.47 (1H, dd, J = 8.0, 2.0 Hz, H-6′), 6.91 (1H, d, J = 8.0 Hz, H-5′), 6.71 (1H, s, H-3), 6.63 (1H, s, H-6), 4.93 (1H, d, J = 7.5 Hz, H-1″), 3.72 (1H, d, J = 10.5 Hz, H-6a″), 3.47 (1H, m, H-6b″), 3.42 (1H, m, H-5″), 3.37 (1H, m, H-2″), 3.31 (1H, m, H-3″), 3.19 (1H, m, H-4″). ^{13}C NMR (125 MHz, DMSO-d_6) δ_C: 182.3 (C-4), 164.3 (C-2), 152.3 (C-5), 151.7 (C-7), 149.9 (C-4′), 145.7 (C-3′), 144.3 (C-9), 127.0 (C-8), 121.6 (C-1′), 119.2 (C-6′), 116.0 (C-5′), 113.6 (C-2′), 105.2 (C-10), 102.7 (C-3), 101.3 (C-1″), 98.7 (C-6), 77.3 (C-5″), 75.7 (C-3″), 73.2 (C-2″), 69.7 (C-4″), 60.7 (C-6″).

3. Results and discussion

Compound **1** was isolated as a yellow amorphous powder. The ESI-MS showed the negative ion peak with m/z = 431.39 $[M-H]^-$, calculated for the molecular formula of $C_{21}H_{20}O_{10}$ with 12 degrees of unsaturation. The ^{13}C NMR spectrum showed the signals of 21 carbons, of which 15 were observed in the downfield region of δ_C 182-98 and the remaining 6 carbons at δ_C 83-61 of a sugar moiety. The ^{13}C NMR data agreed with the structure of a flavonoid glucoside. The ^{13}C NMR spectrum of **1** revealed the signals of a carbonyl group at δ_C 182.6 (C-4), five oxygenated carbons (δ_C 164.4 (C-2), 163.0 (C-7), 161.6 (C-4′), 160.8 (C-5), 156.4 (C-9)), three non-protonated carbons (δ_C 122.1 (C-1′), 105.1 (C-8), and 104.5 (C-10)), and six methine carbons (δ_C 129.5 (C-2′ and C-6′), 116.3 (C-3′ and C-5′), 102.9 (C-3), and 98.6 (C-6)). The ^1H NMR spectrum showed three broad singlet proton signals at δ_H 13.17, 10.80, and 10.35 in the downfield region of three hydroxyl substituents at C-5, C-7, and C-4′. Two singlet protons at δ_H 6.79 and 6.28 were assigned as H-3 and H-6, respectively. The COSY spectrum revealed the correlations of *ortho*-coupled proton signals between H-2′/H-6′ (δ_H 8.03, d, J = 8.8 Hz) and H-3′/H-5′ (δ_H 6.89, d, J = 8.8 Hz). The HMBC spectrum exhibited the correlations from H-3 to C-2/C-4/C-10/C-1′, from H-6 to C-5/C-6/C-7/C-8, from H-2′/H-6′ to C-3′/C-4′/C-5′/C-6′ (or C-2′), from H-3′/H-5′ to C-1′/C-4′/C-5′ (or C-3′). In addition, the anomeric proton at δ_H 4.69 (1H, d, J = 10.0 Hz, H-1″) showed the HMBC correlations to C-7/C-8/C-9/C-2″/C-5″, which demonstrated the sugar moiety was a *C*-glycoside and attached to C-8. Based on the above NMR spectra data and comparison with the spectroscopic data [12], compound **1** was identified as vitexin (apigenin-8-*C*-glucoside).

Compound **2** was isolated as a colorless powder. The negative APCI-MS spectrum data exhibited the ion peak at m/z = 565.00 $[M-H]^-$, consistent with the molecular formula of $C_{26}H_{30}O_{14}$, implying 12 degrees of unsaturation. The ^{13}C NMR spectrum of the compound **2**

showed the presence of 26 carbons, including 14 methines (six aromatic and eight oxymethine carbons), eight non-protonated carbons, four methylene carbons (one methylene at δ_C 44.0 (C-3) and three oxymethylenes at δ_C 75.5 (C-4‴), 65.9 (C-5‴), and 62.3 (C-6″)). All carbon signals appeared in the down-field region from δ_C 198-96 of a C_6-C_3-C_6 flavonoid framework and from δ_C 81-62 of sugar moieties. The ^{1}H NMR and ^{1}H-^{1}H COSY spectra exhibited the correlations between protons H-2 (δ_H 5.39, dd, J = 12.8, 2.8 Hz) and H_2-3 (δ_H 3.19 (1H, dd, J = 17.2, 12.8, H-3a), 2.76 (1H, dd, J = 17.2, 2.8 Hz, H-3b) in an A_2B system, between H-6 (δ_H 6.16, d, J = 2.0 Hz) and H-8 (δ_H 6.17, d, J = 2.0 Hz) in an aromatic *meta*-coupling, and between H-2′/H-6′ (δ_H 7.32, 2H, d, J = 8.4 Hz) and H-3′/H-5′ (δ_H 7.04, 2H, d, J = 8.4 Hz). The pyranose and furanose forms of sugars were determined from the ^{13}C and ^{1}H NMR chemical shift values [14]. The HMBC correlations between H-1″ (δ_H 5.05, d, J = 7.2 Hz) and C-7 (δ_C 166.8) of the aglycon, between H-1‴ (δ_H 5.41, d, J = 1.6 Hz, H-1‴) and C-2″ (δ_C 78.6) showed the presence of a disaccharide chain *O*-[(1→2)-*β*-D-apiofuranosyl]-*β*-D-glucopyranoside. The HMBC correlations of compound **2** were shown in Fig. 2. Based on the NMR spectra data and compared to those of reference data [16], compound **2** was determined as naringenin-7-*O*-(2-*β*-D-apiofuranosyl)-*β*-D-glucopyranoside, which was first reported in *P. lanceolata*.

Compound **3** was obtained as a colorless amorphous powder. The APCI-MS spectral data exhibited a positive-ion peak at m/z = 603.10 $[M+Na]^+$, corresponding to the molecular formula of $C_{27}H_{32}O_{14}$ and 12 degrees of unsaturation. The ^{13}C NMR spectrum of compound **3** showed 27 carbon signals, containing 6 aromatic methine carbons, 7 non-protonated carbons, 11 oxymethine carbons, two methylene carbons (δ_C 62.3 (C-6″), 44.0 (C-3), and one methyl group at δ_C 18.2 (C-6‴). The ^{13}C NMR and ^{1}H NMR data of **3** were similar to those of compound **2** except that the number of ^{13}C NMR signals of **3** was 27 in total, while **2** showed 26 ^{13}C signals. The main difference was that the pentosyl sugar moiety of apiofuranosyl in **2** changed to the hexosyl sugar moiety of rhamnopyranosyl in **3**. The HMBC and COSY correlations were observed as shown in Fig. 2. The HMBC correlations between H-1″ (δ_H 5.10, t, J = 7.2 Hz) and C-7 (δ_C 166.6) of the aglycon, between H-1‴ (δ_H 5.24, dd, J = 4.0, 1.6 Hz, H-1‴) and C-2″ (δ_C 79.2) showed the presence of an *O*-[(1→2)-*α*-L-rhamnopyranosyl]-*β*-D-glucopyranoside sugar chain. Based on the NMR data and compared to those of the reference data [15], compound **3** was determined as 4′,5,7-trihydroxy-flavanone-7-rutinoside or naringin, which is first reported in *P. lanceolata*.

Compound **4** was obtained as a colorless amorphous powder. The UV spectrum of **4** showed absorption maxima at λ_{max} 254.2 and 347.9 nm, which revealed the presence of aromatic and conjugated carbonyl chromophores. The negative ESI-MS spectral data showed the pseudomolecular ion at m/z = 595.07 $[M-H]^-$, corresponding to the molecular formula of $C_{27}H_{32}O_{15}$ with 12 degrees of unsaturation. The increase of 16 mass units in **4** compared to the molecular weight of **3** suggested the addition of a hydroxy group in the structure of **4**. The ^{13}C NMR and ^{1}H NMR data of **4** were similar to those of compound **3** and the difference between compounds **4** and **3** was the change of one aromatic methine (δ_C 116.3, C-3′) in **3** to an oxygenated carbon in the B-ring (δ_C 145.1, C-3′) in **4**. The correlations of *meta*-coupled protons between H-2′ and H-6′ and *ortho*-coupled protons between H-5′ and H-6′ were observed in the ^{1}H NMR spectrum, indicating that the hydroxy group was placed at C-3′ of the B-ring. In addition, the *α*-L-rhamnosyl moiety was attached to the glucose moiety at C-2″ as demonstrated by a HMBC correlation from H-1‴ (δ_H 5.24) to C-2″ (δ_C 77.6), and the *β*-D-glucosyl moiety was linked to C-7 of the aglycon according to the HMBC correlation from H-1″ (δ_H 5.09) to C-7 (δ_C 165.2). On the basis of the spectroscopic data, compound **5** was identified as 3′,4′,5,7-tetrahydroxyflavanone-7-*O*-(*α*-L-rhamnopyranosyl-(1→2))-*β*-D-glucopyranoside or neoeriocitrin, which was isolated from *P. serpens* [4].

Compound **5** was isolated as a yellow amorphous powder. The UV absorptions at λ_{max} 275.6 and 339.8 nm revealed aromatic and conjugated carbonyl chromophores. Its molecular formula was determined as $C_{21}H_{20}O_{12}$ from the negative APCI-MS ion peak at m/z = 463.16 $[M-H]^-$, consistent with 11 degrees of unsaturation. The ^{13}C NMR of compound **2** showed 21 carbon signals, including 10 non-protonated carbons (δ_C 182.3 (C-4), 164.3 (C-2), 152.3 (C-5), 151.7 (C-7), 149.9 (C-4′), 145.7 (C-3′), 144.3 (C-9), 127.0 (C-8), 121.6 (C-1′), and 105.2 (C-10)), 5 aromatic methine carbons (δ_C 119.2 (C-6′), 116.0 (C-5′), 113.6 (C-2′), 102.7 (C-3), and 98.7 (C-6)), 5 oxymethine carbons (δ_C 101.3 (C-1″), 77.3

(C-5″), 75.7 (C-3″), 73.2 (C-2″), and 69.7 (C-4″)), and one oxymethylene carbon δ_C 60.7 (C-6″). All the carbon signals were presented in the downfield region from δ_C 183-98 which was the typical region of C_6-C_3-C_6 flavonoid framework and a sugar moiety. The ^{1}H NMR spectrum of compound **2** showed the *ortho*-coupled aromatic proton signals of H-6′ (δ_H 7.47, dd, J = 8.0, 2.0 Hz) and H-5′ (δ_H 6.91, d, J = 8.0 Hz), and *meta*-coupled proton signals of H-6′ and H-2′ (δ_H 7.48, d, J = 2.0 Hz). Three singlet protons at δ_H 12.37, 6.71, and 6.63 were assigned to 5-OH, H-3 and H-6, respectively. The anomeric proton at δ_H 4.93 (1H, d, J = 7.5 Hz, H-1″) and oxymethine and oxymethylene protons in the range of δ_H 3.10-3.80 demonstrated a β-hexosyl sugar moiety attached to the flavonoid aglycon. The HMBC spectrum exhibited the correlations from 5-OH to C-5/C-6/C-10, from H-3 to C-2/C-4/C-10/C-1″, from H-6 to C-5/C-8/C-10, from H-2′ to C-2/C-3′/C-4′/C-6′, from H-5′ to C-1′/C-3′/C-4′, and from H-6′ to C-2/C-2′/C-4′. Furthermore, the HMBC correlations from H-1″ to C-7, from H-2″ to C-1″/C-3″, from H-3″ to C-2″/C-4″, from H-4″ to C-5″/C-6″, and from H-6″ to C-4″/C-5″ revealed the linkage of the β-D-glucopyranosyl group to C-7. Based on the comparison of the spectral data [13], compound **5** was identified as hypolaetin-7-*O*-β-D-glucopyranoside.

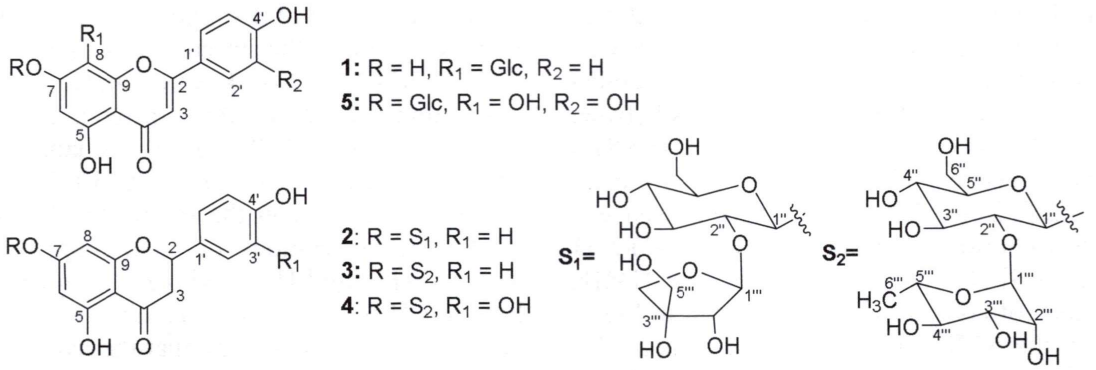

Fig. 1. Chemical structures of compounds **1-5**

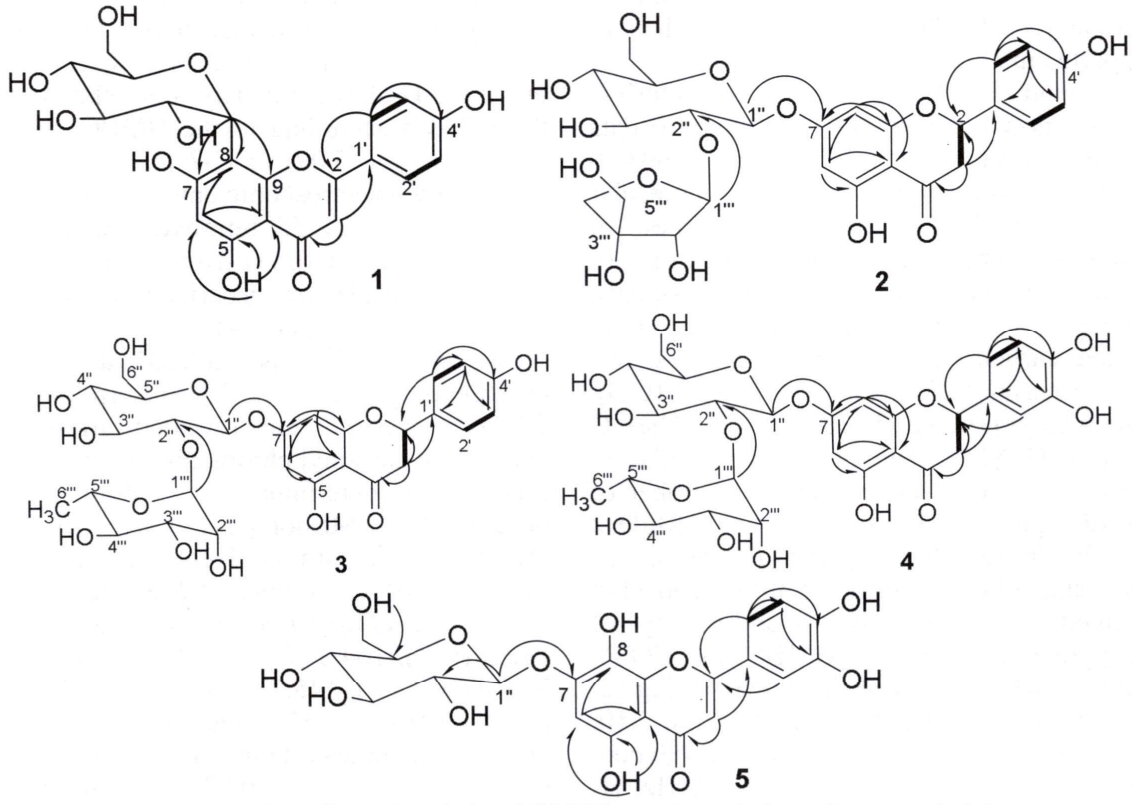

Fig. 2. The key HMBC (⟶) and COSY (▬) correlations of compounds **1-5**

4. Conclusion

In summary, five compounds were isolated from the ethyl acetate fraction of the whole plant of *P. lanceolata* (L.) Farw. Their structures were 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**). Compounds **1-5** are firstly reported from *P. lanceolata* (L.) Farw.

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References

1. Le D. B., Tran V. O. (2007), Botany, *Medical Publishing House*, Hanoi, 207-212 (in Vietnamese). **2.** Shing K. H. (1983), A reclassification of the fern genus *Pyrrosia*, *American Fern Journal*, 73(3), 73-78. **3.** Vo V. C., Tran H. (1999), The useful medicinal plants in Vietnam, *Education Publishing House*, Ho Chi Minh City, Vol 1, 144-145 (in Vietnamese). **4.** Markham K. R., Andersen O. M. (1990), Kaempferol 3-*O*-sophoroside-7-*O*-*α*-L-arabinofuranoside, neohesperidosides and other flavonoids from the fern *Pyrrosia serpens*, *Phytochemistry*, 29(12), 3919-3920. **5.** Yang C., Shi J. G., Mo S. Y., Yang Y. C. (2003), Chemical constituents of *Pyrrosia petiolosa, Journal of Asian Natural Products Research*, 5(2), 143-150. **6.** Wang N., Wang J. H., Li X., Ling J. H., Li N. (2006), Flavonoids from *Pyrrosia petiolosa* (Christ) Ching, *Journal of Asian Natural Products Research*, 8(8), 753-756. **7.** Masuda K., Yamashita H., Shiojima K., Itoh T., Ageta H. (1997), Fern constituents: triterpenoids isolated from rhizomes of *Pyrrosia lingua*. I, *Chemical and Pharmaceutical Bulletin*, 45(4), 590-594. **8.** Yamashita H., Masuda K., Kobayashi T., Ageta H., Shiojima K. (1998), Dammarane triterpenoids from rhizomes of *Pyrrosia lingua*, *Phytochemistry*, 49(8), 2461-2466. **9.** He K., Fan L. L., Wu T. T., Du J. (2018), A new xanthone glycoside from *Pyrrosia sheareri*. *Natural Product Research*, 2982-2987. **10.** Fan Y., Feng H., Liu L., Zhang Y., Xin X., Gao D. (2020), Chemical components and antibacterial activity of the essential oil of six *Pyrrosia* species, *Chemistry and Biodiversity*, 17(10), e200052. **11.** Pham H. H. (2003), Plants in Vietnam, *Young Publishing House*, Ho Chi Minh city, Vol 1, 86-87 (in Vietnamese). **12.** Guimarães C. C., Olivera D. D., Valdevite M., Saltoratto A. L. F., Pereira S. I. V., França S. de C., Pereira A. M. S., Pereira P. S. (2015), The glycosylated flavonoids vitexin, isovitexin, and quercetrin isolated from *Serjania erecta* Radlk (Sapindaceae) leaves protect PC12 cells against amyloid-*β*25-35 peptide-induced toxicity, *Food and Chemical Toxicology*, 86, 88-94. **13.** Karimov A. M., Ostroushko Y. V., Botirov E. K. (2019), Flavone glucosides from the aerial part of *Scutellaria comosa*, *Chemistry of Natural Compounds*, 55, 545-546. **14.** Pawank A. K. (1992). NMR spectroscopy in the structural elucidation of oligosaccharides and glycosides, *Phytochemistry*, 31(10), 3307-3330. **15.** Chang E. J., Lee W. J., Cho S. H., Choi S. W. (2003), Proliferative effects of flavan-3-ols and propelargonidins from rhizomes of *Drynaria fortunei* on MCF-7 and osteoblastic cells, *Archives of Pharmacal Research*, 26(8), 620-630. **16.** Tuntiwachwuttikul P., Pootaeng-on Y., Phansa P., Limpachayaporn P., Charoenchai P., Taylor W. C. (2007), Constituents of the leaves of *Holarrhena pubescens*, *Fitoterapia*, 78(3), 271-273.

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PHYTOCHEMICAL ANALYSIS AND BIOACTIVITY EVALUATION OF *BOEICA POROSA* C.B. CLARKE FROM LAM BINH MOUNTAINOUS REGION, TUYEN QUANG PROVINCE

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Summary

Phytochemical Analysis and Bioactivity Evaluation of *Boeica porosa* C.B. Clarke from Lam Binh Mountainous Region, Tuyen Quang Province

For the first time, a phytochemical and bioactivity analysis of *Boeica porosa* was reported. The total contents of phenolic, flavonoid, saponin, and alkaloid compositions were analysed and the antioxidant, anti-inflammatory effects, α-amylase inhibition and cytotoxicities of extracts and subfractions from *B. porosa* in Lam Binh mountainous area, Tuyen Quang province, were evaluated. Total extracts and subfractions from the aerial part of the plant had higher chemical amounts and exhibited stronger bioactivities than those of the underground part. Ethyl acetate subfraction of the aerial part had