References

1. Ren Y., Tan Q., Heath K., Wu S., Wilson J. R., Ren J., Shriwas P., Yuan C., Tran N. N., Chai H. B., Chen X., Soejarto D. D., Johnson M. E., Cheng X., Burdette J. E., Kinghorn A. D. (2020), Cytotoxic and non-cytotoxic cardiac glycosides isolated from the combined flowers, leaves, and twigs of *Streblus asper*, *Bioorganic & Medicinal Chemistry*, 28(4), 115301. **2.** He R., Deng S., Nie H., Huang Y., Liu B., Yang R., Huang S., Zhou D., Chen H., Li J., Zhang Y. (2017), Two new coumarins from the bark of *Streblus indicus* (Bur.) Corner, *Natural Product Research*, 31(9), 1052-1058. **3.** Nguyen T. N., Dang H. P., Nguyen X. H., Do N. V. T., Le H. T., Le Q. H. T., Nguyen T. T. M. (2021), Tyrosinase inhibitors from the stems of *Streblus ilicifolius*, *Evidence-Based Complementary and Alternative Medicine*, 2021, 5561176. **4.** Huang Y., Huang X., Tian G., Zhang W., Su S., Xu X., Li J., Liu B. (2021), Two new amide glycosides with anti-inflammatory activity from the leaves of *Streblus ilicifolius* (Vidal) Corner, *Natural Product Research*, 202136(6), 1485-1493. **5.** Dej-adisai S., Parndaeng K., Wattanapiromsakul C. (2016), Determination of phytochemical compounds, and tyrosinase inhibitory and antimicrobial activities of bioactive compounds from *Streblus ilicifolius* (S Vidal) Corner, *Tropical Journal of Pharmaceutical Research*, 15(3), 497-506. **6.** Zhang G., Hao L., Zhou D., Liu W., Li C., Su S., Xu X., Huang X., Zhang L. J. (2019), A new phenylpropanoid glycoside from the bark of *Streblus ilicifolius* (Vidal) Corner, *Biochemical Systematics and Ecology*, 87, 103962. **7.** Wang X. W., Mao Y., Wang N. L., Yao X. S. (2008), A new phloroglucinol diglycoside derivative from *Hypericum japonicum* Thunb, *Molecules*, 13, 2796-2803. **8.** Zhang Y., Wang D., Yang L., Zhou D., Zhang J. (2014), Purification and characterization of flavonoids from the leaves of *Zanthoxylum bungeanum* and correlation between their structure and antioxidant activity, *PLoS One*, 9(8), e105725. **9.** Liu H., Mou Y., Zhao J., Wang J., Zhou L., Wang M., Wang D., Han J., Yu Z., Yang F. (2010), Flavonoids from *Halostachys caspica* and their antimicrobial and antioxidant activities, *Molecules*, 15(11), 7933-7945. **10.** Xu T., Fan Z., Lou J., Du Q., Kong Y., Lu Y., Wu X. (2022), Enzymatic synthesis of vitexin glycosides and their activity, *RSC advances*, 12(37), 23839-23844. **11.** Valente L. M. M., Bizarri C. H. B., Liechocki S., Baroza R. S., Paixão D. da, Almeida M. B. S., Benevides P. J. C., Magalhães A., Siani A. C. (2009), Kaempferitrin from *Uncaria guianensis* (Rubiaceae) and its potential as a chemical marker for the species, *Journal of the Brazilian Chemical Society*, 20(6), 1041-1045. **12.** Chen T., Wang P., Du. Y., Shen Y., Li Y. (2012), Preparative isolation and purification of lutonarin and saponarin from barley seedlings by HSCCC, *Journal of Liquid Chromatography & Related Technologies*, 35(18), 2524-2532.

Journal of Medicinal Materials, 2023, Vol. 28, No. 3 (pp. 136 - 141)

CHEMICAL CONSTITUENTS OF THE RHIZOMES OF *DRYNARIA BONII* COLLECTED IN LANG SON PROVINCE

Nguyen Tra My[1], Nguyen Thi Ha Ly[1], Dang Thi Hong Nhung[2], Nguyen Thi Thu[1], Vu Thi Diep[1], Nguyen Thi Hang[1], Phan Van Truong[1], Nguyen The Hung[3], Do Thi Ha[1,*]

[1]National Institute of Medicinal Materials (NIMM), Hanoi, Vietnam;

[2]University of Medicine and Pharmacy, Vietnam National University, Hanoi, Vietnam;

[3]Hanoi University of Pharmacy, Vietnam

*Corresponding author: hado.nimms@gmail.com

(Received April 25th, 2023)

Summary

Chemical Constituents of the Rhizomes of *Drynaria bonii* Collected in Lang Son Province

Seven compounds, including daucosterol (**1**), arisantetralone A (**2**), arisantetralone B (**3**), *p*-hydroxybenzoic acid (**4**), protocatechuic acid (**5**), caffeic acid (**6**), and ferulic acid (**7**) were isolated from the 70% ethanol extract of *Drynaria bonii*. The structures of these compounds were identified using extensive nuclear magnetic resonance spectroscopy (NMR) data, mass spectrometry (MS), and comparison with published literatures. Compounds **2–4**, **6**, and **7** were reported for the first time from this species.

Keywords*: Drynaria bonii*, *Rhizome, Sterol, Lignans, Phenolic acids.*

1. Introduction

The *Drynaria* genus comprises 19 species, seven of which are found in Vietnam [1],[2]. In Vietnam, the medicinal plant *D. bonii* H. Christ is used to treat osteoporosis, bone fractures, tinnitus, and stimulate hair growth [2]. The Vietnamese Pharmacopoeia V (2018) has a monograph on *Rhizoma Drynariae*, which is obtained from the rhizomes of two species of *Drynaria fortunei* (Kunze ex Mett.) J.Sm. and *Drynaria bonii* Christ. [3]. *D. fortunei* has been extensively studied for its chemical composition and quality standardization. This species is specified in the Chinese Pharmacopoeia and Hong Kong Chinese Materia Medica Standards, and naringin is used as a marker for quality control [4],[5]. However, until now, there have been very few studies on the phytochemicals and bioactivity of *D. bonii*. In 2005, Nguyen Tran Giang Huong studied and screened the chemical composition of *D. bonii* rhizomes collected in Lang Son province. The results showed that it contained flavonoids, steroid saponins, tannins, and sugar. The total flavonoid content in the sample was 0.60 ± 0.03%, and the total saponin content was 3.71 ± 0.06% [6]. From 2012 to 2020, Pham Thi Nhat Trinh et al. isolated and determined the structures of some compounds from the rhizomes of *D. bonii* collected in Binh Thuan province, Vietnam. These compounds

were identified as drybonioside, α-tocopherol, 24-methylenecycloartan-3β-ol, triphyllol, chrysophanol, 5-hydroxymethylfurfural, ethyl β-D-fructopyranoside, 3-(5-hydroxymethyl)furan-2-yl)-2-phenylacrylaldehyde, nobiletin, protocatechuic acid, protocatechualdehyde, isoliquiritigenin, rutin, nicotiffein, linoca drynaether A, uracil, 4'-hydroxy-7-methoxyflavan, kaempferol, and indole-3-carboxylic acid [7],[8],[9], [10],[11]. Thus, there are very few studies on the chemical compositions of *D.bonii* in Vietnam until now. Therefore, this study aimed to extraction, isolation and identification the chemical constituents of the rhizomes of *D. bonii* collected in Lang Son province.

2. Materials and methods

2.1. Plant material

Samples of *Drynaria bonii* (Polypodiaceae) were collected in Chi Lang, Lang Son province, Vietnam, in May 2022. The plant was identified by Assoc. Prof. Pham Thanh Huyen and M.Sc. Phan Van Truong (National Institute of Medicinal Materials, NIMM). A voucher specimen (DL-200420) was deposited in the Center of Medicinal Material Resources, NIMM (Hanoi, Vietnam).

2.2. General experimental procedures

NMR spectra were recorded on Bruker Avance Digital 500 and 600 MHz NMR spectrometers (Karlsruhe, Germany) using TMS as an internal standard, *J* in Hz, at 294 K. ESI-MS data were analyzed *via* a Q Exactive Orbitrap Mass Spectrometer. The optical rotation values were recorded with a Jasco P-1020 polarimeter with dichloromethane. *Silica gel* (Merck, 63-200 mm particle size) and YMC gel (75 - 150 μm) were used for column chromatography (CC). Thin-layer chromatography (TLC) was carried out with *silica gel* $60F_{254}$ and RP-$18F_{254}$ plates. Spots were detected under UV light or by spraying with 10% sulfuric acid in 96% ethanol (EtOH) and heating.

2.3. Extraction and isolation

The dried rhizomes of *D. bonii* (10.0 kg) were ground, extracted, and refluxed with 70% EtOH (3 times, 3 hours each time) at 80°C. The extracts were filtered, combined, and evaporated under reduced pressure, yielding a green residue (290.0 g), which was suspended in water and successively separated with *n*-hexane, ethyl acetate (EtOAc) and *n*-butanol (BuOH). The fractionated extracts were combined and evaporated under reduced pressure to obtain the corresponding fractions.

The EtOAc extract (80.0 g) was separated by *silica gel* CC with a gradient mixture of *n*-hexane-acetone (20:1-1:1, v/v) as eluents to give eight fractions (E1-E8) and compound **1** (300.0 mg). Fraction E7 (5.03 g) was fractionated by *silica gel* CC eluting with an isocratic mixture of *n*-hexane-acetone (15:1-1:1, v/v) to obtain fourteen subfractions (E7.1-E7.14). Compounds **2** (8.0 mg) and **3** (12.0 mg) were obtained from subfraction E7.6 (56 mg) by using RP-C_{18} gel CC with an elution mixture of methanol-water (4:1, v/v).

The BuOH extract (50.0 g) was applied to *silica gel* CC with a gradient mixture of ethyl acetate-methanol-water (80:15:5-0:100:0, v/v/v) as eluents to yield ten fractions (B1-B10). Fraction B2 (3.12 g) was separated by RP-C_{18} gel CC eluting with an isocratic mixture of acetone-water (2:3, v/v) to obtain five subfractions (B2.1-B2.5) and compound **4** (50.4 mg). Compound **5** (215.3 mg) was obtained from fraction B3 (3.56 g) by using RP-C_{18} gel CC with an elution mixture of acetone-water (2:3, v/v). Fraction B6 (2.02 g) was subjected to RP-C_{18} gel CC with an isocratic elution of acetone-water (2:3, v/v) to afford two compounds **6** (8.6 mg) and **7** (11.2 mg).

Compound **1** (daucosterol): White powder; ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H: 3.60 (1H, m, H-3), 5.37 (1H, m, H-6), 0.71 (3H, s, H-18), 1.03 (3H, s, H-19), 0.94 (3H, d, *J* = 6.6 Hz, H-21), 0.83 (3H, d, *J* = 6.6 Hz, H-26), 0.85 (3H, d, *J* = 6.6 Hz, H-27), 0.86 (3H, t, *J* = 7.8 Hz, H-29), *Glc*: 4.40 (1H, d, *J* = 7.8 Hz, H-1′), 3.38 (2H, m, H-3', H-4'), 3.27 (1H, m, H-5′), 3.20 (1H, m, H-2′), 3.76 (1H, dd, *J* = 5.4, 12.0 Hz, Ha-6′), 3.86 (1H, dd, *J* = 3.0, 12.0 Hz, Hb-6′).

Compound **2** (arisantetralone A): Pale yellow powder; [α]$_D^{25}$ -35 (*c* 0.15, CH_2Cl_2) ([α]$_D^{25}$ -35 (*c* 0.1, CH_2Cl_2)); ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H: 6.42 (1H, s, H-3), 7.60 (1H, s, H-6), 2.78 (1H, m, H-8), 1.11 (3H, d, *J* = 6.6 Hz, H-9), 6.55 (1H, d, *J* = 2.4 Hz, H-2'), 6.84 (1H, d, *J* = 8.0 Hz, H-5'), 6.54 (1H, dd, *J* = 2.4, 8.0 Hz, H-6'), 3.95 (1H, d, *J* = 6.0 Hz, H-7'), 2.40 (1H, m, H-8'), 0.98 (1H, d, *J* = 7.2 Hz, H-9'), 3.81 (3H, s, 4-OMe), 3.79 (3H, s, 3'-OMe); ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C: 126.3 (C-1), 137.9 (C-2), 111.4 (C-3), 151.4 (C-4), 144.7 (C-5), 112.0 (C-6), 200.0 (C-7), 42.6 (C-8), 11.9 (C-9), 135.9 (C-1'), 111.0 (C-2'), 146.7 (C-3'), 144.4 (C-4'), 114.2 (C-5'), 121.8 (C-6'), 50.5 (C-7'), 42.6 (C-8'), 15.9 (C-9'), 56.0 (4-OMe), 56.0 (3'-OMe); ESI-MS: *m/z* 341.25 $[M-H]^-$.

Compound **3** (arisantetralone B): Pale yellow powder; [α]$_D^{25}$ -35 (*c* 0.15, CH_2Cl_2) ([α]$_D^{25}$ -35 (*c* 0.1, CH_2Cl_2)); ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H: 6.50 (1H, s, H-3), 7.57 (1H, s, H-6), 2.78 (1H, m, H-8), 1.13 (3H, d, *J* = 6.6 Hz, H-9), 6.55 (1H, d, *J* = 1.8 Hz, H-2'), 6.82 (1H, d, *J* = 8.4 Hz, H-5'), 6.54 (1H, dd, *J* = 1.8, 8.4 Hz, H-6'), 3.91 (1H, d, *J* = 5.4 Hz, H-7'), 2.41 (1H, m, H-8'), 0.96 (1H, d, *J* = 6.6 Hz, H-9'), 3.95 (3H, s, 5-OMe), 3.81 (3H, s, 3'-

OMe); ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C: 125.2 (C-1), 140.1 (C-2), 115.5 (C-3), 150.8 (C-4), 145.9 (C-5), 108.1 (C-6), 200.3 (C-7), 43.3 (C-8), 11.7 (C-9), 135.5 (C-1'), 111.1 (C-2'), 146.7 (C-3'), 144.4 (C-4'), 114.2 (C-5'), 121.9 (C-6'), 49.8 (C-7'), 42.0 (C-8'), 16.0 (C-9'), 56.1 (5-OMe), 56.0 (3'-OMe); ESI-MS: *m/z* 341.25 $[M-H]^-$.

Compound **4** (*p*-hydroxybenzoic acid): Pale yellow powder; ^{1}H-NMR (500 MHz, DMSO-d_6) δ_H: 7.78 (2H, d, J = 9.0 Hz, H-2, H-6), 6.81 (2H, d, J = 9.0 Hz, H-3, H-5); ^{13}C-NMR (125 MHz, DMSO-d_6) δ_C: 121.4 (C-1), 131,5 (C-2, C-6), 115.1 (C-3, C-5), 161.6 (C-4), 167.1 (C-7); ESI-MS: *m/z* 137.30 $[M-H]^+$.

Compound **5** (protocatechuic acid): Pale white powder; ^{1}H-NMR (600 MHz, CD_3OD) δ_H: 7.45 (1H, d, J= 1.8 Hz, H-2), 6.81 (1H, d, J = 7.8 Hz, H-5), 7.43 (1H, dd, J = 1.8, 7.8 Hz, H-6); ^{13}C-NMR (150 MHz, CD_3OD) δ_C: 123.1 (C-1), 115.8 (C-2), 146.1 (C-3), 151.5 (C-4), 117.7 (C-5), 123.9 (C-6), 170.2 (C-7); ESI-MS: *m/z* 153.25 $[M-H]^-$.

Compound **6** (caffeic acid): Pale white powder; ^{1}H-NMR (500 MHz, CD_3OD) δ_H: 7.05 (1H, d, J= 2.0 Hz, H-2), 6.80 (1H, d, J = 8.0 Hz, H-5), 6.95 (1H, dd, J = 2.0, 8.0 Hz, H-6), 7.55 (1H, d, J = 15.6 Hz, H-7), 6.24 (1H, d, J = 15.6 Hz, H-8); ^{13}C-NMR (125 MHz, CD_3OD) δ_C: 127.8 (C-1), 115.6 (C-2), 146.8 (C-3), 149.4 (C-4), 116.5 (C-5), 122.8 (C-6), 147.0 (C-7), 115.1 (C-8), 171.0 (C-9); ESI-MS: *m/z* 179 $[M-H]^-$.

Compound **7** (ferulic acid): Pale white powder; ^{1}H-NMR (500 MHz, CD_3OD) δ_H: 7.19 (1H, d, J= 1.0 Hz, H-2), 6.83 (1H, d, J = 8.0 Hz, H-5), 7.08 (1H, dd, J = 1.0, 8.0 Hz, H-6), 7.62 (1H, d, J = 16.0 Hz, H-7), 6.33 (1H, d, J = 16.0 Hz, H-8), 3.91 (3H, s, 3-OCH_3); ^{13}C-NMR (125 MHz, CD_3OD) δ_C: 127.8 (C-1), 111.8 (C-2), 149.4 (C-3), 150.5 (C-4), 115.9 (C-5), 123.9 (C-6), 146.9 (C-7), 116.5 (C-8), 170.9 (C-9), 56.6 (3-OCH_3); ESI-MS: *m/z* 193.20 $[M-H]^-$.

2.4. HPLC analysis of D. bonii

A Shimadzu HPLC system, equipped with a Nexera X2 LC-30AD pump solvent system, an Autosampler SIL-30AC, and an SPD-M20A, was applied. Separation was performed using an Agilent C_{18} column (250 × 4.6 mm, 5 µm) at a temperature of 25°C. The mobile phase consisted of acetonitrile (ACN) and formic acid 0.1% in water using a gradient elution of 0-10 min (5-20% ACN), 10-40 min (20-90% ACN), and 40-45 min (90 - 5% ACN). The solvent flow rate was maintained at 1.0 mL/min. The detection was performed at a wavelength of 254 nm.

Sample solution: weigh 1.0 g of the powdered sample and place it in a 100 mL round-bottomed flask. Add 25 mL of 70% ethanol and reflux the mixture for 30 minutes. Cool down to room temperature and filter through a 0.45 µm filter prior to HPLC analysis.

Reference compound solution: weigh 1 mg of each reference compound and dissolve in 5 mL of 70% ethanol.

3. Results and discussion

3.1. Extraction and isolation of compounds from the rhizome of D. bonii

Seven compounds were isolated from the 70% ethanol extract of the rhizomes of *D. bonii* (**1**-**7**) (Fig. 1). The structures of 7 compounds were elucidated by comparing their spectroscopic data to published values.

Compound **1** was obtained as a white powder. The UV absorptions were observed at 254 and 366 nm. Analysis of the ^{1}H-NMR spectrum revealed the presence of an olefinic proton at δ_H 5.37 (1H, m, H-6), a hydroxymethine group at δ_H 3.60 (1H, m, H-3), 6 methyl groups, including 2 singlet signals at δ_H 0.71 (3H, s, H-18) and 1.03 (3H, s, H-19), 3 doublet signals at δ_H 0.94 (3H, d, J = 6.6 Hz, H-21), 0.83 (3H, d, J = 6.6 Hz, H-26), and 0.85 (3H, d, J = 6.6 Hz, H-27), and a triplet signal at δ_H 0.86 (3H, t, J = 7.8 Hz, H-29), indicating a sterol structure of **1**. Furthermore, the compound was found to have signals of the β-D-glucopyranose moiety with the presence of an anomeric proton [δ_H 4.40 (1H, d, J = 7.8 Hz, H-1′)], a hydroxymethylene group [δ_H 3.76 (1H, dd, J = 5.4, 12.0 Hz, Ha-6′) and 3.86 (1H, dd, J = 3.0, 12.0 Hz, Hb-6′)], and four hydroxymethine groups [δ_H 3.20 (1H, m, H-2′), 3.38 (2H, m, H-3', H-4'), and 3.27 (1H, m, H-5′)], indicating that it was a sterol glucoside. Thus, the structure of **1** was identified as daucosterol by comparing the NMR spectra [12].

Compound **2** was obtained as a pale-yellow powder with a molecular formula of $C_{20}H_{22}O_5$, which was determined through ESI-MS analysis, revealing a quasi-molecular ion at *m/z* 341.25 $[M-H]^-$. The ^{1}H-NMR spectrum displayed signals characteristic of 2 methyl groups at δ_H 1.11 (3H, d, J = 6.6 Hz, H-9) and 0.98 (1H, d, J = 7.2 Hz, H-9′), 5 methine protons of the aromatic ring at δ_H 6.42 (1H, s, H-3), 7.60 (1H, s, H-6), 6.55 (1H, d, J = 2.4 Hz, H-2′), 6.84 (1H, d, J = 8.0 Hz, H-5′), and 6.54 (1H, dd, J = 2.4; 8.0 Hz, H-6′), 2 methoxy groups at δ_H 3.81 (3H, s, 4-OMe), 3.79 (3H, s, 3′-OMe), and 3 methine groups at δ_H 2.40 (1H, m, H-8′), 2.78 (1H, m, H-8), and 3.95 (1H, d, J = 6.0 Hz, H-7′). The ^{13}C-NMR spectrum combined with the HSQC spectrum revealed 20 carbon signals, including 2 methyl groups [δ_C 11.9 (C-9) and 15.9(C-9′)], 2 methoxy groups [δ_C 56.0 (4-OMe, 3′-OMe)], 3 aliphatic methine

carbons [δ_C 50.5 (C-7′) and 42.6 (C-8, C-8′)], 1 ketone carbon [δ_C 200.0 (C-7)], and 12 carbons of two aromatic rings (δ_C 111.0 - 151.4). Further HMBC analysis spectra revealed interactions of H-9 (δ_H 1.11) with C-7 (δ_C 200.0)/ C-8 (δ_C 42,6)/ C-8' (δ_C 42.6) and H-9' (δ_H 0.98) with C-7' (δ_C 50.5)/ C-8'/ C-8, indicating the presence of a tetralone group. The HMBC analysis of the interactions of H-7' (δ_H 3.95) with C-1' (δ_C 135.9)/ C-2' (δ_C 111.0)/ C-6' (δ_C 121.8) revealed that one aryl group was attached to C-7'. Moreover, this aryl group had three aromatic protons (δ_H 6.54, 6.55, and 6.84) and two oxygen-bearing carbons in the downfield region (δ_C 144.4 and 146.7). Additionally, the HMBC interaction between H-2' (δ_H 6.55), H-5' (δ_H 6.84), and the methoxy group protons (δ_H 3.79) with C-3' (δ_C 146.7) indicated that the methoxy group was located at C-3'. The location of the second methoxy group was determined to be at C-4 of the remaining aryl ring through HMBC interactions between H-6 (δ_H 7.60) and C-4 (δ_C 151.4) and between the methoxy proton (δ_H 3.81) and C-4 (δ_C 151.4). The NMR spectral indicated that **2** was an aryltetralone lignan [13]. The relative configuration of **2** at C-7′, C-8′, and C-8 was determined by the NOESY experiment. Analysis of NOESY correlations of H-7' (δ_H 3.95)/H-9 (δ_H 1.11) and H-9 (δ_H 1.11)/H-9' (δ_H 0.98) revealed that H-7' and the two methyl groups were positioned on the same face and were assigned the β-configuration. Thus, based on spectral analysis and comparison with the reported literature [13], the structure of **2** was elucidated as arisantetralone A.

Compound **3** was obtained as a pale-yellow powder. Compound **3** exhibited a *quasi*-molecular ion peak at *m/z* 341.25 $[M-H]^-$ in the ESI-MS spectrum. Based on the NMR data and the quasi-molecular peak, the molecular formula of **3** was suggested to be $C_{20}H_{22}O_5$. The NMR spectrum of **3** was similar to that of **2**, except for some small shifts in the chemical shifts of C-2 (-2.2 ppm), C-3 (-4.1 ppm), and C-6 (+3.9 ppm), indicating a change in the location of the methoxy group from C-4 to C-5. This was confirmed by the HMBC interactions between C-5 (δ_C 145.9) and H-3 (δ_H 6.50)/ methoxy protons (δ_H 3.95). The NOESY correlations between H-7' (δ_H 3.91), H-9 (δ_H 1.13), and H-9' (δ_H 0.96) provided evidence for the same relative configuration as that observed in compound **2**. Therefore, based on the above analysis, **3** was determined to be arisantetralone B [13] (Fig. 1).

Compound **4** was obtained as a pale-yellow powder. The molecular formula of **4** was suggested as $C_7H_6O_3$ based on a *pseudo*-molecular ion peak at *m/z* 137.30 $[M-H]^-$ and NMR spectra. The ^{1}H-NMR spectrum showed two pairs of proton signals of an AA'BB' system at δ_H 7.78 (2H, d, J = 9.0 Hz, H-2, H-6) and 6.81 (2H, d, J = 9.0 Hz, H-3, H-5). The ^{13}C-NMR spectrum showed signals of 5 carbons, in which the carbon signal at δ_C 167.7 (C-9) was specific for the carboxyl group. The signal at δ_C 161.6 (C-4) indicated that the aromatic ring has been substituted by a hydroxy group. Comparing the NMR spectra [14], the structure of **4** was identified as *p*-hydroxybenzoic acid (Fig. 1).

Compound **5** was obtained as a pale-yellow powder. Its molecular formula was suggested as $C_7H_6O_4$ based on a *pseudo*-molecular ion peak at *m/z* 153.25 $[M-H]^-$ and NMR spectra. The ^{1}H-NMR spectrum of **5** showed three aromatic proton signals of an ABX system at δ_H 6.81 (1H, d, J = 7.8 Hz, H-5), 7.45 (1H, d, J = 1.8 Hz, H-2), and 7.43 (1H, dd, J = 1.8, 7.8 Hz, H-6). The ^{13}C-NMR spectrum exhibited 7 carbon signals, including a carboxyl carbon [δ_C 170.2 (C-7)], 3 carbons not bonded to hydrogen [δ_C 123.1 (C-1), 146.1 (C-3), and 151.5 (C-4)] and 3 methine carbons [δ_C 115.8 (C-2), 117.7 (C-5), and 123.9 (C-6)], also showing the appearance of the ABX system in the structure of **5**. Thus, **5** was elucidated as protocatechuic acid by comparison of its NMR data with those published [15].

Compound **6** was obtained as a pale-yellow powder. The molecular formula of **6** was found to be $C_9H_8O_4$ *via* the NMR data and a *pseudo*-molecular ion peak at *m/z* 179 $[M-H]^-$. In the ^{1}H-NMR spectrum, 3 proton signals of an ABX system appeared at δ_H 7.05 (1H, d, J = 2.0 Hz, H-2), 6.80 (1H, d, J = 8.0 Hz, H-5), and 6.95 (1H, dd, J = 2.0, 8.0 Hz, H-6). In addition, there were also signals of a *trans*-double bond at δ_H 6.24 (1H, d, H-8) and 7.55 (1H, d, H-7) with a large coupling constant J = 15.6 Hz. The ^{13}C-NMR spectrum showed the signals of 9 carbons, in which the carbon signal at δ_C 171.0 (C-9) was specific for the carboxyl group. The signals at δ_C 146.8 (C-3) and 149.4 (C-4) indicated that the aromatic ring was substituted with two hydroxy groups. Considering these observations in combination with the reported data [16], **6** was determined to be caffeic acid.

Compound **7** was isolated as a white powder, and the ESI-MS spectrum displayed a *pseudo*-molecular ion peak at *m/z* 193.20 $[M-H]^-$. Based on the NMR and MS data, the molecular formula of **7** was determined to be $C_{10}H_{10}O_4$. The ^{1}H-NMR spectrum of **7** exhibited similarities to that of **6**, except for the presence of an additional methoxy

group at δ_H 3.91 and δ_C 56.6 (3-OCH_3). The position of the methoxy group was determined at C-3 through the HMBC interactions between protons at δ_H 3.91 (OCH_3)/ H-2 (δ_H 7,19)/ H-5 (δ_H 6.83) and C-3 (δ_C 149.4). Based on spectral analysis and by comparison with the literature [17], **7** was identified as ferulic acid.

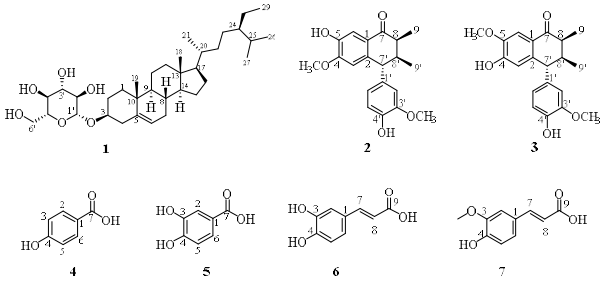

Fig. 1. Chemical structures of compounds (**1-7**) isolated from the rhizomes of *D. bonii*

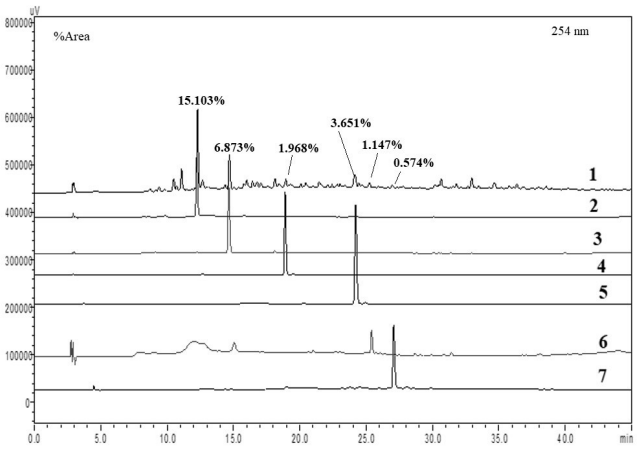

Fig. 2. HPLC chromatogram of *D. bonii* and the reference compounds

*(**1**- Drynaria bonii sample; **2**- protocatechuic acid; **3**- p-hydroxybenzoic acid; **4**- ferulic acid; **5**- caffeic acid; **6**- arisantetralone A; **7**- arisantetralone B)*

Seven compounds, including a sterol glucoside (**1**), two lignans (**2-3**), and four phenolic acids (**4-7**) were isolated from *D. bonii* rhizomes, and the structures of these compounds were identified. Compounds **2-4**, **6**, and **7** were reported for the first time in this species. These compounds have demonstrated biological activities similar to those of medicinal herbs. Daucosterol (**1**), a common phytosterol in herbal medicine, has shown potential pharmacological activities, such as anticancer, neuroprotective, antioxidant, anti-inflammatory, antidiabetic, and immunomodulatory effects [18]. Protocatechuic acid (**5**) has also been found to have various pharmacological activities, including anti-inflammatory, antioxidant, anti-hyperglycemic, antibacterial, anticancer, anti-aging, anti-atherogenic, anti-tumoral, anti-asthma, antiulcer, antispasmodic, and neurological properties [19]. Additionally, caffeic acid (**6**) has anti-inflammatory, anti-cancer, and antiviral activities [20], while both caffeic acid (**6**) and ferulic acid (**7**) exhibit antioxidant activity [21],[22].

3.2. HPLC analysis of isolated compounds in the rhizome of D. bonii

HPLC-DAD method was applied to qualitatively analyze the six isolated compounds (**2-7**) in the 70% EtOH extract of *D. bonii* rhizome. The HPLC-DAD conditions were presented in Section 2.4. The results are shown in Fig. 2.

The results showed that the peaks of four compounds (ferulic acid, caffeic acid, arisantetralone A, and arisantetralone B) appeared with low signal intensity in the chromatogram of the *D. bonii* sample. At UV 254 nm, the percentage of peak areas of the compounds were protocatechuic acid (15.103%), p-hydroxybenzoic acid (6.873%), ferulic acid (1.968%), caffeic acid (3.651%), arisantetralone A (1.147%), and arisantetralone B (0.574%). The peak signal of protocatechuic acid and *p*-hydroxybenzoic acid in the chromatogram of the *D. bonii* sample migh be the main components in the rhizome of *D. bonii*.

4. Conclusion

In this study, seven compounds, daucosterol (**1**), arisantetralone A (**2**), arisantetralone B (**3**), *p*-hydroxybenzoic acid (**4**), protocatechuic acid (**5**), caffeic acid (**6**), and ferulic acid (**7**), were isolated from the 70% EtOH extract of *D. bonii*. Among them, compounds **2-4**, **6**, and **7** were the first reported in *D. bonii*.

Acknowledgments: *This work was supported by the National Institute of Medicinal Materials - Ministry of Health (Vietnam) on the project (Contract No. 20/2022/HĐ-ĐTCS-PTTC) "Isolation, fingerprintanalysis, and development of HPLC method for the quantitative determination of the main component in Drynaria bonii".*

References

1. Janssen T., Schneider H. (2005), Exploring the evolution of humus collecting leaves in *Drynarioid ferns* (Polypodiaceae, Polypodiidae) based on phylogenetic evidence, *Plant Systematics and Evolution*, 252, 175-197. **2**. Do Tat Loi (2004), *Medicinal plants and Remedy of Vietnam*, Publisher Medicine, Hanoi, 491-192. **3**. Ministry of Health (2017), *Vietnamese Pharmacopoeia V*, Medical Publishing House, Hanoi, Vietnam, 1124-1125. **4**. Chinese Pharmacopoeia Commission (2015), *Pharmacopoeia of the People's Republic of China*, Vol 1A, 351. **5**. Department of Health (2015), *Standards HongKong Chinese Materia Medica Drynariae Rhizoma*, Vol 7, 206-215. **6**. Nguyen Tran Giang Huong (2005), Study on effects of traditional processing methods on chemical composition and

biological activity of *Drynaria bonii* rhizome, *Journal of Medicinal Materials*, 10(5), 158-164. **7**. Pham Thi Nhat Trinh, Le Tien Dung, Nguyen Cong Hao, Pham Thanh Thao (2012), Terpenoid and anthraquinone compounds from the rhizomes *Drynaria bonii* H. Christ, Polypodiaceae, *Journal of Chemistry*, 50(1), 54-57. **8**. Pham Thi Nhat Trinh, Mai Đinh Tri, Huynh Thi My Nuong, Le Tien Dung (2015), Drybonioside, a new glucoside from the *Drynaria bonii* H. Christ rhizomes, *Natural Product Research*, 29(2), 137-140. **9**. Pham Thi Nhat Trinh, Mai Dinh Tri, Nguyen Huu An, Pham Ngoc An, Phan Nhat Minh, Le Tien Dung (2015), Phenolic compounds from the rhizomes of *Drynaria bonii*, *Chemistry of Natural Compounds*, 51, 476-479. **10**. Pham Thi Nhat Trinh, Mai Dinh Tri, Dang Chi Hien, Nguyen Huu An, Phan Nhat Minh, Pham Ngoc An, Le Tien Dung (2016), A new flavan from the *Drynaria bonii* H. Christ rhizomes, *Natural Product Research*, 30(7), 761-767. **11**. Pham Thi Nhat Trinh, Bach Long Giang, Tong Thanh Danh, Quach Tong Hung, Cao Van Du, Nguyen Trong Tuan (2020), Phytoconstituents of the rhizome of *Drynaria bonii* and the effect of phytoconstituents on the cell proliferation of osteoblast MG-63 cells, *IOP Conference Series: Materials Science and Engineering*, IOP Publishing, 022079. **12**. Mouffok S., Haba H., Lavaud C., Long C., Benkhaled M. (2012), Chemical constituents of *Centaurea omphalotricha* Coss. & Durieu ex Batt. & Trab, *Records of Natural Products*, 6(3), 292-295. **13**. Cheng Y. B., Chang M. T., Lo Y. W., Ho C. J., Kuo Y. C., Chien C. T., Chen S. Y., Liou S. S., Kuo Y. H., Shen Y. C. (2009), Oxygenated lignans from the fruits of *Schisandra arisanensis*, *Journal of Natural Products*, 72(9), 1663-1668. **14**. Pham Duc Nhuan, Nguyen Thi Thao Ly, Nguyen Dieu Lien Hoa, Nguyen Mau Vu (2015), Chemical constituents of the bark of *Crescentia cujete*, *Journal of Analytical Sciences*, 20(3), 274-277. **15**. Rho T., Yoon K. D. (2017), Chemical constituents of *Nelumbo nucifera* seeds, *Natural Product Sciences*, 23(4), 253-257. **16**. Nanda P., Goldha F., Nova S., Nofrizal, Amri B., Dayar A. (2021), Isolation and antibacterial properties of phenyl acrylic acid derivatives from *Balanophora elongata* Blume, *Journal of Mathematical and Fundamental Sciences*, 53(2), 231-242. **17**. Pinheiro P. G., Santiago G. M., da Silva F. E., de Araújo A. C., de Oliveira C. R., Freitas P. R., Rocha J. E., de Araújo Neto J. B., da Silva M. M., Tintino S. R., Siyadatpanah A. (2022), Ferulic acid derivatives inhibiting *Staphylococcus aureus* tetK and MsrA efflux pumps, *Biotechnology Reports*, 34, e00717. **18**. El Omari N., Jaouadi I., Lahyaoui M., Benali T., Taha D., Bakrim S., El Menyiy N., El Kamari F., Zengin G., Bangar S. P., M. Lorenzo J. (2022), Natural sources, pharmacological properties, and health benefits of daucosterol: versatility of actions, *Applied Sciences*, 12(12), 5779. **19**. Khan A. K., Rashid R., Fatima N., Mahmood S., Mir S., Khan S., Jabeen N., Murtaza G. (2015), Pharmacological activities of protocatechuic acid, *Acta Poloniae Pharmaceutica*, 72(4), 643-650. **20**. Touaibia M., Jean-Francois J., Doiron J. (2011), Caffeic acid, a versatile pharmacophore: an overview, *Mini Reviews in Medicinal Chemistry*, 11(8), 695-713. **21**. Graf E. (1992), Antioxidant potential of ferulic acid, *Free Radical Biology and Medicine*, 13(4), 435-448. **22**. Gülçin İ. (2006), Antioxidant activity of caffeic acid (3,4-dihydroxycinnamic acid), *Toxicology*, 217(2-3), 213-220.

Journal of Medicinal Materials, 2023, Vol. 28, No. 3 (pp. 141 - 145)

CHEMICAL COMPOSITION OF THE AERIAL PARTS OF *PIPER SARMENTOSUM* ROXB., PIPERACEAE

Nguyen Thi Duyen[1,*], Tran Thi Tuyet[2], Hoang Thi Ngoc Anh[2], Nguyen Thi Ngoc Bich[2], Truong Trong Huy[3], Nguyen Van Tai[1], Vu Thi Hanh Yen[4], Do Thi Dinh[5]

[1]National Institute of Medicinal Materials (NIMM), Hanoi, Vietnam; [2]Dai Nam University, Hanoi, Vietnam; [3]Hanoi University of Science, Hanoi, Vietnam; [4]Institute of Drug Quality Control in Ho Chi Minh City, Vietnam; [5]Hai Duong Central College of Pharmacy, Vietnam

*Corresponding author: nguyenduyen6784@gmail.com

(Received March 26th, 2023)

Summary

Chemical Composition of the Aerial Parts of *Piper sarmentosum* Roxb., Piperaceae

From dichloromethane and ethyl acetate extracts of *Piper sarmentosum* Roxb., six compounds were isolated (**1-6**). Their chemical structures were identified as α-asarone (**1**), 5-phenyl-3-pentenamide (**2**), piperlotine B (**3**), quercetin (**4**), rutin (**5**), and β-sitosterol (**6**), based on spectral data and literature references. Compound **2** was isolated from this plant for the first time.

Keywords: *Piper sarmentosum*, *Piperlotine B, 5-phenyl-3-pentenamide.*

1. Introduction

Piper sarmentosum is a popularly cultivated plant in Vietnam, with traditional medicinal uses for rheumatism, digestive issues, lumbago, digestive troubles, vomiting, diarrhea, and others [1]. Scientific studies have shown its potential for antiplatelet aggregation, antioxidant, and antituberculosis activities [2],[3],[4], as well as a possible treatment for hypertension and diabetes mellitus [5]. The phytochemical studies have identified essential oils, alkaloids, flavonoids, lignans, and sterols present in the plant [2],[6],[8]. In this study, we isolated and identified six compounds from the aerial parts of *P. sarmentosum* Roxb. (Fig.1), α-asarone (**1**), 5-phenyl-3-pentenamide (**2**), piperlotine B (**3**), quercetin (**4**), rutin (**5**), and β-sitosterol (**6**). This is the first report of these compounds being isolated from *P. sarmentosum* cultivated in Vietnam.

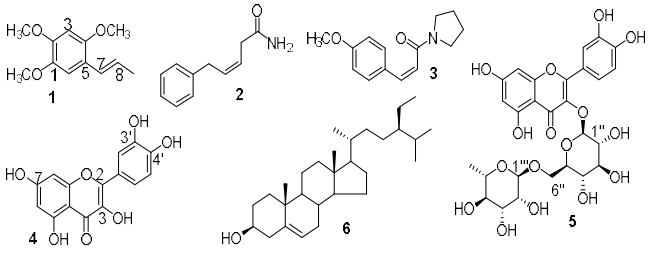

Fig. 1. Chemical structures of compounds (**1-6**)