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FLAVONOIDS FROM THE AERIAL PART OF *STREBLUS ILICIFOLIUS* (VIDAL) CORNER, MORACEAE

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Summary

Flavonoids from the Aerial Part of *Streblus ilicifolius* (Vidal) Corner, Moraceae

Streblus ilicifolius (Vidal) Corner (Moracae) has been used in traditional medicine in Vietnam for the treatment of inflammation and pimple. From the ethyl acetate fraction of the aerial part of *Streblus ilicifolius*, eight compounds were isolated and identified as vincetoxicoside B (**1**), afzelin (**2**), quercitrin (**3**), vitexin (**4**), quercetin (**5**), kaempferitrin (**6**), quercetin 3,7-*O*-*α*-L-dirhamnopyranoside (**7**), and saponarin (**8**). The structures of **1-8** were elucidated by spectroscopic methods (MS, 1D, and 2D NMR).

Keywords: *Streblus ilicifolius*, Moraceae, *Flavonoid, Quercetin.*

1. Introduction

The genus *Streblus*, which belongs to the Moraceae family, comprises over 25 species in Southeast Asia and Africa [1]. Several *Streblus* species, such as *S. asper*, *S. zeylanicus*, and *S. indicus* have been used in traditional medicine for the treatment of dysentery, toothache, and hepatitis B virus infection. The main components of the genus *Streblus* were lignans, flavonoids, and coumarins, which exhibited cytotoxicity, anti-microbial, and anti-inflammatory activities [2]. Among them, *S. ilicifolius* was found in Central Vietnam, and has been used as an anti-inflammatory and anti-pimple herb in traditional medicine in Vietnam [3]. Previous phytochemical investigations of *S. ilicifolius* reported the presence of amide glycosides [4], phenolics [3],[5], phenylpropanoid glycosides, and lignan glycosides [6]. To the best of our knowledge, the study of the phytochemistry and biological activities of *S. ilicifolius* was limited in Vietnam. In this study, the extraction and isolation of flavonoids from the ethyl acetate extract of *S. ilicifolius* were reported herein. Their structures were determined by spectroscopic data and compared to those reported in the literature.

2. Experimetal procedures

2.1. Materials and chemicals

The aerial part of *S. ilicifolius* was collected in March 2021, in Gia Lai province, Vietnam. A voucher specimen (SI032021.GL) has been deposited at the University of Medicine and Pharmacy at Ho Chi Minh City (UMP) and was identified by Dr. Vo Van Leo - Department of Pharmacognosy, UMP. The solvents were used for isolation and extraction: ethanol 96%, *n*-hexane, ethyl acetate, chloroform, methanol, and *n*-butanol (Scharlau, Spain) in analytical reagent grade.

2.2. General experimental procedures

Silica gel (43-63 µm, Merk) and Sephadex LH-20 (GE Healthcare, Sweden) were used for isolation and purification the isolated compounds. TLC was performed using normal phase *silica gel* Merck 60 F_{254} plates. Spots were detected by UV light (254 nm and 365 nm), 1% $FeCl_3$ solution/ethanol 95%, and vanillin-sulphuric acid/absolute ethanol. ESI-MS spectra were recorded using Xevo TQ-S micro mass spectrometer (Waters, USA). NMR spectra were measured on a Bruker Neo Avance 400 MHz using TMS as an internal standard. NMR solvents were purchased from Sigma-Aldrich (USA).

2.3. Extraction and isolation

The dried aerial parts of *S. ilicifolius* (4.5 kg) were percolated with 96% EtOH. The extract was concentrated under vacuum pressure with a rotary evaporator. The crude extract was suspended in water and successively partitioned with different solvents: *n*-hexane (H, 125 g), ethyl acetate (E, 103 g), and saturated *n*-BuOH (B, 43 g), respectively. The ethyl acetate fraction (30 g) was separated on an open column chromatography (CC) with normal phase using a stepwise gradient elution with EtOAc-MeOH (100:0, 98:2, 95:5, 9:1, 8:2, 7:3, 5:5, 0:100, v/v) to give 12 sub-fractions (E1-E12), according to their TLC profiling. Sub-fraction E3 (0.84 g) was loaded on Sephadex LH-20 CC, eluting with MeOH (100%) to give four sub-fractions (E3.1-E3.4). The precipitate from sub-fraction E3.4 was purified using MeOH (100%) to yield compound **1** (6.0 mg). Sub-fraction E3.4 was subjected to a

silica gel column and eluted with gradient solvent systems of $CHCl_3$-MeOH (100:0, 9:1, 98:2, 97:3, 95:5, 90:10, 80:20, 0:100) to yield compounds **2** (4.3 mg) and **3** (16.4 mg), respectively. Sub-fraction E4 (1.2 g) was subjected to Sephadex LH-20 CC (MeOH 100%) to give four sub-fractions (E4.1-E4.4). Sub-fraction E4.3 was crystalized using MeOH (100%) to yield compound **4** (12.2 mg). Sub-fraction E4.4 was separated by Sephadex LH-20 CC (MeOH 100%) to give eight sub-fractions (E4.4.1-E4.4.8). Sub-fraction E4.4.8 was purified by crystalizing (MeOH 100%) to yield compound **5** (22.6 mg). Sub-fraction E5 (1.2 g) was fractionated by Sephadex LH-20 CC (MeOH 100%) to give four sub-fractions (E5.1-E5.4). Sub-fraction E5.3 (200 mg) was purified by Sephadex LH-20 CC (MeOH 100%) to give three sub-fractions (E5.3.1-E5.3.3). Compounds **6** (8.2 mg) and **7** (15.6 mg) were separated by preparative TLC from sub-fraction E5.3.3 (26.3 mg), respectively. Sub-fraction E10 (1.2 g) was loaded on Sephadex LH-20 CC (MeOH 100%) to give four sub-fractions (E10.1-E10.4). Sub-fraction E10.4 (250 mg) was purified using Sephadex LH-20 CC (MeOH 100%) to give three sub-fractions (E10.4.1-E10.4.2). Compound **8** (11.5 mg) was crystallized from sub-fraction E10.4.2 by using MeOH (100%).

Vincetoxicoside B (**1**): pale-yellow amorphous powder. Molecular formula: $C_{21}H_{20}O_{11}$. ESI-MS: *m/z* 447.012 $[M–H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H: 12.49 (1H, s, 5-O<u>H</u>), 9.51 (1H, s, 4'-O<u>H</u>), 7.72 (1H, d, *J* = 2.4 Hz, H-2'), 7.58 (1H, dd, *J* = 8.4, 2.4 Hz, H-6'), 6.89 (1H, d, *J* = 8.4 Hz, H-5'), 6.79 (1H, d, *J* = 2.4 Hz, H-8), 6.42 (1H, d, *J* = 2.4 Hz, H-6), 5.55 (1H, d, *J* = 2.0 Hz, H-1''), 5.14 (1H, s, 2"-O<u>H</u>), 4.92 (1H, s, 4"-O<u>H</u>), 4.80 (1H, s, 3"-O<u>H</u>), 3.84 (1H, m, H-2"), 3.64 (1H, dd, *J* = 9.2, 2.4 Hz, H-3"), 3.44 (1H, m, H-5"), 3.32 (1H, m, H-4"), 1.13 (3H, d, *J* = 6.4 Hz, H-6"). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 175.9 (C-4), 161.3 (C-7), 160.3 (C-5), 155.9 (C-9), 147.8 (C-4'), 147.4 (C-2), 145.0 (C-3'), 136.0 (C-3), 121.7 (C-1'), 119.6 (C-6'), 115.0 (C-2'), 114.6 (C-5'), 104.5 (C-10), 98.3 (C-1"), 97.8 (C-6), 93.6 (C-8), 71.5 (C-4"), 70.1 (C-3"), 70.0 (C-5"), 69.7 (C-2"), 17.3 (C-6").

Afzelin (**2**): yellow amorphous powder. Molecular formula: $C_{21}H_{20}O_{10}$. ESI-MS: *m/z* 431.050 $[M–H]^-$. ^{1}H NMR (400 MHz, CD_3OD) δ_H: 7.77 (2H, dt, *J* = 8.8, 2.4 Hz, H-2'/H-6'), 6.93 (2H, dt, *J* = 8.8, 2.4 Hz, H-3'/H-5'), 6.38 (1H, d, *J* = 2.0 Hz, H-8), 6.20 (1H, d, *J* = 2.0 Hz, H-6), 5.37 (1H, d, *J* = 1.6 Hz, H-1"), 4.28 (1H, dd, *J* = 3.2, 1.6 Hz, H-2"), 3.76 (1H, m, H-3"), 3.37 (1H, m, H-4"), 3.34 (1H, m, H-5"), 0.92 (3H, d, *J* = 6.0 Hz, H-6"). ^{13}C NMR (100 MHz, CD_3OD) δ_C: 178.2 (C-4), 164.9 (C-7), 161.8 (C-5), 160.2 (C-4'), 157.9 (C-2), 157.2 (C-9), 134.8 (C-3), 130.5 (C-2'/C-6'), 121.3 (C-1'), 115.2 (C-3'/C-5'), 104.4 (C-10), 102.1 (C-1"), 98.6 (C-6), 93.5 (C-8), 71.8 (C-4"), 70.8 (C-5"), 70.7 (C-3"), 70.5 (C-2"), 16.3 (C-6").

Quercitrin (**3**): pale-yellow amorphous powder. Molecular formula: $C_{21}H_{20}O_{11}$. ESI-MS: *m/z* 447.032 $[M–H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H: 12.66 (1H, s, 5-O<u>H</u>), 7.30 (1H, d, *J* = 2.4 Hz, H-2'), 7.25 (1H, dd, *J* = 8.4, 2.4 Hz, H-6'), 6.87 (1H, d, *J* = 8.4 Hz, H-5'), 6.39 (1H, d, *J* = 2.0 Hz, H-8), 6.21 (1H, d, *J* = 2.0 Hz, H-6), 5.25 (1H, d, *J* = 1.6 Hz, H-1"), 3.97 (1H, m, H-2"), 3.50 (1H, m, H-3"), 3.22 (1H, m, H-4"), 3.13 (1H, m, H-5"), 0.81 (3H, d, *J* = 6.0 Hz, H-6"). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 178.2 (C-4), 164.7 (C-7), 161.8 (C-5), 157.8 (C-9), 156.9 (C-2), 148.9 (C-4'), 145.7 (C-3'), 134.7 (C-3), 121.6 (C-6'), 121.2 (C-1'), 116.1 (C-5'), 115.9 (C-2'), 104.6 (C-10), 102.3 (C-1"), 99.2 (C-6), 94.1 (C-8), 71.6 (C-4"), 71.1 (C-3"), 70.8 (C-5"), 70.5 (C-2"), 17.9 (C-6").

Vitexin (**4**): pale-yellow amorphous powder. Molecular formula: $C_{21}H_{20}O_{10}$. ESI-MS: *m/z* 431.394 $[M–H]^-$. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 13.17 (1H, s, 5-O<u>H</u>), 10.35 (1H, s, 4'-O<u>H</u>), 10.89 (1H, s, 7-O<u>H</u>), 8.03 (2H, d, *J* = 8.8 Hz, H-2'/H-6'), 6.89 (2H, d, *J* = 8.8 Hz, H-3'/H-5'), 6.78 (1H, s, H-3), 6.27 (1H, s, H-6), 4.69 (1H, d, *J* = 9.5 Hz, H-1"), 4.60 (1H, s, 6"-O<u>H</u>), 3.79 (1H, m, H-2"), 3.76 (1H, m, H-6a"), 3.53 (1H, d, H-6b"), 3.38 (1H, m, H-4"), 3.25 (2H, m, H-3" and H-5"). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 182.0 (C-4), 163.8 (C-2), 162.4 (C-7), 161.0 (C-4'), 160.3 (C-5), 155.9 (C-9), 128.9 (C-2'/C-6'), 121.5 (C-1'), 115.7 (C-3'/C-5'), 104.5 (C-8), 103.9 (C-10), 102.3 (C-3), 98.0 (C-6), 81.7 (C-5"), 78.6 (C-3"), 73.3 (C-1"), 70.7 (C-2"), 70.4 (C-4"), 61.2 (C-6").

Quercetin (**5**): yellow amorphous powder. Molecular formula: $C_{15}H_{10}O_7$. ESI-MS: *m/z* 301.005 $[M–H]^-$. ^{1}H-NMR (400 MHz, CD_3OD) δ_H: 7.73 (1H, d, *J* = 2.4 Hz, H-2'), 7.63 (1H, dd, *J* = 8.4, 2.4 Hz, H-6'), 6.88 (1H, d, *J* = 8.4 Hz, H-5'), 6.38 (1H, d, *J* = 2.0 Hz, H-8), 6.18 (1H, d, *J* = 2.0 Hz, H-6). ^{13}C NMR (100 MHz, CD_3OD) δ_C: 176.0 (C-4), 164.2 (C-7), 161.1 (C-5), 156.9 (C-9), 147.4 (C-2), 146.6 (C-4'), 144.9 (C-3'), 135.9 (C-3), 122.8 (C-1'), 120.3 (C-6'), 114.8 (C-5'), 114.6 (C-2'), 103.1 (C-10), 97.8 (C-6), 93.0 (C-8).

Kaempferitrin (**6**): yellow amorphous powder. Molecular formula: $C_{27}H_{30}O_{14}$. ESI-MS: *m/z* 577.388 $[M–H]^-$. ^{1}H NMR (400 MHz, CD_3OD) δ_H: 7.77 (2H, dd, *J* = 8.8, 2.0 H-2'/H-6'),

6.89 (2H, dd, *J* = 8.8, 2.0, H-3'/H-5'), 6.71 (1H, d, *J* = 2.0 Hz, H-8), 6.45 (1H, d, *J* = 2.0 Hz, H-6), 5.56 (1H, d, *J* = 1.6 Hz, H-1'''), 5.38 (1H, d, *J* = 1.6 Hz, H-1''), 4.22 (1H, dd, *J* = 3.6, 1.6 Hz, H-2''), 4.02 (1H, dd, *J* = 3.6, 2.0 Hz, H-2'''), 3.83 (1H, dd, *J* = 9.2, 3.6, Hz, H-3'''), 3.76 (1H, dd, *J* = 9.2, 3.6, Hz, H-3''), 3.60 (1H, m, H-5'''), 3.46 (1H, m, H-5''), 3.35 (1H, m, H-4''), 3.05 (1H, m, H-4'''), 1.26 (3H, d, *J* = 6.0 Hz, H-6'''), 0.95 (3H, d, *J* = 6.0 Hz, H-6''). ^{13}C NMR (100 MHz, CD_3OD) δ_C: 178.2 (C-4), 162.9 (C-4'), 162.0 (C-7), 161.6 (C-5), 158.8 (C-2), 156.6 (C-9), 134.8 (C-3), 130.6 (C-2'/C-6'), 119.5 (C-1'), 116.0 (C-3'/C-5'), 106.1 (C-10), 102.1 (C-1''), 99.1 (C-6), 98.4 (C-1'''), 94.2 (C-8), 72.2 (C-4'''), 71.8 (C-4''), 70.7 (C-3''), 70.7 (C-5''), 70.6 (C-3'''), 70.5 (C-2''), 70.3 (C-2'''), 69.9 (C-5'''), 16.6 (C-6''), 16.7 (C-6''').

Quercetin-3,7-*O*-L-dirhamnopyranoside (**7**): yellow amorphous powder. Molecular formula: $C_{27}H_{30}O_{15}$. ESI-MS: *m/z* 593.091 $[M-H]^-$. ^{1}H-NMR (400 MHz, DMSO-d_6) δ_H: 7.36 (1H, d, *J* = 2.4 Hz, H-2'), 7.33 (1H, dd, *J* = 8.4, 2.4 Hz, H-6'), 6.90 (1H, d, *J* = 8.4 Hz, H-5'), 6.72 (1H, d, *J* = 2.4 Hz, H-8), 6.46 (1H, d, *J* = 2.4 Hz, H-6), 5.56 (1H, d, *J* = 1.6 Hz, H-1'''), 5.37 (1H, d, *J* = 1.6 Hz, H-1''), 4.22 (1H, m, H-2''), 4.01 (1H, m, H-2'''), 3.83 (1H, dd, *J* = 9.6, 3.2 Hz, H-3'''), 3.76 (1H, dd, *J* = 9.6, 3.2 Hz, H-3''), 3.60 (1H, m, H-5'''), 3.50 (1H, m, H-5''), 3.47 (1H, m, H-4'''), 3.35 (1H, m, H-4''), 1.26 (3H, d, *J* = 6.0 Hz, H-6'''), 0.95 (3H, d, *J* = 6.0 Hz, H-6''). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 178.4 (C-4), 162.1 (C-7), 161.6 (C-5), 158.6 (C-2), 156.7 (C-9), 149.4 (C-4'), 145.4 (C-3'), 135.0 (C-3), 121.6 (C-6'), 120.9 (C-1'), 115.5 (C-2'), 115.1 (C-5'), 106.1 (C-10), 102.1 (C-1''), 99.1 (C-6), 98.5 (C-1'''), 94.2 (C-8), 72.2 (C-4'''), 71.9 (C-4''), 70.7 (C-3''), 70.7 (C-5''), 70.7 (C-3'''), 70.5 (C-2''), 70.3 (C-2'''), 69.9 (C-5'''), 16.7 (C-6'''), 16.3 (C-6'').

Saponarin (**8**): pale-yellow amorphous powder. Molecular formula: $C_{27}H_{30}O_{15}$. ESI-MS: *m/z* 593.067 $[M-H]^-$. ^{1}H NMR (400 MHz, DMSO-d_6) δ_H: 13.52 (1H, s, 5-OH), 10.42 (1H, s, 4'-OH), 7.98 (2H, dd, *J* = 8.8, 2.4 Hz, H-2'/H-6'), 6.94 (2H, dd, *J* = 8.8, 2.4 Hz, H-3'/H-5'), 6.90 (1H, s, H-3), 6.87 (1H, s, H-8), 4.98 (1H, d, *J* = 7.2 Hz, H-1'''), 4.66 (1H, d, *J* = 9.6 Hz, H-1''), 3.94 (1H, m, H-2''), 3.77 (1H, m, H-6a'''), 3.60 (2H, m, H-6''), 3.53 (1H, m, H-6b'''), 3.56 (1H, m, H-5'''), 3.24 (1H, m, H-3''), 3.17 (1H, m, H-5''). ^{13}C NMR (100 MHz, DMSO-d_6) δ_C: 182.0 (C-4), 164.0 (C-2), 162.3 (C-7), 161.2 (C-4'), 159.2 (C-5), 156.3 (C-9), 128.5 (C-2'/C-6'), 120.8 (C-1'), 115.9 (C-3'/C-5'), 110.4 (C-6), 104.8 (C-10), 103.1 (C-3), 101.1 (C-1'''), 93.6 (C-8), 80.8 (C-5''), 78.8 (C-3''), 77.1 (C-5'''), 75.6 (C-3'''), 73.7 (C-2'''), 72.5 (C-1''), 70.7 (C-2''), 69.5 (C-4'''), 69.4 (C-4''), 60.5 (C-6'''), 60.2 (C-6'').

3. Results and discussion

Compound **1** was isolated as a pale-yellow amorphous powder. The ESI-MS with an $[M-H]^-$ ion at *m/z* 447.012, suggested a molecular formula of $C_{21}H_{20}O_{11}$ with 12 degrees of unsaturation. The ^{13}C NMR spectrum showed the signals of 21 carbons, including 15 carbons in the downfield region at δ_C 176-93, and hexosyl sugar moiety. The ^{13}C NMR spectrum of **1** revealed signals of a carbonyl group at δ_C 175.9 (C-4), seven oxygenated carbons (δ_C 161.3 (C-7), 160.3 (C-5), 155.9 (C-9), 147.8 (C-5′), 147.4 (C-2), 145.0 (C-3′), and 136.0 (C-3)), two non-protonated carbons (δ_C 121.7, C-1′ and 104.5, C-10), and ten methine carbons (including five aromatic methines at δ_C 97.8 (C-6), 93.6 (C-8), 119.6 (C-6′), 115.0 (C-2′), 114.6 (C-5′), and five oxymethine carbons at δ_C 98.3 (C-1″), 71.5 (C-4″), 70.1 (C-3"), 70.0 (C-5"), 69.7 (C-2")), and methyl group δ_C 17.3 (C-6"). The ^{13}C-NMR spectra of compound **1** showed a typical flavonol pattern with a quercetin. The ^{1}H NMR and ^{1}H-^{1}H COSY spectra exhibited the *ortho*-coupled aromatic proton signals of H-6′ (δ_H 7.58, dd, *J* = 8.4, 2.4 Hz) and H-5′ (δ_H 6.89, d, *J* = 8.4 Hz), two *meta*-coupled proton signals of H-6′ and H-2′ (δ_H 7.72, d, *J* = 2.4 Hz), and H-6 (δ_H 6.42, d, *J* = 2.4 Hz) and H-8 (δ_H 6.79, d, *J* = 2.4 Hz). In addition, the ^{1}H-NMR spectrum displayed the signals of sugar moiety at δ_H 3.32-3.84 (H-2"-H-5"), a methyl at δ_H 1.13 (3H, d, *J* = 6.4 Hz, H-6"), and a doublet corresponding to the anomeric proton H-1" (δ_H 5.55, d, *J* = 2.0 Hz), which characterized an *α*-L-rhamnose moiety. The HMBC spectrum revealed the correlations from 5-OH (δ_H 12.49, s) to C-5/C-6/C-10, from H-6 to C-5/C-7/C-8/C-10, from H-8 to C-4/C-6/C-7/C-9/C-10, from H-2′ to C-2/C-1′/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′ (Fig. 2). In the HMBC spectrum, the anomeric proton correlated with carbon signal at δ_C 161.3 (C-7), which exhibited the linkage of the *α*-L-rhamnopyranosyl to C-7. Based on the above evidence and detailed analyses of the NMR spectra, compound **1** was determined as quercetin 7-*O*-*α*-L-rhamnopyranoside or vincetoxicoside B [7].

Compound **2** was obtained as a pale-yellow amorphous powder. Its molecular formula was determined as $C_{21}H_{20}O_{10}$ from the negative ESI-MS ion peak at *m/z* 431.050 $[M-H]^-$, consistent with 12 degrees of unsaturation. The ^{13}C NMR spectrum showed signals of 21 carbons, including 15 carbons of flavonol and a sugar

moiety. Six carbon signals of the sugar moiety of **2** were similar to those of **1**. The decrease of 16 mass units in **2** compared to the molecular weight of **1** and the ^{13}C NMR spectrum of **2** showed the presence of a symmetric B-ring structure, which is demonstrated by the loss of a hydroxy group in **2**. The ^{1}H NMR spectrum of **2** showed signals of *meta*-coupled protons between H-8 (δ_H 6.38 (1H, d, J = 2.0 Hz) and H-6 (δ_H 6.20, d, J = 2.0 Hz) and the signal of two pair of aromatic symmetric protons at δ_H 7.77 (2H, dt, J = 8.8, 2.4 Hz, H-2' and H-6') and 6.93 (2H, dt, J = 8.8, 2.4 Hz, H-3' and H-5') were assigned to the structure of B-ring. The α-L-rhamnopyranosyl sugar moiety was linked to C-3 according to the HMBC correlation from H-1″ (δ_H 5.37) to C-3 (δ_C 134.8). The key HMBC correlations of **2** were shown in Fig. 2. Therefore, the structure of compound **2** was determined as kaempferol-3-*O*-α-L-rhamnoside or afzelin [8].

Compound **3** was isolated as a yellow amorphous powder. The molecular formula was $C_{21}H_{20}O_{11}$ based on the negative ESI-MS spectrum with an ion peak at *m/z* 447.032 $[M-H]^-$. The ^{1}H and ^{13}C NMR spectra of compound **3** were closely related to those of **1**. The main difference was that the rhamnopyranosyl moiety attached to C-3 (δ_C 134.7) of **3** instead of C-7 in **1**, which was demonstrated by HMBC correlation between H-1" (δ_H 5.25, d, J = 1.6 Hz) and C-3 (δ_C 134.7). The key HMBC and COSY correlations of **3** were shown in Fig. 2. In comparison with the published data in the literature [9], compound **3** was identified as quercetin 3-*O*-α-L-rhamnopyranoside or quercitrin.

Compound **4** was isolated as an amorphous yellow powder. The negative ESI-MS spectrum showed a pseudomolecular ion peak at *m/z* 431.394 $[M-H]^-$, corresponding to the molecular formula of $C_{21}H_{20}O_{10}$. The ^{13}C NMR and HSQC spectra showed the presence of 19 carbon signals including of two symmetric carbon signals at δ_C 128.9 (C-2'/C-6') and 115.7 (C-3'/C-5'), which was inferred to a flavonoid glycoside. The ^{13}C and ^{1}H NMR spectra of **4** were close to those of **2**, except for the absence of hydroxyl group at C-3 (δ_C 102.3) in **4**. Additionally, the sugar moiety was changed to glucopyranosyl in **4** instead of rhamnopyranosyl in **2** with the aid of carbon signal C-6" (δ_C 61.2) and proton H_2-6 (δ_C 3.76 (1H, m, H-6a") and 3.53 (1H, d, H-6b")). The configuration of sugar moiety was identified as β-D-glucopyranoside by the coupling constant of J = 9.5 Hz. In the HMBC spectrum, the anomeric proton at δ_H 4.69 (H-1") correlated with the carbons at δ_C 104.5 (C-8), 162.4 (C-7), and 155.9 (C-9), which was confirmed the glycosidic linkage of 8-*C*-glucosyl unit on the A-ring of flavonoid. In comparison with the published data in the literature, compound **4** was determined as apigenin-8-*C*-β-D-glucopyranoside or vitexin [10].

Compound **5** was isolated as a yellow amorphous powder. It has the molecular formula $C_{15}H_{10}O_7$ determined by the negative ESI-MS ion peak at *m/z* 301,005 $[M-H]^-$. The ^{13}C NMR and HSQC data showed 15 carbon signals in the downfield region of δ_C 176-93 of a flavone aglycon. The ^{13}C and ^{1}H-NMR spectra data of **5** were similar to those of **3**, except for the absence of sugar moiety linked to carbon C-3 in **5**. The key HMBC and ^{1}H-^{1}H COSY correlations of **5** were shown in Fig. 2. From the above evidence of NMR spectra and ESI-MS spectrum data, compound **5** was identified as quercetin in comparison with the spectroscopic data reported in the literature [9].

Compound **6** was isolated as a yellow amorphous powder. The ESI-MS spectral data exhibited a negative-ion peak at *m/z* 577.388 $[M-H]^-$, corresponding to the molecular formula of $C_{27}H_{30}O_{14}$ and 12 degrees of unsaturation. The ^{13}C NMR spectrum of compound **6** showed 27 carbon signals, containing one carbonyl group (δ_C 178.2, C-4), 6 aromatic methine carbons (δ_C 130.6 (C-2'/C-6'), 116.0 (C-3'/C-5'), 99.1 (C-6), and 94.2 (C-8)), 8 non-protonated carbons (δ_C 162.9 (C-4'), 162.0 (C-7), 161.6 (C-5), 158.8 (C-2), 156.6 (C-9), 134.8 (C-3), 119.5 (C-1'), and 106.1 (C-10)), 10 oxymethine carbons (δ_C 102.1 (C-1"), 98.4 (C-1'"), 72.2 (C-4"'), 71.8 (C-4"), 70.7 (C-3"), 70.7 (C-5"), 70.6 (C-3"'), 70.5 (C-2"), 70.3 (C-2"'), and 69.9 (C-5"')), and two methyl groups (δ_C 16.6 (C-6″) and 16.7 (C-6‴)). The ^{1}H NMR and ^{1}H-^{1}H COSY spectra showed the correlations of a pair of symmetric protons signals between protons H-2′/H-6′ (δ_H 7.77, dd, J = 8.8, 2.0 Hz) and H-3′/H-5′ (δ_H 6.91, dd, J = 8.8, 2.0 Hz) in an aromatic *ortho*/*meta*-coupled protons, and a *meta*-coupled proton signals between H-6 (δ_H 6.45, d, J = 2.0 Hz) and H-8 (δ_H 6.71, d, J = 2.0 Hz). The HMBC correlations between H-1″ (δ_H 5.38, d, J = 1.6 Hz) and C-3 (δ_C 134.8), and between H-1‴ (δ_H 5.56, d, J = 1.6 Hz) and C-7 (δ_C 162.0), exhibited the presence of an -3,7-*O*-α-L-dirhamnopyranoside sugar chain. The key HMBC and ^{1}H-^{1}H COSY correlations were observed as shown in Fig. 2. Based on the comparison of the spectral data [11], compound **6** was identified as kaempferol-3,7-*O*-α-L-dirhamnopyranoside or kaempferitrin.

Compound **7** was isolated as a yellow amorphous powder. Its molecular formula

$C_{27}H_{29}O_{15}$ was determined by the negative ESI-MS ion peak at *m/z* 593.091 $[M-H]^-$. The ^{13}C and ^1H NMR spectra data of **7** resembled to those of **6** except for the additional hydroxy group in the structure of **7**. The ^1H NMR and ^1H-^1H COSY spectra showed the presence of *ortho*-coupled proton signals between H-6′ (δ_H 7.33, dd, J = 8.4, 2.4 Hz) and H-5′ (δ_H 6.90, d, J = 8.4 Hz) and a *meta*-coupled proton signals between H-6′ and H-2′ (δ_H 7.36, d, J = 2.4 Hz), which suggested the hydroxy group attached to C-3′ of B-ring. The HMBC and COSY correlations were observed as shown in Fig. 2. Thus, compound **7** was elucidated as quercetin-3,7-*O*-*α*-L-dirhamnopyranoside [9],[11].

Compound **8** was obtained as a yellow amorphous powder. Its molecular formula $C_{27}H_{29}O_{15}$ was established by negative ESI-MS with an $[M-H]^-$ ion peak at *m/z* 593.067. The ^{13}C NMR spectrum showed the presence of 27 carbon signals, including one carbonyl (δ_C 182.0, C-4), 16 methines (six aromatics and ten oxymethine carbons), eight non-protonated carbons (δ_C 164.0 (C-2), 162.3 (C-7), 161.2 (C-4'), 159.2 (C-5), 156.3 (C-9), 120.8 (C-1'), 110.4 (C-6), and 104.8 (C-10)), two oxymethylene carbon at δ_C 60.5 (C-6‴) and 60.2 (C-6″). All carbon signals appeared in the down-field region from δ_C 182-93 of a C_6-C_3-C_6 flavonoid framework and from δ_C 80-60 of two sugar moieties. The ^1H NMR and ^1H-^1H COSY spectra revealed the signals of a pair of symmetric protons between H-2′/H-6′ (δ_H 7.98, dd, J = 8.8, 2.4 Hz) and H-3′/H-5′ (δ_H 6.94, dd, J = 8.8, 2.4 Hz) in an aromatic *ortho*/*meta*-coupled protons, and a two singlet signals of H-3 at δ_H 6.90 and H-8 at δ_H 6.87. The pyranose forms of sugar moieties were determined to be *α*-D-glucopyranoside by the coupling constants of anomeric protons at δ_H 4.66 (1H, d, J = 9.6 Hz, H-1″) and δ_H 4.98 (1H, d, J = 7.2 Hz, H-1‴), and the chemical shift values of oxymethylene at C-6″ (δ_C 60.2, δ_H 3.60, H_2-6″) and C-6‴ (δ_C 60.5, 3.77 (1H, m, H-6a‴), and 3.53 (1H, m, H-6b‴). In the HMBC spectrum, the anomeric proton signals of glucose moiety at δ_H 4.66 (H-1″) correlated with the carbon signals at δ_C 159.2 (C-5), 110.4 (C-6), 162.3 (C-7) and the correlation between H-1‴ (δ_H 4.98) and C-7 (δ_C 162.3) was observed. Therefore, the glycosidic linkages of the 6-*C*-glucopyranosyl and 7-*O*-glucopyranosyl on the A-ring of the flavonoid were confirmed. The key HMBC correlations of compound **8** were shown in Fig. 2. Based on the NMR spectra data and compared to those of reference data published in the literature [12], compound **8** was determined as apigenin-6-C-7-*O*-*β*-*D*-diglucopyranoside or saponarin.

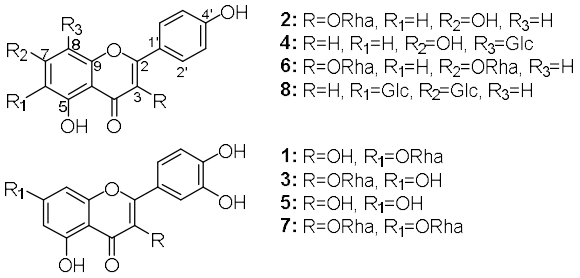

Fig. 1. Chemical structures of the isolated compound **1-8**

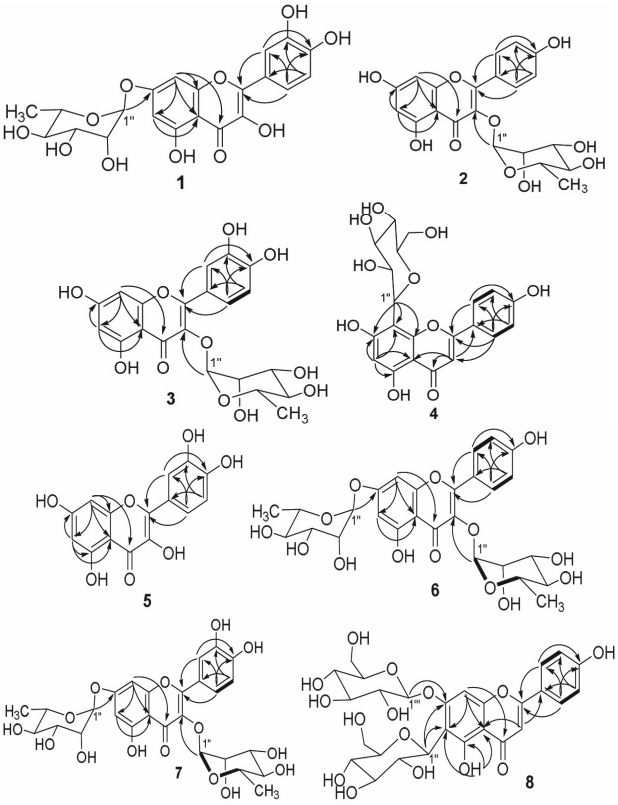

Fig.2. The key HMBC (⟶) and COSY (▬) correlations of compounds **1-8**

4. Conclusion

From the ethyl acetate extract of the aerial parts of *S. ilicifolius* (Vidal) Corner, eight flavonoids were isolated by using various chromatographic methods. Their structures were elucidated as vincetoxicoside B (**1**), afzelin (**2**), quercitrin (**3**), vitexin (**4**), quercetin (**5**), kaempferitrin (**6**), quercetin-3,7-*O*-*α*-L-dirhamnopyranoside (**7**), and saponarin (**8**) by detailed analysis of 1D and 2D NMR spectral data and in comparison with the NMR data published in the literature. Compounds **1-8** were reported here for the first time from the species *S. ilicifolius*. Among them, vitexin and saponarin were isolated for the first time in the genus *Streblus*.

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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.

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

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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