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Journal of Medicinal Materials, 2023, Vol. 28, No. 6 (pp. 351 - 357)

CHEMICAL CONSTITUENTS FROM TWIGS AND LEAVES OF *JASMINUM SUBTRIPLINERVE* BLUME.

Nguyen Tra My[1], Nguyen Thi Thu[1], Nguyen Thi Ha Ly[1], Vu Thi Diep[1], Tran Huyen Trang[1], Pham Thi Hien[1], Hoang Thi Dieu Huong[1], Nguyen Thi Thuy[2], Nguyen Minh Khoi[1], Do Thi Ha[1,*]

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

[2]Hanoi Medical University Hospital, Vietnam

*Corresponding author: hado.nimms@gmail.com

(Received August 17th, 2023)

Summary

Chemical Constituents from Twigs and Leaves of *Jasminum subtriplinerve* Blume.

Seven compounds, including verbascoside (**1**), *p*-hydroxybenzoic acid (**2**), protocatechuic acid (**3**), vanillic acid (**4**), kaempferol (**5**), quercetin (**6**), and *β*-sitosterol (**7**) were isolated from the ethyl acetate extract of *Jasminum subtriplinerve* twigs and leaves. The structures of these compounds were identified using extensive nuclear magnetic resonance spectroscopy (NMR) data, mass spectrometry (MS), and comparison with published literature. Compounds **4** and **6** were reported for the first time from the *Jasminum* genus. Furthermore, the quantification of verbascoside (**1**) in *J. subtriplinerve* was performed using the HPLC-DAD method. The determined contents of verbascoside in the leaves, twigs, and combined twigs and leaves samples of *J. subtriplinerve* were 2.77%, 0.46%, and 0.74%, respectively. These results suggest that verbascoside might be used as a marker for standardizing the quality of *J. subtriplinerve*'s twigs and leaves.

Keywords: *Jasminum subtriplinerve* Blume., *Phenylethanoid glycoside, Flavonoids, Phenolic acids, Verbascoside.*

1. Introduction

Jasminum subtriplinerve Blume., a species of Oleaceae, is widely distributed worldwide, growing naturally in the mountains and midlands of countries such as India, Myanmar, Cambodia, Laos, and the southern provinces of China [1]. In Vietnam, *J. subtriplinerve* can be found in numerous provinces across mountains, midlands, and plains, including Lao Cai, Hoa Binh, Hanoi, Nghe An, Ha Tinh, Quang Tri, and Thua Thien Hue [2]. Traditional medicine employs *J. subtriplinerve* leaves, which are primarily utilized to manage irregular menstruation in postpartum women with high fever, lymphadenitis, mastitis, breast abscess, leukorrhea, rheumatism-induced bone and joint pain, scabies, impetigo, and pruritic skin conditions [3]. The plant serves various medicinal purposes, such as supporting liver function, promoting bile secretion, aiding digestion, improving appetite, facilitating sleep, and demonstrating antibacterial and anti-inflammatory properties [4]. Investigations into chemical composition and biological activities reveal that *J. subtriplinerve* twigs and leaves contain phenylethanoid glycosides (e.g., verbascoside), terpenoids, flavonoids (e.g., kaempferol), phenolic acids (e.g., *p*-hydroxybenzoic acid, protocatechuic acid), and steroids (e.g., *β*-sitosterol) [5]. These constituents contribute to the plant's antibacterial, anti-inflammatory, antioxidant, liver-protective, cytotoxic, and antipyretic effects [1],[5],[6],[7],[8],[9].

J. subtriplinerve is widely distributed in Vietnam and offers various benefits. However, the Vietnamese Pharmacopoeia V does not currently provide chemical marker(s) for standardizing the quality of this species. Therefore, this study focuses on the extraction, isolation, and structural determination of compounds from the twigs and leaves of *J. subtriplinerve*. The aim is to gather valuable data that can be used to select chemical markers for standardizing the quality of *J. subtriplinerve* twigs and leaves.

2. Materials and methods

2.1. Plant material

Twigs and leaves of *Jasminum subtriplinerve* Blume. (Oleaceae) were collected in Huong Son, My Duc, Vietnam, in February 2022. The plant was identified by MSc Nguyen Van Hieu and BSc Le Thi Tu Linh (National Institute of Medicinal Materials, NIMM). A voucher specimen (DL-040222) was deposited in the Center of Medicinal Material Resources, NIMM (Hanoi, Vietnam).

2.2. General experimental procedures

NMR spectra were recorded on a 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. *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 or by spraying with 10% sulfuric acid in 96% ethanol (EtOH) and heating. Verbascoside was purchased from Chemfaces, China (CAS 61276-17-3, Lot No: CFS202202, Purity: 99.1%).

2.3. Extraction and isolation

The dried twigs and leaves of *J. subtriplinerve* (10.0 kg) were extracted and refluxed with 80% EtOH (3 times, 3 hours each time) at 80°C. The extracts were filtered, combined, and evaporated under reduced pressure, yielding a green residue (1.5 kg), which was suspended in water and successively separated with *n*-hexane and ethyl acetate (EtOAc). 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 dichloromethane - acetone (20:1 - 1:1, v/v) as eluents to give six fractions (E1 - E6) and compound **7** (300.0 mg). Fraction E3 (3.03 g) was fractionated by *silica gel* CC eluting with an isocratic mixture of ethyl acetate - methanol - water (90:9:1 - 70:25:5, v/v/v) to obtain five subfractions (E3.1 - E3.5). Compound **1** (200.6 mg) was obtained from subfraction E3.3 (1.46 g) by using RP-C_{18} gel CC with an elution mixture of methanol - water (1:1, v/v). Fraction E6 (5.12 g) was fractionated by Sephadex CC eluting with an isocratic mixture of methanol - water (1:1, v/v) to obtain four fractions (E6.1 - E6.4). Subfraction E6.3 (2.94 g) was applied to RP-C_{18} gel CC with an isocratic elution of acetone - water (4:1, v/v) to afford three compounds **2** (12.1 mg), **3** (203.1 mg), and **4** (8.3 mg). Two compounds **5** (10.5 mg) and **6** (12.7 mg) were obtained from subfraction E6.4 (315.8 mg) by using RP-C_{18} gel CC with an isocratic elution of acetone - water (4:1, v/v).

Compound **1**: Pale yellow powder; ^{1}H-NMR (500 MHz, CD_3OD) δ_H: 7.07 (1H, d, J = 2.0 Hz, H-2), 6.80 (1H, d, J = 8.5 Hz, H-5), 6.98 (1H, dd, J = 8.5, 2.0 Hz, H-6), 7.61 (1H, br d, J = 16.0 Hz, H-7), 6.30 (1H, br d, J = 16.0 Hz, H-8), 6.72 (1H, d, J = 2.0 Hz, H-2'), 6.70 (1H, d, J = 8.0 Hz, H-5'), 6.59 (1H, dd, J = 8.0, 2.0 Hz, H-6'), 2.82 (2H, m, H-7'), 3.75 (1H, m, H-8'a), 4.07 (1H, m, H-8'b), 4.40 (1H, d, J = 8.0 Hz, H-1''), 3.41 (1H, dd, J = 9.0, 8.0 Hz, H-2''), 3.84 (1H, t, J = 9.0 Hz, H-3''), 4.94 (1H, m, H-4''), 3.54 (4H, H-5'', H-6'', H-3''', H-5'''), 3.63 (1H, m, H-6''), 5.21 (1H, d, J = 1.5 Hz, H-1'''), 3.94 (1H, m, H-2'''), 3.29 (1H, m, H-4'''), 1.11 (3H, d, J = 6.0 Hz, H-6'''); ^{13}C-NMR (125 MHz, CD_3OD) δ_C: 127.7 (C-1), 114.7 (C-2), 146.8 (C-3), 149.8 (C-4), 117.1 (C-5), 123.2 (C-6), 148.0 (C-7), 115.3 (C-8), 168.3 (C-9), 131.5 (C-1'), 116.3 (C-2'), 146.1 (C-3'), 144.7 (C-4'), 116.5 (C-5'), 121.3 (C-6'), 36.6 (C-7'), 72.4 (C-8'), 103.0 (C-1''), 76.2 (C-2''), 81.7 (C-3''), 70.6 (C-4''), 76.1 (C-5''), 62.4 (C-6''), 104.2 (C-1'''), 72.3 (C-2'''), 72.1 (C-3'''), 73.8 (C-4'''), 70.4 (C-5'''), 18.4 (C-6'''); ESI-MS: *m/z* 623.2 $[M-H]^-$, 647.2 $[M+Na]^+$.

Compound **2**: Pale yellow powder; ^{1}H-NMR (500 MHz, DMSO-d_6) δ_H: 7.79 (2H, d, J = 9.0 Hz, H-2, H-6), 6.82 (2H, d, J = 9.0 Hz, H-3, H-5); ^{13}C-NMR (125 MHz, DMSO-d_6) δ_C: 121.3 (C-1), 131,5 (C-2, C-6), 115.1 (C-3, C-5), 161.5 (C-4), 167.1 (C-7); ESI-MS: *m/z* 137.30 $[M-H]^+$.

Compound **3**: Pale white powder; ^{1}H-NMR (500 MHz, CD_3OD) δ_H: 7.46 (1H, d, J = 1.5 Hz, H-2), 6.81 (1H, d, J = 8.5 Hz, H-5), 7.44 (1H, dd, J = 8.5, 1.5 Hz, H-6); ^{13}C-NMR (125 MHz, CD_3OD) δ_C: 123.3 (C-1), 115.8 (C-2), 146.0 (C-3), 151.5 (C-4), 117.8 (C-5), 123.9 (C-6), 170.3 (C-7); ESI-MS: *m/z* 153.25 $[M-H]^-$.

Compound **4**: White powder; ^{1}H-NMR (500 MHz, CD_3OD) δ_H: 7.60 (1H, d, J= 1.5 Hz, H-2), 6.79 (1H, d, J = 8.5 Hz, H-5), 7.53 (1H, dd, J = 8.5, 1.5 Hz, H-6), 3.89 (3H, s, 3-OCH_3); ^{13}C-NMR (125 MHz, CD_3OD) δ_C: 128.3 (C-1), 114.0 (C-2), 148.2 (C-3), 150.8 (C-4), 115.4 (C-5), 124.6 (C-6), 174.5 (C-7), 56.3 (3-OCH_3).

Compound **5**: Pale yellow powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ_H: 6.18 (1H, d, J= 1.8 Hz, H-6), 6.43 (1H, d, J = 1.8 Hz, H-8), 8.04 (2H, d, J = 9.0 Hz, H-2', H-6'), 6.92 (2H, d, J = 9.0 Hz, H-3', H-5'), 12.47 (1H, s, 5-OH); ^{13}C-NMR (150 MHz, DMSO-d_6) δ_C: 146.8 (C-2), 135.6 (C-3), 175.9 (C-4), 160.7 (C-5), 98.2 (C-6), 164.0 (C-7), 93.5 (C-8), 156.2 (C-9), 103.0 (C-10), 121.7 (C-1'), 129.5 (C-2', C-6'), 115.4 (C-3', C-5'), 159.2 (C-4'); ESI-MS: *m/z* 285.15 $[M-H]^-$.

Compound **6**: Pale yellow powder; ^{1}H-NMR (600 MHz, DMSO-d_6) δ_H: 6.18 (1H, d, J= 1.8 Hz, H-6), 6.40 (1H, d, J = 1.8 Hz, H-8), 7.67 (1H, d, J = 2.4 Hz, H-2'), 6.87 (1H, d, J = 8.4 Hz, H-5'), 7.53 (1H, dd, J = 8.4, 2.4 Hz, H-6'), 9.57 (1H, s, 3-OH), 12.47 (1H, s, 5-OH), 10.77 (1H, s, 7-OH), 9.34 (2H, s, 3'-OH, 4'-OH); ^{13}C-NMR (150 MHz, DMSO-d_6) δ_C: 146.8 (C-2), 135.7 (C-3), 175.8 (C-4), 160.7 (C-5), 98.1 (C-6), 163.9 (C-7), 93.3 (C-8), 156.1 (C-9), 103.0 (C-10), 121.9 (C-1'), 115.0 (C-2'), 145.0 (C-3'), 147.7 (C-4'), 115.6 (C-5'), 119.9 (C-6').

Compound **7**: White powder; ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H: 3.52 (1H, m, H-3), 5.35 (1H, m, H-6), 0.68 (3H, s, H-18), 1.01 (3H, s, H-19), 0.92 (3H, d, J = 6.6 Hz, H-21), 0.81 (3H, d, J = 6.6 Hz, H-26), 0.84 (3H, d, J = 6.6 Hz, H-27), 0.85 (3H, t, J = 7.2 Hz, H-29).

2.4. Quantitative analysis of verbascoside (1) in twigs and leaves of J. subtriplinerve using an HPLC-DAD method

Verbascoside (**1**) in the twigs and leaves of *J. subtriplinerve* was quantitatively analyzed by an HPLC-DAD method, following published references [10].

Verbascoside standard stock solution (1240 mg/ml): Weigh accurately 12.4 mg of verbascoside, and dissolve it in 10 ml of methanol (HPLC grade).

Verbascoside standard solution for the assay: Measure accurately the volume of the verbascoside standard stock solution, and dilute it with methanol to produce a series of solutions of 620, 310, 155, 77.5, 38.8, 19.4, and 9.7 µg/ml.

Test solution: Weigh accurately 1.00 g of the sample and place it in a 50-ml round-bottomed flask, then add 15 ml of methanol. Sonicate the mixture three times for 120 min each time times at 40°C. Filter and transfer the filtrate to a 50-ml volumetric flask, and make up to the mark with methanol. Filter through a 0.45-µm PTFE filter.

HPLC-DAD analysis: A Shimadzu LC-20AD pump system, equipped with an Autosampler SIL-20A HT and an SPD-M20A PDA detector, was applied. Separation was performed using an Agilent C_{18} column (250 × 4.6 mm, 5 µm) at a temperature of 30°C. The mobile phase consisted of acetonitrile/0.01% TFA (**A**) and water/0.01% TFA (**B**) using a gradient elution of 0-30 min (10-30% **A**), 30-36 min (30-80% **A**), 36-41 min (80-10% **A**), 41-50 min (10% **A**). The solvent flow rate was maintained at 0.5 ml/min. Detection was performed at a wavelength of 330 nm.

The percentage of verbascoside in the tested sample was calculated as:

$$X\ (\%\ \text{or g/100g dry weight}) = \frac{C \times V \times 100 \times 100}{m \times 10^6 \times (100-a)}$$

where C is the concentration of the analyzed compound in the sample solution determined using the calibration curve (µg/ml), V is the volume of the sample solution (ml), m is the weight of the tested sample used to prepare the test solution (mg), and a is the moisture of the sample (%).

3. Results and discussion

Seven compounds were isolated from the 80% ethanol extract of *J. subtriplinerve* twigs and leaves (**1-7**) (**Fig. 1**). From this extract, the chemical structures of 7 compounds were elucidated by comparing their spectroscopic data to those of published values.

Compound **1** was obtained as a pale yellow powder with a molecular formula of $C_{29}H_{35}O_{15}$, which was determined through ESI-MS analysis, revealing a *quasi*-molecular ion at *m/z* 623.2 $[M-H]^-$ and 647.2 $[M+Na]^+$. The ^{1}H-NMR spectrum revealed that **1** contained a phenylethoxy group with characteristic signals at δ_H 6.72 (1H, d, J = 2.0 Hz, H-2′), 6.70 (1H, d, J = 8.0 Hz, H-5′), 6.59 (1H, dd, J = 8.0, 2.0 Hz, H-6′), 2.82 (2H, m, H-7′), and 3.75 (1H, m, H-8′a) and 4.07 (1H, m, H-8′b). Signals at δ_H 7.07 (1H, d, J = 2.0 Hz, H-2), 6.80 (1H, d, J = 8.5 Hz, H-5) and 6.98 (1H, dd, J = 8.5, 2.0 Hz, H-6) along with signals at δ_H 7.61 (1H, br d, J = 16.0 Hz) and 6.30 (1H, br d, J = 16.0 Hz) characterized a *trans*-caffeoyl group. Additionally, the ^{1}H-NMR spectrum of **1** featured signals of anomeric protons at δ_H 4.40 (1H, d, H-1″) and δ_H 5.20 (1H, d, H-1‴) corresponding to

glucose and rhamnose units, respectively. The large coupling constant (J = 8.0 Hz) characterized the β configuration of the D-glucopyranoyl unit. The α configuration of the L-rhamnopyranosyl moiety was determined through the coupling constant J = 1.5 Hz. The ^{13}C-NMR and DEPT spectra of **1** exhibited signals of 29 carbons, including 8 carbons of the phenylethanoid group (δ_C 146.1, 144.7, 131.5, 121.3, 116.5, 116.3, 72.1 and 36.6), 9 carbons of the *trans*-caffeoyl group (δ_C 168.3, 149.8, 148.0, 146.8, 127.7, 123.2, 117.1, 115.3, and 114.7), 6 carbons of the β-glucopyranosyl unit (δ_C 103.0, 81.7, 76.2, 76.1, 70.6, and 62.4), and 6 carbons of the α-rhamnopyranosyl moiety (δ_C 104.2, 73.8, 72.3, 72.1, 70.4, and 18.4). The HMBC spectra demonstrated the interaction between proton H-1'''$_{Rha}$ (δ_H 5.20) and C-3"$_{Glc}$ (δ_C 81.7), confirming the position of the α-L-rhamnopyranosyl moiety at C-3"$_{Glc}$. The position of the β-D-glucopyranosyl moiety was determined through interactions between H-1"$_{Glc}$ (δ_H 4.40) and C-8′ (δ_C 115.3), and H-4"$_{Glc}$ (δ_H 4.94) and C-9 (δ_C 168.3). Based on spectral analysis and comparison with the reference literature [11], it can be concluded that compound **1** was acteoside, otherwise known as verbascoside.

Compound **2** was isolated as a pale yellow powder. Its molecular formula was deduced to be $C_7H_6O_3$ based on the presence of a *pseudo*-molecular peak at *m/z* 137.30 [M-H]$^-$ in conjunction with NMR spectra. The ^{1}H-NMR spectrum exhibited two pairs of proton signals corresponding to an AA'BB' system at δ_H 7.79 (2H, d, J = 9.0 Hz, H-2, H-6) and 6.82 (2H, d, J = 9.0 Hz, H-3, H-5). The ^{13}C-NMR spectrum displayed resonances for 5 carbons, with the signal at δ_C 167.1 (C-7) being indicative of the presence of a carboxyl group. Additionally, the signal at δ_C 161.5 (C-4) signified the presence of a hydroxy group on the aromatic ring. Upon comparing the NMR spectra with the reference data [12], the structure of **2** was assigned as *p*-hydroxybenzoic acid.

Compound **3** was isolated as a pale white powder. The molecular formula of **3** was proposed as $C_7H_6O_4$ based on a *pseudo*-molecular ion peak observed at *m/z* 153.25 [M-H]$^-$, in conjunction with NMR spectra. The ^{1}H-NMR spectrum of **3** exhibited signals of three aromatic protons arranged in an ABX system, appearing at δ_H 7.46 (1H, d, J = 1.5 Hz, H-2), 6.81 (1H, d, J = 8.5 Hz, H-5), and 7.44 (1H, dd, J = 8.5, 1.5 Hz, H-6). The ^{13}C-NMR spectrum revealed resonances for 7 carbons, encompassing 1 carboxylic carbon [δ_C 170.3 (C-7)], 3 non-protonated carbons [δ_C 123.3 (C-1), 146.0 (C-3), and 151.5 (C-4)], and 3 methine carbons [δ_C 115.8 (C-2), 117.8 (C-5), and 123.9 (C-6)]. This carbon arrangement corroborated the presence of the ABX system within the structure of **3**. Consequently, it was determined that **3** was protocatechuic acid, as confirmed by comparing its NMR data with published references [13].

Compound **4** was obtained as a white powder. The ^{1}H-NMR spectrum of **4** revealed resonance signals of 3 aromatic protons at δ_H 7.60 (1H, d, J = 1.5 Hz, H-2), 6.79 (1H, d, J = 8.5 Hz, H-5), and 7.53 (1H, dd, J = 8.5, 1.5 Hz, H-6) belonging to an ABX system, along with 1 resonance signal of a methoxy group at δ_H 3.89 (3H, s). The ^{13}C-NMR spectrum of **4** exhibited 8 carbon signals, including 1 carbonyl carbon (δ_C 174.5), 2 oxygen-linked aromatic carbons (δ_C 148.2 and 150.8, 1 aromatic carbon (δ_C 128.3), 3 methine carbons (δ_C 114.0, 115.4, and 124.6), and 1 methoxy group (δ_C 56.3). The position of the methoxy group was confirmed through HMBC interactions between protons H-2 (δ_H 7.60) and H-5 (δ_H 6.79) with C-3 at δ_C 148.2), while no HMBC interaction was observed between proton H-6 (δ_H 6.18) and carbon at δ_C 148.2. The comparison of the NMR spectral data of **4** with vanillic acid [14] exhibited similarity. Therefore, **4** can be identified as vanillic acid.

Compound **5** was obtained as a pale yellow powder with a molecular formula of $C_{15}H_{10}O_6$, which was determined through ESI-MS analysis, revealing a quasi-molecular ion at *m/z* 285.15 [M-H]$^-$. The ^{1}H-NMR spectrum of **5** exhibited a doublet signal pair at δ_H 6.18 (1H, d, J = 1.8 Hz) and 6.43 (1H, d, J = 1.8 Hz), characteristic of the H-6 and H-8 protons of the aromatic A-ring, and a signal pair at δ_H 8.04 (2H, d, J = 9.0 Hz) and 6.92 (2H, d, J = 9.0 Hz), characteristic of the *p*-substituted aromatic B-ring. Additionally, a signal of the OH group at C-5 (δ_H 12.47) was observed in the ^{1}H-NMR spectrum. The ^{13}C-NMR spectrum of **5** displayed 13 carbon signals, with signals at δ_C 129.5 and 115.4 being twice as intense as the others, indicating the presence of a *p*-substituted B-ring and suggesting the structure of **5** as a flavonoid. Considering these observations in

combination with the reported data [15], compound **5** was determined to be kaempferol.

Compound **6** was isolated as a pale yellow powder. The ^{1}H-NMR spectrum of **6** exhibited two doublet signals at δ_H 6.18 (1H, d, J = 2.4 Hz) and 6.40 (1H, d, J = 1.8 Hz), characteristic of the H-6 and H-8 protons of the flavonoid A-ring; three signals at δ_H 7.67 (1H, d, J = 2.4 Hz), 6.87 (1H, d, J = 8.4 Hz), and 7.53 (1H, dd, J = 8.4, 2.4 Hz), characteristic of the ABX system of the B-ring. Additionally, the proton spectrum revealed signals indicative of hydroxyl groups [δ_H 9.57 (1H, s, 3-OH), 12.47 (1H, s, 5-OH), 10.77 (1H, s, 7-OH), and 9.34 (2H, s, 3'-OH, 4'-OH)]. The ^{13}C-NMR spectrum of **6** displays 15 carbon signals, including a ketone at δ_C 175.8 (C-4), five methine carbons at δ_C 98.1 (C-6), 93.3 (C-8), 115.0 (C-2'), 115.6 (C-5'), 119.9 (C-6'), and nine non-hydrogen-bonded carbons at δ_C 146.8 (C- 2), 135.7 (C-3), 160.7 (C-5), 163.9 (C-7), 156.1 (C-9), 103.0 (C-10), 121.9 (C-1'), 145.0 (C-3'), and 147.7 (C-4'). Based on spectral analysis and by comparison with the reported literature [16], **6** was identified as quercetin.

Compound **7** was isolated as a white powder. Analysis of the ^{1}H-NMR spectrum showed the presence of an olefinic proton at δ_H 5.35 (1H, m, H-6), a hydroxymethine group at δ_H 3.52 (1H, m, H-3), along with 6 methyl groups. These included two singlet signals at δ_H 0.68 (3H, s, H-18) and 1.01 (3H, s, H-19), three doublet signals at δ_H 0.92 (3H, d, J = 6.6 Hz, H-21), 0.81 (3H, d, J = 6.6 Hz, H-26), and 0.84 (3H, d, J = 6.6 Hz, H-27), as well as a triplet signal at δ_H 0.85 (3H, t, J = 7.2 Hz, H-29). These findings collectively indicated the presence of a sterol structure. Therefore, based on a comparative analysis of the NMR spectra [17], the structure of **7** was determined to be β-sitosterol.

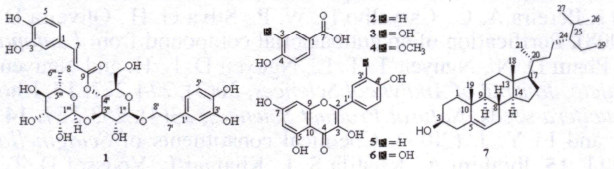

Fig. 1. Chemical structures of compounds **1-7**

Seven compounds, including a caffeoyl phenylethanoid glycoside (**1**), three phenolic acids (**2-4**), two flavonoids (**5-6**), and a sterol (**7**), were isolated from *J. subtriplinerve*, and the structures of these compounds were identified. Compounds **4** and **6** were reported for the first time in this species. These compounds have demonstrated biological activities similar to those of medicinal herbs. β-Sitosterol (**7**), a common phytosterol in herbal medicine, has shown potential pharmacological activities such as anti-inflammatory, apoptosis induction, chemoprotective or chemopreventive effects, hypocholesterolemic action, angiogenic properties, genotoxicity modulation, analgesic bioassay, anthelminthic and anti-mutagenic effects, immunomodulation, prostatic cancer treatment, antioxidant benefits, benign prostatic hyperplasia management, neuroprotection, and anti-diabetic effects [18]. Protocatechuic acid (**3**) has also been found to possess 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]. Vanillic acid (**4**) exhibited antioxidant, anti-inflammatory, immuno-stimulating, neuroprotective, hepatoprotective, cardioprotective, and antiapoptotic activities [20]. Additionally, kaempferol (**5**) and quercetin (**6**), common flavonoids in medicinal herbs, offer various pharmacological effects, including anti-inflammatory, anti-cancer, and antioxidant activities [21],[22].

Verbascoside (**1**) was the compound with the highest isolated mass among the seven compounds, suggesting that it may constitute the majority of the chemical composition of *J. subtriplinerve*'s twigs and leaves. Consequently, an HPLC-DAD method was employed to determine the verbascoside content in *J. subtriplinerve*. The calibration curve followed the equation y = 13775x - 25714 (R^2 = 0.9997), where y represents the area of the analyzed peak, and x signifies its concentration (μg/mL). With an R^2 value exceeding 0.99, it can be concluded that this method exhibits linearity (**Fig. 2**). The contents of verbascoside in the leaves, twigs, and combined twigs and leaves samples of *J. subtriplinerve* were found to be 2.77%, 0.46%, and 0.74%, respectively (**Fig. 3**).

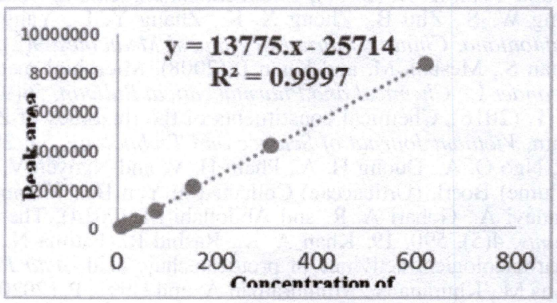

Fig. 2. The calibration curve of verbascoside

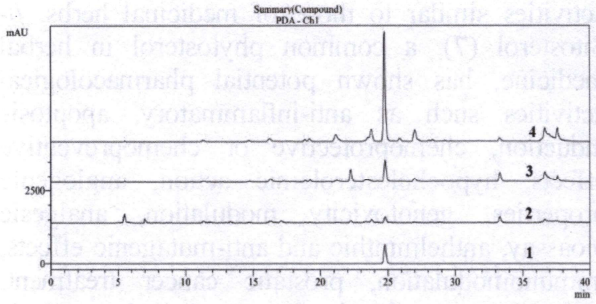

Fig. 3. HPLC-DAD chromatogram analysis of verbascoside in *J. subtriplinerve* (1-verbascoside, 2-the twigs sample, 3-the twigs and leaves sample, 4-the leaves sample)

Furthermore, verbascoside (**1**) has demonstrated remarkable biological activities, such as antibacterial, antitumor, hepatoprotective, anti-inflammatory, analgesic, and antioxidant effects [23],[24],[25],[26]. The primary activities of verbascoside characterized the principal effects and uses of *J. subtriplinerve* and represented one of the three compounds that exhibited the strongest antioxidant activity in *J. subtriplinerve* [6]. Variations in the concentration of verbascoside can impact the quality of medicinal herbs. Therefore, considering the quantitative data on verbascoside content in the herbal extract and its specific biological effects in *J. subtriplinerve*, we recommend using verbascoside as a marker for evaluating the quality of this plant.

4. Conclusion

In this study, seven compounds, including verbascoside (**1**), *p*-hydroxybenzoic acid (**2**), protocatechuic acid (**3**), vanillic acid (**4**), kaempferol (**5**), quercetin (**6**), and *β*-sitosterol (**7**) were isolated from the ethyl acetate extract of *J. subtriplinerve*. Among them, compounds **4** and **6** were the first reported in *J. subtriplinerve*. Furthermore, verbascoside (**1**) is a major compound with levels of 2.77%, 0.46%, and 0.74% in the leaves, twigs, and combined twigs and leaves samples, respectively, and can be used as a chemical marker for standardizing the quality of the twigs and leaves of *J. subtriplinerve*.

Acknowledgments: *This work was supported by the National Institute of Medicinal Materials - Ministry of Health (Vietnam) on the project (Contract No. 18/2022/HĐ-ĐTCS-PTTC) "Isolation and development of HPLC method for the quantitative determination of the main component in Jasminum subtriplinerve".*

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Journal of Medicinal Materials, 2023, Vol. 28, No. 6 (pp. 357 - 360)

THE PREPARATION OF DIOSMIN FROM HESPERIDIN *VIA* THREE STEPS

Nguyen Thi Ha[1,*], Hoang Thi Ngoc Anh[2], Nguyen Minh Khoi[1], Pham Hai Long[1], Nguyen Thi Thuy Loan[3]

[1]National Institute of Medicinal Materials, Hanoi, Vietnam; [2]Dai Nam University, Vietnam; [3]Edupharm International Joint Stock Company, Hanoi, Vietnam

*Corresponding author: nguyenthihavdl2005@gmail.com

(Received May 19th, 2023)

Summary

The Preparation of Diosmin from Hesperidin *via* three Steps

Diosmin (diosmetin 7-*O*-rutinoside), the natural flavonoids mainly found in *Citrus* fruits, is used for the treatment of hemorrhoids or chronic venous diseases. In this report, we studied the semi-synthesis of diosmin from hesperidin through three steps. In the first step, hydroxyl functional groups of hesperidin were protected, then acetyl-hesperidin (**1**) was continuously oxidized on the C-3 position, diosmin was obtained by deacetylation reaction. The structure of compounds **1-3** was confirmed by 1D, 2D-NMR, and LC-ESI MS spectrum.

Keywords: *Diosmin, Diosmetin 7-O-rutinoside, Hesperidin, Oxidation, Deacetylated.*

1. Introduction

Chronic venous insufficiency, varicose veins of the lower extremities, and hemorrhoids are quite common diseases today. Chronic venous insufficiency is a condition that occurs when the veins lose their ability to return blood to the heart due to impaired function of the venous valves of the superficial and/or deep venous systems [1]. If tiny valves in these veins are weak or damaged, blood can flow backward and pool in the veins, causing the veins to stretch or twist, that is varicose veins [2]. Same here, the veins are swollen in your anus and lower rectum, called piles [3]. In the world, 300 million people suffering from these diseases of all ages, and the annual cost is about 1 billion USD per year. About 40% of people over 50 years old in Vietnam suffer from it, and 8.2% of these are treated [4]. Many treatments are given such as the use of drugs to treat symptoms, diuretics, and oriental medicine [5]. Several naturally extracted or semi-synthetic flavonoids are like Phlebodia (600 mg diosmin), Flebosmin (300 mg diosmin), Diosmin Arrow (600 mg diosmin) and Daflon (combination of diosmin: hesperidin in a weight ratio of 450 mg: 50 mg) have been used for the treatment of hemorrhoids or chronic venous diseases. They increase flexibility, strengthen, and protect blood vessel walls, reduce capillary permeability, increase venous tone, enhance lymphatic drainage, and inhibit inflammatory mediators [6].

Diosmin is a natural flavonoid mainly present in the peel of some *Citrus* fruits, such as oranges and lemons [7]. Diosmin is a flavonoid that possesses antioxidant and anti-inflammatory properties. Although it has many interesting properties, the content of diosmin in natural plants is very low, and the cost of direct extraction is extremely high, so it is usually prepared by the semi-synthesis from hesperidin which is distributed in the genus *Citrus* is about 6% in *Citrus* fruits [8]. Therefore, the study on the semi-synthesis of diosmin from hesperidin has been of interest to scientists. In the world, many research processes were reported for semi-synthetic diosmin from hesperidin at the laboratory scale [9-12]. According to Cremades, diosmin was synthesized by using *N*-bromosuccinimide for the bromination of acetyl hesperidin in the presence of benzoyl peroxide in chloroform and obtained 44% yield [13]. In another report, hesperidin was treated with aqueous sodium hydroxide in the presence of iodine and pyridine at 100°C to obtain diosmin with a yield of 66% [10]. In this study, we reported a process for the preparation of diosmin from hesperidin. The process involves the