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PHENOLICS FROM THE BRANCHES AND LEAVES OF *EHRETIA ASPERULA* ZOLL. & MORITZI COLLECTED IN VIETNAM

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Summary

Six compounds, including 3,4-dihydroxybenzaldehyde (**1**), caffeic acid (**2**), rosmarinic acid (**3**), salvianolic acid B (**4**), benzyl *β*-D-glucopyranoside (**5**), and astragalin (**6**), were isolated from the ethyl acetate extract of *E. asperula*. Their structures were elucidated based on extensive nuclear magnetic resonance spectroscopy (NMR) data and mass spectrometry (MS), as well as by comparison with published literature. Among them, compounds **1** and **5** are reported for the first time from the *Ehretia* genus. These compounds were isolated to identify key chemical markers for evaluating the quality of *E. asperula* in Vietnam.

Keywords: *Ehretia asperula; Phenolics; Rosmarinic acid*, *Salvianolic acid B.*

1. Introduction

Ehretia asperula Zoll. & Mor. (Boraginaceae) is a valuable medicinal herb traditionally used by the Muong ethnic group in Hoa Binh province, Vietnam. This species has also been found in several Southeast Asian countries, including China, Thailand, Myanmar, and Vietnam. According to traditional medicine, *E. asperula* is known for its diuretic properties and is used to treat boils, ulcers, and for detoxification [1]. In recent years, its use in supporting the treatment of liver diseases such as hepatitis, jaundice, and especially cancer has become increasingly popular, to the extent that it is often referred to as the "cancer plant" [1],[2],[3]. In addition, it exhibits anti-inflammatory, detoxifying, cooling, liver-protective, circulation-regulating, and immune-enhancing effects [2]. Modern pharmacological studies have shown that extracts and compounds isolated from *E. asperula* possess cytotoxic and antioxidant activities [2], as well as anti-inflammatory [4], antimicrobial [2], retinal-protective [5], antidiabetic, antihypertensive, and anti-acne effects [2],[5]. Phytochemical investigations have revealed the presence of flavonoids, saponins, tannins, reducing sugars, amino acids, polysaccharides, fats, sterols, and coumarins [6]. However, these studies have not yet clarified the characteristic chemical constituents of this species, although triterpenoids [5], flavonoids [5],[7],[8], and rosmarinic acid derivatives [5],[8] have been reported.

Because of its diverse traditional and pharmacological applications, *E. asperula* is now widely used in various health care products in Vietnam. However, its quality has not been strictly controlled, and previous studies in Vietnam have not yet provided a comprehensive phytochemical profile of this species. Moreover, confusion has existed between *Ehretia asperula* and *Celastrus hindsii* in local usage. Therefore, this study aims to isolate and identify the major chemical constituents of *E. asperula* collected in Vietnam to provide chemical evidence for its quality evaluation.

2. Materials and methods

2.1. Plant materials

Branches and leaves of *Ehretia asperula* Zoll. & Mor. were collected from Phu Dong, Hanoi, in July 2025. The plant was identified by Dao Viet

Quoc (Center for Medicinal Materials Resources, NIMM). The voucher specimen (NIMM 29130) is deposited at the Center for Medicinal Materials Resources, NIMM (Hanoi, Vietnam).

2.2. General experimental procedures

NMR spectra were recorded on Bruker 500 and 600 MHz spectrometers (Bruker, Karlsruhe, Germany) using tetramethylsilane (TMS) as an internal standard. HR-ESI-MS spectra were acquired on an Agilent 6530 Accurate-Mass QTOF LC/MS system. The optical rotation values were recorded with a Jasco P-1020 polarimeter with solvent methanol. Column chromatography (CC) was performed using *silica gel* (0.040–0.063 mm, Merck), YMC ODS-A (12 nm, S-75 μm, YMC Co., Ltd., Japan), and MCI gel CHP20P (75–150 μm, Mitsubishi Chemical, Japan). Thin-layer chromatography (TLC) was carried out on pre-coated *silica gel* 60 F_{254} (0.25 mm, Merck) and *silica gel* 60 RP-18 $F_{254}S$ (0.25 mm, Merck) plates. Spots were visualized under ultraviolet (UV) light, followed by spraying with 10% sulfuric acid in ethanol and heating.

2.3. Extraction and isolation

The dried branches and leaves of *E. asperula* (10 kg) were pulverized and extracted by maceration with 70% ethanol (3 × 3 days, 1:7, kg/L). The combined extracts were filtered to remove plant residues and concentrated under reduced pressure to recover the solvent, yielding 1.31 kg of crude extract. This extract (1 kg) was suspended in hot water (1:1, w/v) and successively partitioned with solvents of increasing polarity (*n*-hexane and ethyl acetate, respectively), each applied three times at a ratio of 1:1 (v/v). Concentration of the combined layers under reduced pressure afforded 14.36 g of the *n*-hexane extract, 52.30 g of the ethyl acetate extract, and an aqueous residue.

The ethyl acetate extract (50 g) was subjected to *silica gel* CC and eluted with 100% dichloromethane (DCM), followed by gradient systems of DCM–EtOAc (70:1 → 10:1, v/v) and DCM–acetone (5:1 → 1:2, v/v), yielding ten fractions (E1–E10). Then, fraction E4 (1.4 g) was further separated by *silica gel* CC using a gradient of DCM–MeOH (30:1 → 10:1, v/v), affording three subfractions (E4.1–E4.3). Subfraction E4.2 (739.6 mg) was purified by RP-C_{18} CC with MeOH–H_2O (1:3, v/v) to yield compound **1** (10 mg). Subsequently, fraction E7 (7.01 g) was chromatographed on *silica gel* CC using a gradient of DCM–MeOH (30:1 → 1:1, v/v), yielding ten subfractions (E7.1–E7.10). Subfraction E7.3 (2.03 g) was subjected to RP-C_{18} CC with MeOH–H_2O (1:2, v/v) as the eluent, producing four smaller subfractions (E7.3.1–E7.3.4). Subfraction E7.3.1 (623.3 mg) was further purified by *silica gel* CC with EtOAc–MeOH–H_2O (8:1:1, v/v/v), yielding two compounds, **2** (1.4 mg) and **3** (65.3 mg). Then, fraction E9 (7.29 g) was separated into six subfractions (E9.1–E9.6) by MCI gel CC using a gradient of acetone–H_2O (1:3 → 1:1, v/v). Subfraction E9.4 (775.1 mg) was subjected to *silica gel* CC with EtOAc–MeOH–H_2O (15:1:1, v/v/v), yielding five smaller subfractions (E9.4.1–E9.4.5). Further purification of subfraction E9.4.2 (277.4 mg) by RP-C_{18} CC with MeOH–H_2O (1:2, v/v) afforded compound **6** (19.1 mg). Subfraction E9.2 (1.13 g) was chromatographed on RP-C_{18} CC with MeOH–H_2O (1:2, v/v), yielding six subfractions (E9.2.1–E9.2.6). Compound **5** (2 mg) was isolated from subfraction E9.2.3 (102.3 mg) via RP-C_{18} CC with MeOH–H_2O (1:2, v/v). Subfraction E9.5 (786.9 mg) was fractionated by MCI gel CC with MeOH–H_2O (1:2, v/v) to obtain two subfractions (E9.5.1 and E9.5.2). Subsequent purification of subfraction E9.5.1 (312.8 mg) by Sephadex LH-20 CC with MeOH–H_2O (1:2, v/v) yielded compound **4** (10 mg).

Compound 1: pale-yellow solid; HR-ESI-MS *m/z* 139.03919 $[M+H]^+$ (calcd. for $C_7H_7O_3^+$, 139.03897); ^{1}H-NMR (600 MHz, CD_3OD) δ_H (ppm): 7.31 (1H, d, J = 1.8 Hz, H-2), 6.91 (1H, d, J = 7.8 Hz, H-5), 7.30 (1H, dd, J = 7.8, 1.8 Hz, H-6), 9.69 (1H, s, H-7); ^{13}C-NMR (125 MHz, CD_3OD) δ_C (ppm): 130.8 (C-1), 115.4 (C-2), 147.2 (C-3), 153.7 (C-4), 116.3 (C-5), 126.4 (C-6), 193.1 (C-7).

Compound 2: yellow solid; HR-ESI-MS *m/z* 181.04977 $[M+H]^+$ (calcd. for $C_9H_9O_4^+$, 181.04954); ^{1}H-NMR (600 MHz, CD_3OD) δ_H (ppm): 7.03 (1H, d, J = 1.8 Hz, H-2), 6.77 (1H, d, J = 8.4 Hz, H-5), 6.96 (1H, dd, J = 8.4, 1.8 Hz, H-

6), 7.51 (1H, d, J = 15.6 Hz, H-7), 6.22 (1H, d, J = 15.6 Hz, H-8); ^{13}C-NMR (125 MHz, CD_3OD) δ_C (ppm): 127.8 (C-1), 115.6 (C-2), 146.8 (C-3), 149.5 (C-4), 116.6 (C-5), 122.8 (C-6), 147.0 (C-7), 115.1 (C-8), 171.0 (C-9).

Compound 3: pale-yellow powder; HR-ESI-MS m/z 359.07780 $[M-H]^-$ (calcd. for $C_{18}H_{15}O_8^-$, 359.07724); $[\alpha]_D^{20}$ = +90.9, (c = 0.66, MeOH) [$[\alpha]_D^{20}$ = +22.9, (c = 0.2, MeOH) [9]]; ^{1}H-NMR (600 MHz, CD_3OD) δ_H (ppm): 7.05 (1H, d, J = 2.4 Hz, H-2), 6.71 (1H, d, J = 8.4 Hz, H-5), 6.94 (1H, dd, J = 8.4, 1.8 Hz, H-6), 7.54 (1H, d, J = 15.6 Hz, H-7), 6.28 (1H, d, J = 15.6 Hz, H-8), 6.79 (1H, d, J = 2.4 Hz, H-2′), 6.80 (1H, d, J = 7.8 Hz, H-5′), 6.64 (1H, dd, J = 7.8, 2.4 Hz, H-6′), 3.00 (1H, dd, J = 15.0, 9.0 Hz, H-7′), 3.10 (1H, dd, J = 15.0, 3.6 Hz, H-7′), 5.16 (1H, dd, J = 9.0, 3.6 Hz, H-8′); ^{13}C-NMR (150 MHz, CD_3OD) δ_C (ppm): 127.8 (C-1), 115.2 (C-2), 146.7 (C-3), 149.5 (C-4), 116.5 (C-5), 123.0 (C-6), 147.2 (C-7), 115.1 (C-8), 168.9 (C-9), 130.3 (C-1′), 117.6 (C-2′), 146.0 (C-3′), 145.0 (C-4′), 116.3 (C-5′), 121.8 (C-6′), 38.3 (C-7′), 76.3 (C-8′), 176.5 (C-9′).

Compound 4: yellowish amorphous powder; HR-ESI-MS m/z 719.16159 $[M+H]^+$ (calcd. for $C_{36}H_{31}O_{16}^+$, 719.16066); $[\alpha]_D^{20}$ = +71.4, (c = 0.70, MeOH) [$[\alpha]_D^{18}$ = +92, (c = 0.07, EtOH) [10]]; ^{1}H-NMR (500 MHz, CD_3OD) δ_H (ppm): 6.83 (1H, d, J = 8.5 Hz, H-5), 7.16 (1H, d, J = 8.5 Hz, H-6), 7.53 (1H, d, J = 16.0 Hz, H-7), 6.21 (1H, d, J = 16.0 Hz, H-8), 6.52 (1H, d, J = 2.0 Hz, H-2′), 6.54 (1H, d, J = 8.0 Hz, H-5′), 6.31 (1H, dd, J = 8.0, 2.0 Hz, H-6′), 3.01 (2H, m, H-7′, H-7″), 3.07 (1H, dd, J = 14.5, 4.5 Hz, H-7′), 5.18 (2H, m, H-8′, H-8″), 6.74 (1H, d, J = 2.0 Hz, H-2″), 6.70 (1H, d, J = 8.0 Hz, H-5″), 6.62 (1H, dd, J = 8.0, 2.0 Hz, H-6″), 2.83 (1H, dd, J = 14.5, 10.0 Hz, H-7″), 6.77 (1H, d, J = 2.0 Hz, H-2‴), 6.75 (1H, d, J = 8.0 Hz, H-5‴), 6.65 (1H, dd, J = 8.0, 2.0 Hz, H-6‴), 5.86 (1H, d, J = 5.0 Hz, H-7‴), 4.36 (1H, d, J = 5.0, H-8‴); ^{13}C-NMR (125 MHz, CD_3OD) δ_C (ppm): 124.6 (C-1), 126.4 (C-2), 149.1 (C-3), 145.1 (C-4), 118.3 (C-5), 122.2 (C-6), 143.6 (C-7), 116.5 (C-8,), 168.0 (C-9), 128.9 (C-1′), 117.3 (C-2′), 145.9 (C-3′), 145.1 (C-4′), 116.4 (C-5′), 121.7 (C-6′), 37.9 (C-7′), 75.5 (C-8′), 172.5 (C-9′), 129.2 (C-1″), 117.6 (C-2″), 146.1 (C-3″), 145.3 (C-4″), 116.4 (C-5″), 122.1 (C-6″), 37.5 (C-7″), 74.6 (C-8″), 173.6 (C-9″), 133.6 (C-1‴), 113.3 (C-2‴), 146.6 (C-3‴), 146.8 (C-4‴), 116.5 (C-5‴), 118.4 (C-6‴), 88.3 (C-7‴), 58.0 (C-8‴), 172.3 (C-9‴).

Compound 5: pale-yellow solid; HR-ESI-MS m/z 271.11795 $[M+H]^+$ (calcd. for $C_{13}H_{19}O_6^+$, 271.11761); ^{1}H-NMR (600 MHz, CD_3OD) δ_H (ppm): 7.44 (2H, d, J = 7.2 Hz, H-2, H-6), 7.34 (2H, t, J = 7.8 Hz, H-3, H-5), 7.29 (1H, t, J = 7.2 Hz, H-4), 4.68 (1H, d, J = 12.0 Hz, H-7), 4.94 (1H, d, 12.0 Hz, H-7), 4.37 (1H, d, J = 7.8 Hz, H-1′), 3.26–3.38 (3H, m, H-2′–H-5′), 3.71 (1H, dd, J = 12.0, 6.0 Hz, H-6′), 3.91 (1H, dd, J = 12.0, 2.4 Hz, H-6′); ^{13}C-NMR (150 MHz, CD_3OD) δ_C (ppm): 139.1 (C-1), 129.3 (C-2, C-6), 129.2 (C-3, C-5), 128.7 (C-4), 71.7 (C-7), 103.3 (C-1′), 75.2 (C-2′), 78.1 (C-3′), 71.8 (C-4′), 78.0 (C-5′), 62.8 (C-6′).

Compound 6: yellow powder; HR-ESI-MS m/z 449.10843 $[M+H]^+$ (calcd. for $C_{21}H_{21}O_{11}^+$, 449.10784); ^{1}H-NMR (600 MHz, CD_3OD) δ_H (ppm): 6.18 (1H, d, J = 1.8 Hz, H-6), 6.36 (1H, s, H-8), 8.06 (2H, d, J = 8.4 Hz, H-2′, H-6′), 6.90 (2H, d, J = 9.0 Hz, H-3′, H-5′), 5.19 (1H, d, J = 7.2 Hz, H-1″), 3.47 (1H, dd, J = 9.0, 7.2 Hz, H-2″), 3.44 (1H, t, J = 9.0 Hz, H-3″), 3.33 (1H, m, H-4″), 3.22 (1H, m, H-5″), 3.55 (1H, dd, J = 12.0, 5.4 Hz, H-6″), 3.70 (1H, dd, J = 12.0, 2.4 Hz, H-6″); ^{13}C-NMR (150 MHz, CD_3OD) δ_C (ppm): 158.9 (C-2), 135.4 (C-3), 179.2 (C-4), 162.9 (C-5), 100.7 (C-6), 168.2 (C-7), 95.3 (C-8), 158.6 (C-9), 105.1 (C-10), 122.8 (C-1′), 132.2 (C-2′, C-6′), 116.1 (C-3′, C-5′), 161.6 (C-4′), 104.4 (C-1″), 75.7 (C-2″), 78.0 (C-3″), 71.3 (C-4″), 78.3 (C-5″), 62.6 (C-6″).

2.4. HPLC analysis of E. asperula

The HPLC analysis was performed using a Shimadzu system equipped with a Nexera X2 LC-30AD solvent delivery unit, an Autosampler SIL-30AC, and an SPD-M20A photodiode array detector. Separation was carried out on an Agilent C_{18} column (250 × 4.6 mm, 5 μm). The mobile phase consisted of acetonitrile (ACN) and water, with a gradient elution program as follows: 0–60 min, 5–50% ACN. The flow rate was 1.0 mL/min, with detection at 330 and 210 nm.

For the sample preparation, 1.0 g of powdered branches and leaves was placed in a 100 mL round-bottomed flask, followed by the addition of 25 mL of 70% ethanol. The mixture was refluxed for 30 minutes, then cooled to room temperature and filtered through a 0.45 μm membrane filter before HPLC analysis.

For the reference standard preparation, 1 mg of each isolated compound was accurately weighed and dissolved in 5 mL of 70% ethanol.

3. Results and discussion

3.1. Structural elucidation of compounds isolated from E. asperula

Six compounds (**1**–**6**, Fig. 1) were isolated from the ethyl acetate fraction of *E. asperula*, and their structures were elucidated based on spectroscopic data and comparison with published literature.

Compound **1** was isolated as a pale-yellow solid. Its molecular formula was established as $C_7H_6O_3$ by HR-ESI-MS at *m/z* 139.03919 $[M+H]^+$ (calcd. for $C_7H_7O_3^+$, 139.03897). The ^{1}H-NMR spectrum showed three ABX-type aromatic proton signals at δ_H 7.31 (1H, d, J = 1.8 Hz, H-2), 6.91 (1H, d, J = 7.8 Hz, H-5), and 7.30 (1H, dd, J = 7.8, 1.8 Hz, H-6). In addition, a signal corresponding to an aldehyde proton was observed at δ_H 9.69 (1H, s, H-7). The ^{13}C-NMR spectrum displayed seven carbon signals, including three methine carbons (δ_C 115.4, 116.3, and 126.4), three non-protonated carbons (δ_C 130.8, 147.2, and 153.7), and one carbon at δ_C 193.1 (C-7) characteristic of an aldehyde group. Based on these spectroscopic data and comparison with the literature [11], compound **1** was identified as 3,4-dihydroxybenzaldehyde.

Compound **2** was obtained as a yellow solid. The molecular formula, $C_9H_8O_4$, of **2** was determined based on its HR-ESI-MS ion peak at *m/z* 181.04977 $[M+H]^+$ (calcd. for $C_9H_9O_4^+$, 181.04954). Similar to compound **1**, the ^{1}H-NMR spectrum of compound **2** also exhibited an ABX spin system corresponding to three aromatic protons at δ_H 7.03 (1H, d, J = 1.8 Hz, H-2), 6.77 (1H, d, J = 8.4 Hz, H-5), and 6.96 (1H, dd, J = 8.4, 1.8 Hz, H-6). In addition, two downfield doublets at δ_H 6.22 (1H, d, H-8) and 7.51 (1H, d, H-7) with a large coupling constant (J = 15.6 Hz) indicated the presence of a *trans*-configured double bond. The ^{13}C-NMR spectrum displayed nine carbon signals, including five methine carbons (δ_C 115.1, 115.6, 116.6, 122.8, and 147.0), three non-protonated carbons (δ_C 127.8, 146.8, and 149.5), and a resonance at δ_C 171.0 (C-9), characteristic of a carboxylic acid group. By comparison with the literature [12] and based on the above spectral evidence, compound **2** was identified as caffeic acid.

Compound **3** was purified as a yellow solid, and its molecular formula, $C_{18}H_{16}O_8$, was determined by HR-ESI-MS (*m/z* 359.07780 $[M–H]^–$; calcd. for $C_{18}H_{15}O_8^–$, 359.07724). The ^{1}H-NMR spectrum showed 11 proton signals. In the aliphatic region, two doublets of doublets at δ_H 3.10 (1H, dd, J = 15.0, 3.6 Hz, H-7′) and 3.00 (1H, dd, J = 15.0, 9.0 Hz, H-7′) were assigned to a methylene group, while a hydroxymethine proton at δ_H 5.16 (1H, dd, J = 9.0, 3.6 Hz) corresponded to H-8′. Six aromatic protons forming two ABX spin systems appeared at δ_H 7.05 (1H, d, J = 2.4 Hz, H-2), 6.71 (1H, d, J = 8.4 Hz, H-5), 6.94 (1H, dd, J = 8.4, 1.8 Hz, H-6), and 6.79 (1H, d, J = 2.4 Hz, H-2′), 6.79 (1H, d, J = 7.8 Hz, H-5′), 6.64 (1H, dd, J = 7.8, 2.4 Hz, H-6′). Additionally, two downfield doublets at δ_H 6.28 (1H, d, J = 15.6 Hz, H-8) and 7.54 (1H, d, J = 15.6 Hz, H-7) indicated the presence of a *trans*-configured double bond, as observed in caffeic acid. The ^{13}C-NMR spectra exhibited 18 carbon signals. The resonances between δ_C 115.1 and 149.5 corresponded to aromatic carbons forming two ABX systems. Two carbonyl carbons were observed at δ_C 176.5 and 168.9, attributed to the carboxylic and ester groups, respectively. These spectroscopic data, together with comparison to the reported values [13], established the structure of compound **3** as rosmarinic acid.

Compound **4** was obtained as a yellowish amorphous powder. Its molecular formula, $C_{36}H_{30}O_{16}$, was established based on HR-ESI-MS (*m/z* 719.16159 $[M+H]^+$; calcd. for $C_{36}H_{31}O_{16}^+$, 719.16066). The ^{1}H-NMR spectral data of **4** showed signals corresponding to three ABX-type aromatic rings [I: δ_H 6.52 (1H, d, J = 2.0 Hz, H-

2′), 6.54 (1H, d, J = 8.0 Hz, H-5′), and 6.31 (1H, dd, J = 8.0, 2.0 Hz, H-6′); II: δ_H 6.74 (1H, d, J = 2.0 Hz, H-2″), 6.70 (1H, d, J = 8.0 Hz, H-5″), and 6.62 (1H, dd, J = 8.0, 2.0 Hz, H-6″); III: δ_H 6.77 (1H, d, J = 2.0 Hz, H-2‴), 6.75 (1H, d, J = 8.0 Hz, H-5‴), and 6.65 (1H, dd, J = 8.0, 2.0 Hz, H-6‴)], an AB-type aromatic ring [δ_H 6.83 (1H, d, J = 8.5 Hz, H-5) and 7.16 (1H, d, J = 8.5 Hz, H-6)], and two *trans*-olefinic protons [δ_H 7.53 (1H, d, J = 16.0 Hz, H-7) and 6.21 (1H, d, J = 16.0 Hz, H-8)]. Additionally, three oxymethine protons were observed at δ_H 5.18 (2H, m, H-8′, H-8″) and 5.86 (1H, d, J = 5.0 Hz, H-7‴), along with four methylene protons at δ_H 2.83 (1H, dd, J = 14.5, 10.0 Hz, H-7″), 3.01 (2H, m, H-7′, H-7″), and 3.07 (1H, dd, J = 14.5, 4.5 Hz, H-7′), and one methine proton at δ_H 4.36 (1H, d, J = 5.0, H-8‴). The ^{13}C-NMR spectrum displayed 36 carbon signals, including four carbonyl carbons, twenty-four aromatic carbons, two olefinic carbons, three oxymethines, two methylenes, and one methine. The coupling constant ($^3J_{7‴,8‴}$ = 5.0 Hz) in the benzofuran ring suggested a *trans* configuration. On the basis of spectroscopic data, together with comparison with the literature [14], compound **4** was determined as salvianolic acid B.

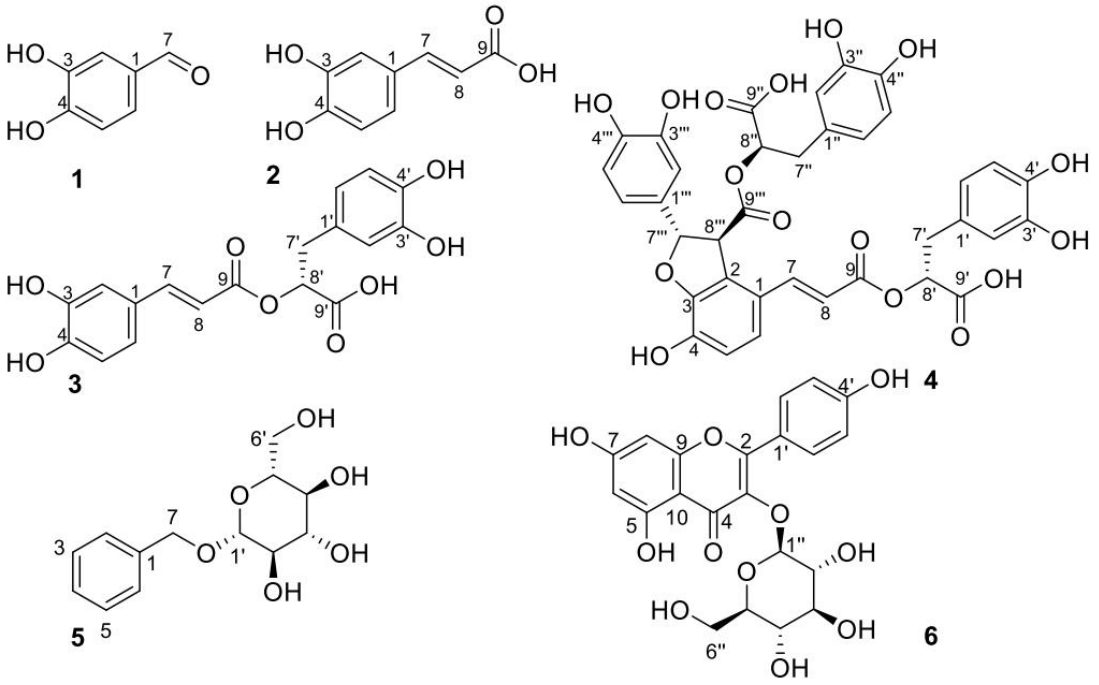

Fig. 1. Chemical structures of isolated compounds (1–6) from *E. asperula*

Compound **5** was isolated as a pale-yellow solid, with the molecular formula $C_{13}H_{18}O_6$, as determined by HR-ESI-MS (*m/z* 271.11795 $[M+H]^+$; calcd. for $C_{13}H_{19}O_6^+$, 271.11761). The ^{1}H-NMR spectrum displayed six aromatic proton signals at δ_H 7.44 (2H, br d, J = 7.2 Hz), 7.34 (2H, t, J = 7.8 Hz), and 7.29 (1H, t, J = 7.2 Hz), together with two methylene protons at δ_H 4.68 (1H, d, J = 12.0 Hz) and 4.94 (1H, d, J = 12.0 Hz), suggesting the presence of a phenyl group. In addition, an anomeric proton signal was observed at δ_H 4.37 (1H, d, J = 7.8 Hz), along with resonances of hydroxymethylene protons at δ_H 3.71 (1H, dd, J = 12.0, 6.0 Hz) and 3.91 (1H, dd, J = 12.0, 2.4 Hz), and hydroxymethine protons at δ_H 3.26–3.38 (m), indicating the presence of a β-glucopyranosyl moiety. The ^{13}C-NMR spectrum showed 13 carbon signals, including seven carbons corresponding to the phenyl group at δ_C 139.1, 129.3 (×2C), 129.2 (×2C), 128.7, and 71.7, and six

carbons belonging to the β-glucopyranosyl unit at δ_C 103.3, 78.1, 78.0, 75.2, 71.8, and 62.8. All spectral data were consistent with those reported for benzyl-*O*-β-D-glucopyranoside [15].

Compound **6**, a yellow powder, was assigned the molecular formula $C_{21}H_{20}O_{11}$ based on HR-ESI-MS (*m/z* 449.10843 $[M+H]^+$; calcd. for $C_{21}H_{21}O_{11}^+$, 449.10784) and NMR data. The NMR data indicated that compound **6** is a flavonoid glycoside. The ^{1}H-NMR spectrum showed four aromatic protons forming an AA′BB′ system of ring B at δ_H 8.06 (2H, d, J = 8.4 Hz) and 6.90 (2H, d, J = 9.0 Hz), together with two *meta*-coupled protons of ring A at δ_H 6.36 (1H, s) and 6.18 (1H, d, J = 1.8 Hz), suggesting a kaempferol skeleton. The presence of a β-glucopyranosyl moiety was confirmed by the anomeric proton signal at δ_H 5.19 (1H, d, J = 7.2 Hz) and the resonances of hydroxymethylene protons at δ_H 3.55 (1H, dd, J = 12.0, 5.4 Hz) and 3.70 (1H, dd, J = 12.0, 2.4 Hz), together with hydroxymethine protons at δ_H 3.47 (1H, dd, J = 9.0, 7.2 Hz), 3.44 (1H, t, J = 9.0 Hz), 3.33 (1H, m), and 3.22 (1H, m). The ^{13}C-NMR spectrum exhibited 21 carbon signals, including 15 carbons of the aglycone and 6 carbons of the sugar moiety. The location of the sugar unit at C-3 was determined based on the chemical shift changes at C-2 ($\Delta\delta$ = +10.8 ppm), C-3 ($\Delta\delta$ = −1.7 ppm), and C-4 ($\Delta\delta$ = +1.8 ppm) of ring C compared with those of kaempferol [16]. Comparison of the NMR data with those reported in the literature [17] confirmed the structure of compound **6** as kaempferol 3-*O*-glucoside (astragalin).

3.2. HPLC qualitative analysis of isolated compounds from E. asperula

The HPLC-DAD method was employed to qualitatively analyze six isolated compounds (**1**–**6**) in the 70% ethanol extract of the branches and leaves of *E. asperula*. The analytical conditions were described in Section 2.4, and the chromatographic results are shown in Fig. 2.

The chromatogram revealed that four compounds, including 3,4-dihydroxybenzaldehyde, caffeic acid, benzyl β-D-glucopyranoside, and astragalin, appeared with relatively low signal intensities in the *E. asperula* sample. In contrast, rosmarinic acid and salvianolic acid B exhibited the most prominent peaks in the chromatogram, suggesting that these may be the major phenolic constituents in the branches and leaves of *E. asperula*.

The isolated compounds are widely distributed in nature and have been reported to exhibit diverse biological activities. Among them, 3,4-dihydroxybenzaldehyde exerts neuroprotective effects by attenuating cerebral ischemia/reperfusion injury, restoring mitochondrial integrity, and improving neurological function and ATP levels [18]. It also demonstrates potent anti-allergic activity by suppressing mast cell degranulation and inhibiting the release of cytokines such as IL-4, IL-6, and TNF-α in allergic models [19]. Similarly, caffeic acid is well known for its antioxidant, antimicrobial, anticancer, and anti-inflammatory properties [20]. It displays cardioprotective potential by modulating enzymes such as ACE and AChE and shows antidiabetic activity through inhibition of α-amylase and α-glucosidase, thereby contributing to glucose homeostasis in type 2 diabetes [21]. Rosmarinic acid (RA) exhibits a broad spectrum of biological effects, including antioxidant, anti-inflammatory, antiviral, and neuroprotective activities. It has also been shown to improve cognitive performance and ameliorate kidney disorders such as mesangioproliferative glomerulonephritis [22]. Salvianolic acid B is clinically relevant for the treatment of cardiovascular and cerebrovascular diseases owing to its potent antioxidant, antiplatelet, and antithrombotic properties [23]. Moreover, it demonstrates antidiabetic effects by lowering blood glucose and insulin resistance while promoting glycogen synthesis in diabetic models [24]. Benzyl β-D-glucopyranoside was isolated from this genus for the first time, and its pharmacological properties have not yet been investigated. In contrast, astragalin is a well-

studied natural flavonoid with multiple therapeutic activities, including anti-inflammatory, antioxidant, neuroprotective, cardioprotective, and anticancer effects. It alleviates inflammatory conditions such as asthma and arthritis by inhibiting NF-κB signaling and suppressing cytokines like TNF-α and IL-6 [25].

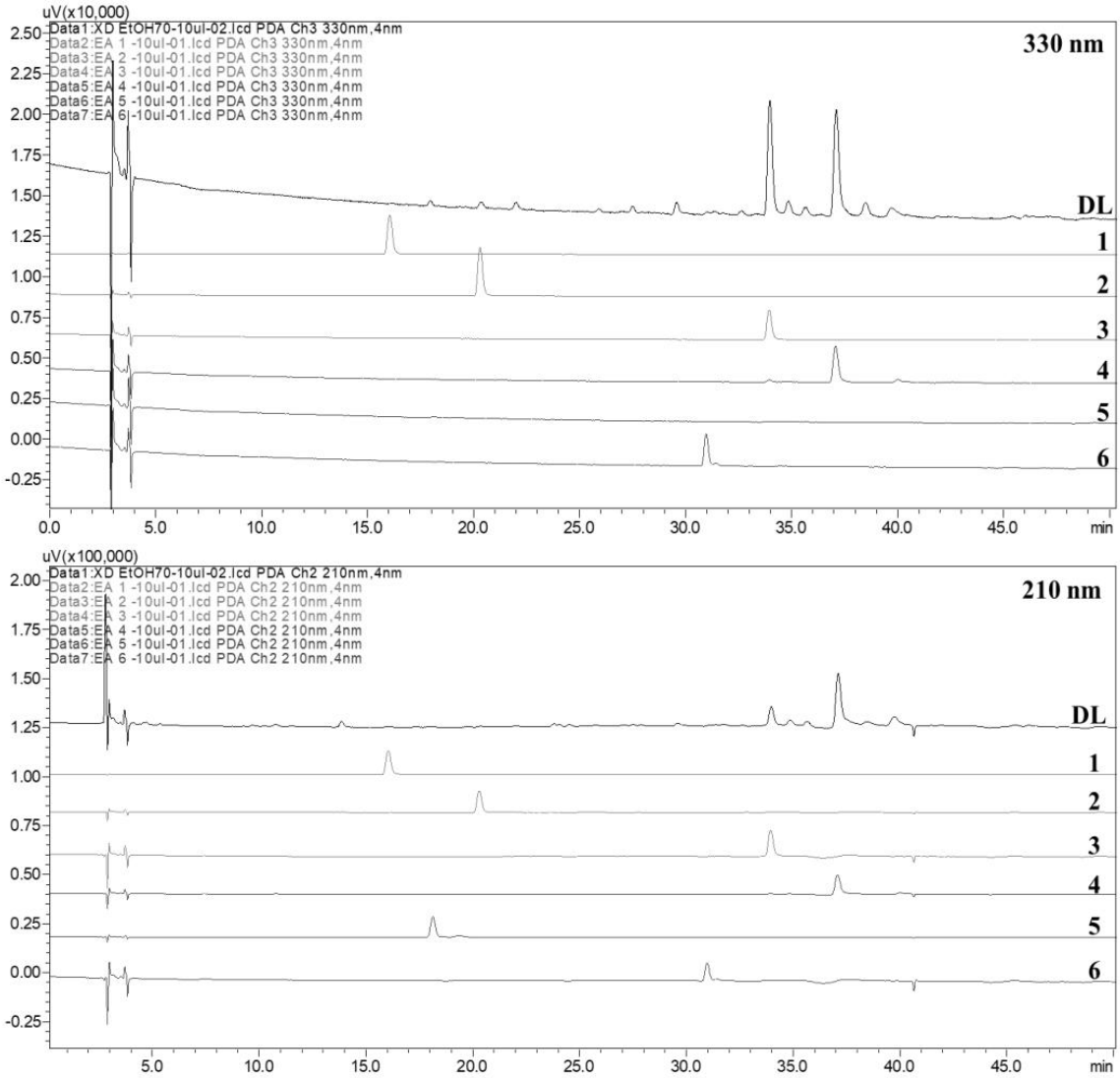

Fig. 2. HPLC chromatogram fingerprint of *E. asperula*
*(**DL-** E. asperula sample; **1-** 3,4-dihydroxybenzaldehyde; **2-** caffeic acid; **3-** rosmarinic acid; **4-** salvianolic acid B; **5-** benzyl β-D-glucopyranoside; **6-** astragalin)*

Recent studies have demonstrated that rosmarinic acid and salvianolic acid B are the major polyphenolic constituents of *E. asperula* leaves. Le Thi Tam et al. (2021) quantified rosmarinic acid at 7.782 ± 0.221 mg/g and salvianolic acid B at 13.439 ± 0.375 mg/g, the latter being the most abundant compound among the analyzed constituents [5]. In addition, Nguyen

Thi Thu Hang (2024) reported the presence of rosmarinic acid in both *in vitro* and *ex vitro* cultured leaves, with contents of 0.019% and 0.700%, respectively [26]. Fingerprint chromatographic analysis further confirmed that rosmarinic acid and salvianolic acid B are the major compounds in *E. asperula*. According to WHO guidelines [27], the general requirements for markers include clear identity, specificity, and selectivity when using the specified analytical method(s). They should be present in traceable quantity for identification or in sufficient quantity for assay. Markers should also be easily obtained, stable under the specified storage conditions, and easily detected and quantified using analytical methods. These findings indicate that rosmarinic acid and salvianolic acid B are characteristic and predominant components, and therefore were proposed as marker compounds for the establishment of an in-house standard for this species.

4. Conclusion

In summary, six compounds were isolated from the branches and leaves of *E. asperula*, and their structures were elucidated by spectroscopic analyses, including ESI-MS, 1D-NMR, and 2D-NMR, and comparison with published data. These compounds were identified as 3,4-dihydroxybenzaldehyde (**1**), caffeic acid (**2**), rosmarinic acid (**3**), salvianolic acid B (**4**), benzyl *β*-D-glucopyranoside (**5**), and astragalin (**6**). Among them, compounds **1** and **5** were isolated for the first time from the *Ehretia* genus. Furthermore, rosmarinic acid (**3**) and salvianolic acid B (**4**) are proposed as potential chemical markers for the quality evaluation of *E. asperula*. These findings expand the chemical profile of *E. asperula* and provide valuable information for future phytochemical and pharmacological studies on this species.

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