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IRIDOID GLYCOSIDES, CHLOROGENIC ACID, AND ITS ACYL DERIVATIVES FROM ROOTS OF *DIPSACUS ASPER* WALL EX DC. IN VIETNAM

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Summary

Iridoid Glycosides, Chlorogenic Acid, and its Acyl Derivatives from Roots of *Dipsacus asper* Wall ex DC. in Vietnam

From the ethyl acetate fraction of *Dipsacus asper* Wall. ex DC. (DA) roots, five compounds were isolated, including sweroside (**1**), 6'-*O*-acetylsweroside (**2**), chlorogenic acid (**3**), 4,5-di-*O*-caffeoyl quinic acid (**4**), and methyl 4,5-di-*O*-caffeoylquinate (**5**). Their structures were verified through the analysis of NMR and MS spectral data in comparison with those published in the literatures. Compound **2** was isolated for the first time from *D. asper* Wall.

Keywords: *Dipsacus asper*, *Tuc doan nhon*, *sweroside, Chlorogenic acid, Quinic acid.*

1. Introduction

Dipsacus asper Wall. ex DC. (synonym: *Dipsacus inermis* Wall.) (DA) is a perennial herb belonging to the Dipsacaceae family and is naturally distributed in mountainous regions of China, Korea, and Japan. In Vietnam, it grows naturally in the northwestern provinces such as Lai Chau, Lao Cai, and Ha Giang [1]. The root of this plant is commonly used in traditional medicine to treat various conditions such as back pain, knee fatigue, and pregnancy [2]. Modern pharmacological studies have also shown that DA root extracts exhibit diverse bioactivities, including anti-osteoporosis, neuroprotective, anti-

uterine contraction, hepatoprotective, anti-inflammatory, antioxidant, cardioprotective [3],[4],[5],[6],[7]. Studies on the chemical composition of the roots have shown the presence of various types of compounds, including iridoid, triterpenoid saponins, alkaloids, phenolic compounds, lignin, and fatty acids [8],[9]. Although DA is distributed and used in Vietnam as traditional medicine, to our best knowledge, there have been no studies on the chemical composition of this plant in Vietnam until now. In this study, we describe the isolation and structural elucidation of compounds from the roots of DA grown in Vietnam.

2. Experimental

2.1. Plant materials

The plant materials were collected from Lai Chau province, Vietnam, in April 2022, and identified as DA by MSc. Phan Van Truong. A voucher specimen with the number NIMM-19206 has been deposited at the Center for Medicinal Plant Resources, National Institute of Medicinal Materials (NIMM). The samples were prepared by chopping, dried in the shade, then in an oven at 50°C, ground to raw powder, and stored in sealed plastic bags in a cool, dry place away from direct sunlight.

2.2. General experimental procedures

The NMR data were obtained using Bruker AM 500 MHz and 600 MHz FT-NMR spectrometers with TMS as the internal standard. The electrospray ionization mass spectrometry (ESI-MS) spectra were recorded on an Agilent 1260 Series Single Quadrupole LC/MS Systems (Agilent, USA). Optical rotations were measured on a Jasco P-2000 Polarimeter serial A060161232. Column chromatography was performed using *silica gel* (Merck, Darmstadt, Germany particle size 63 - 200 μm or 40 - 63 μm), reversed-phase YMC ODS-AA12S75 (75 μm), and Sephadex® LH-20 (GE Healthcare). High Performanee Liquid Chromatography (HPLC) was carried out on a preparative HPLC Shimadzu LC20-AP with a Supelco Analytical Discovery® HS C_{18} (250 × 21.2 mm, 10 μm) column. Thin-layer chromatography (TLC) was conducted on DC-Alufolien 60 F_{254} pre-coated plates (Merck 1.05715). The spots were detected by UV radiation or by spraying with 10% H_2SO_4/EtOH followed by heating or 5% $FeCl_3$/EtOH.

2.3. Extraction and isolation

The dried roots of DA (4.5 kg) were extracted three times with 75% ethanol (40 L × 3 times, 4 days/time) at room temperature. The resulting ethanol extract was filtered, and solvents were removed under reduced pressure. The remaining extract was suspended in water and fractionated with dichloromethane (DCM), ethyl acetate, and *n*-butanol. The corresponding dichloromethane (50.12 g), ethyl acetate (79.5 g), and *n*-butanol (250 g) fractions were obtained after evaporation of the solvents in vacuum conditions.

The ethyl acetate fraction (65 g) was fractionated using a *silica gel* column eluted with a gradient solvent system of DCM-MeOH (100:0-0:100, v/v) to give 16 fractions (TDE1-TDE16). Fraction TDE7 (3 g) was subjected to a *silica gel* column and eluted with a gradient solvent system of DCM-MeOH (100:1-0:100, v/v) to yield 11 sub-fractions (TDE7.1-TDE7.11). Sub-fraction TDE7.11 (0.1 g) was then subjected to preparative HPLC using a solvent system of acetonitrile and 0.01% TFA in water (0 - 10' min: 20 - 25% ACN, 10 - 40' min: 25% - 40% ACN, 8 mL/min, UV 210 nm and 254 nm) to give compound **1** (10.3 mg, t_R= 12.8 min). Fraction TDE11 (1.42 g) was subjected to a *silica gel* column and eluted with a gradient solvent system of DCM-MeOH (100:0-0:100, v/v) to yield 5 sub-fractions (TDE11.1-TDE11.5). Sub-fraction TDE11.1 (0.85 g) was further fractionated using column chromatography on Sephadex LH-20 with MeOH and yielded 4 sub-fractions (TDE11.1.1-TDE11.1.4). Sub-fraction TDE11.1.1 (65 mg) was then subjected to preparative HPLC using a solvent system of acetonitrile and 0.01% TFA in water (0-15' min: 20%-30% ACN, 15-50' min: 30%-45% ACN, 8 mL/min, UV 190 nm and 254 nm) to obtain compound **2** (12.2 mg, t_R = 22.8 min). Sub-fraction TDE11.1.4 (69.8 mg) was subjected to preparative HPLC with a solvent system of acetonitrile and 0.01% TFA in water (0 - 10' min: 20% - 35% ACN, 10-45' min: 35% - 45% ACN, 8 mL/min, UV 190 nm and 254 nm) which resulted in the isolation of compounds **4** (10.2 mg, t_R = 27.3 min) and **5** (14.5 mg, t_R = 23.3 min). Lastly, fraction TDE16 (5.21 g) was subjected to *silica gel* column chromatography and eluted with a gradient solvent system of EtOAc-MeOH-H_2O (100:1:0.1-0:100:0.1, v/v/v) to obtain 10 sub-fractions (TDE16.1-TDE16.10). Sub-fraction TDE16.5 (1.97 g) was further separated using *silica gel* column chromatography and eluted with a gradient solvent system of EtOAc-MeOH (100:0-0:100, v/v) to give 6 sub-fractions (TDE16.5.1-TDE16.5.6). Sub-fraction TDE16.5.3 (0.4 g) was then subjected to column chromatography on Sephadex LH-20 using MeOH as the eluent to obtain 4 sub-fractions (TDE16.5.3.1-

TDE16.5.3.3). From sub-fraction TDE16.5.3.1 (52 mg), a precipitate was obtained upon filtration and recrystallization in methanol, yielding compound **3** (14 mg).

Compound 1 (sweroside)*: Colorless oil;* $[\alpha]^{22}$ = -6.12 (*c* = 0.01, MeOH). ESI-MS: *m/z* 359.05 $[M+H]^+$; ^{1}H-NMR (600 MHz, CD_3OD), δ_H (ppm): 5.57 (1H, d, *J* = 1.8 Hz, H-1), 7.61 (1H, d, *J* = 2.4 Hz, H-3), 3.17 (1H, m, H-5), 1.79 (1H, m, Ha-6), 1.72 (1H, ddd, *J* = 13.8, 12.6, 4.2 Hz, Hb-6), 4.47 (1H, m, Ha-7), 4.39 (1H, ddd, *J* = 12.0, 11.4, 2.4 Hz, Hb-7), 5.58 (1H, dt, *J* = 17.4, 10.2 Hz, H-8), 2.72 (1H, ddd, *J* = 9.6, 5.4, 1.2 Hz, H-9), 5.31 (1H, dd, *J* = 17.4, 1.2 Hz, Ha-10), 5.29 (1H, dd, *J* = 10.2, 1.8 Hz, Hb-10). *Glc*: 4.71 (1H, d, *J* = 8.4 Hz, H-1'), 3.22 (1H, dd, *J* = 9.6, 8.4 Hz, H-2'), 3.38 (1H, t, *J* = 9.0 Hz, H-3'), 3.32-3.36 (2H, m, H-4', H-5'), 3.90 (1H, dd, *J* = 12.0, 2.4 Hz, Ha-6'), 3.68 (1H, dd, *J* =12.0, 6.0 Hz, Hb-6'). ^{13}C-NMR (150 MHz, CD_3OD), δ_C (ppm): 99.7 (C-1), 153.9 (C-3), 106.0 (C-4), 28.4 (C-5), 25.9 (C-6), 69.7 (C-7), 133.3 (C-8), 43.8 (C-9), 120.8 (C-10), 168.5 (C-11), *Glc*: 99.7 (C-1'), 74.7 (C-2'), 77.9 (C-3'), 71.5 (C-4'), 78.4 (C-5'), 62.7 (C-6').

Compound 2 (6'-*O*-acetylsweroside)*: Colorless oil*; ESI-MS: *m/z* 362.7 $[M-2H_2O-H]^-$. ^{1}H-NMR (600 MHz, CD_3OD), δ_H (ppm): 5.41 (1H, d, J = 1.8 Hz, H-1), 7.60 (1H, s, H-3), 3.15-3.18 (1H, m, H-5), 1.79-1.82 (1H, m, Ha-6), 1.71-1.76 (1H, m, Hb-6), 4.48-4.49 (1H, m, Ha-7), 4.37-4.39 (1H, m, Hb-7), 5.54-5.60 (1H, m, H-8), 2.71-2.74 (1H, m, H-9), 5.35 (1H, dd, *J* = 16.8, 1.2 Hz, Ha-10), 5.29 (1H, dd, *J* =10.2, 1.2 Hz, Hb-10), *Glc*: 4.70 (1H, d, *J* = 7.8 Hz, H-*1'*), 3.20-3.22 (1H, m, H-2'), 3.52-3.55 (1H, m, H-3'), 3.37-3.42 (1H, m, H-4'), 3.68-3.70 (1H, m, H-5'), 4.41-4.43 (1H, m, Ha-6'), 4.25 (1H, dd, *J* = 12.0, 6.0 Hz, Hb-6'), 2.08 (3H, s, CH_3); ^{13}C-NMR (150 MHz, CD_3OD), δ_C (ppm): 98.3 (C-1), 153.8 (C-3), 106.1 (C-4), 28.4 (C-5), 25.9 (C-6), 69.7 (C-7), 133.3 (C-8), 43.9 (C-9), 120.9 (C-10), 168.4 (C-11), Glc: 99.9 (C-1'), 74.6 (C-2'), 75.6 (C-3'), 71.4 (C-4'), 77.7 (C-5'), 64.6 (C-6'), 172.7 (6′-COOCH3), 20.7 (6′-COOCH3).

Compound 3 (chlorogenic acid)*: White power*; mp. 208-211°C. ESI-MS: *m/z* 393.20 $[M+K]^+$; ^{1}H-NMR (600 MHz, DMSO-d_6), δ_H *(ppm):* 1.77-1.84 (2H, m, H-2), 3.90 (1H, d, *J* = 2.4 Hz, H-3), 3.47 (1H, m, H-4), 5.16 (1H, m, H-5), 1.98 (1H, dd, *J* = 2,4; 13.8 Hz, Ha-6), 1.60 (1H, d, J = 13.8 Hz, Hb-6), 7.04 (1H, s, H-*2'*), 6.72 (1H, d, *J* = 7.8 Hz, H-5'), 6.93 (1H, d, *J* = 7.8 Hz, H-6'), 7.45 (1H, d, *J* = 15.6 Hz, H-7'), 6.22 (1H, d, *J* = 15.6 Hz, H-8'); ^{13}C-NMR (150 MHz, DMSO-d_6), δ_C (ppm):75.3 (C-1), 39.8 (C-2), 71.6 (C-3), 73.3 (C-4), 71.5 (C-5), 38.1 (C-6), 176.5 (C-7), 125.1 (C-1'), 114.7 (C-2'), 146.1 (C-3'), 149.2 (C-4'), 115.9 (C-5'), 121.2 (C-6'), 144.8 (C-7'), 114.3 (C-8'), 166.4 (C-9').

Compound 4 (4,5-di-*O*-caffeoylquinic acid)*: Colorless oil;* ESI-MS: *m/z* 515.6 $[M-H]^-$.^{1}H-NMR (600 MHz, CD_3OD), δ_H (ppm): 2.11 (1H, m, Hb-2), 2.24-2.26 (1H, m, Ha-2), 4.37 (1H, m, H-3), 5.12 (1H, dd, *J* = 3.6, 7.8 Hz, H-4), 5.57 (1H, m, H-5), 2.27-2.36 (2H, m, 2H-6), 6.77-6.78 (2H, m, H-5', H-5"), 6.92-6.96 (2H, m, H-6', H-6"), 7.52 (1H, d, *J* = 15.6 Hz, H-7'), 6.18 (1H, d, *J* = 15.6 Hz, H-8'), 7.02-7.05 (2H, dd, *J* =1.8 Hz, H-2', H-2"), 6.77-6.78 (2H, m, H-5', H-5"), 7.62 (1H, d, *J* = 15.6 Hz, H-7"), 6.31 (1H, d, *J* = 15.6 Hz, H-8"); ^{13}C-NMR (150 MHz, CD_3OD), δ_C (ppm): 75.8 (C-1), 38.5 (C-2), 69.1 (C-3), 75.2 (C-4), 69.3 (C-5), 38.5 (C-6), 174.8 (C-7), 127.7 (C-1'), 115.2 (C-2', C-2"), 146.8 (C-3', C-3"), 149.7 (C-4'), 116.5 (C-5', C-5"), 123.1 (C-6', C-6"), 147.6 (C-7'), 114.6 (C-8'), 167.9 (C-9'), 127.6 (C-1"), 149.8 (C-4"), 123.1 (C-6"), 147.7 (C-7"), 114.8 (C-8"), 168.5 (C-9").

Compound 5 (methyl 4,5-di-*O*-caffeoylquinate)*: Yellow oil;* ESI-MS: *m/z* 529.0 $[M-H]^-$; ^{1}H-NMR (600 MHz, CD_3OD), δ_H (ppm): 2.27 (2H, m, H-2), 4.37 (1H, m, H-3), 5.12 (1H, m, H-4), 5.56 (1H, m, H-5), 2.35 (1H, m, H-6a), 2.11 (1H, dd, *J* =13,8, 6,0 Hz, H-6b), 3.74 (3H, s, $COOCH_3$), 7.05 (2H, d, *J* = 1.8 Hz, H-2', H-2"), 6.77 (2H, d, *J* = 7.8 Hz, H-5', H-5"), 6.94 (2H, m, H-6', H-6"),7.60 (1H, d, *J* = 16.2 Hz, H-7'), 6.30 (1H, d, *J* = 16.2 Hz, H-8'), 7.51 (1H, d, *J* = 16.2 Hz, H-7"), 6.18 (1H, d, *J* =16.2 Hz, H-8"). ^{13}C-NMR (150 MHz, CD_3OD), δ_C (ppm): 75.8 (C-1), 38.6 (C-2), 68.6 (C-3), 74.9 (C-4), 69.1 (C-5), 38.3 (C-6), 175.2 ($COOCH_3$), 53.1 ($COOCH_3$), 127.7 (C-1'), 115.2 (C-2', C-2"), 146.8 (C-3'), 149.7 (C-4'), 116.5 (C-5'), 123.1 (C-6', C-6"), 147.7 (C-7', C-7"), 114.7 (C-8'), 167.9 (C-9'), 127.6 (C-1"), 146.8 (C-3"), 149.7 (C-4"), 116.5 (C-5"), 114.6 (C-8"), 168.5 (C-9").

3. Results and discussions

Compound **1** was obtained as *a* colorless oil with a predicted molecular formula of $C_{16}H_{22}O_9$ by ESI-MS *(m/z* 359 $[M+H]^+$). The ^{1}H-NMR spectrum showed proton signals for the olefin at δ_H 7.61 (1H, d, *J* = 2.4 Hz, H-3), and one terminal vinyl group at δ_H 5.58 (1H, dt, *J* =17.4, 10.2 Hz, H-8), 5.31 (1H, dd, *J* = 17.4, 1.2 Hz, Ha-10) and 5.29 (1H, dd, *J* = 10.2, 1.8 Hz, Hb-10). Furthermore, the ^{1}H NMR spectrum also reveals the presence of an anomeric proton *at* δ_H 4.71 (1H, d, *J* = 8.4 Hz, H-1′) along with

hydroxymethine and hydroxymethylene protons at δ_H 3.22 (1H, dd, J = 9.6, 8.4 Hz, H-2′), 3.38 (1H, t, J = 9.0 Hz, H-3′), 3.32-3.36 (2H, m, H-4′, H-5′), 3.90 (1H, dd, J = 12.0, 2.4 Hz, Ha-6'), and 3.68 (1H, dd, J = 12.0, 6.0 Hz, Hb-6'), suggesting the presence of an *β*-glucopyranose moiety in the structure of **1**. The ^{13}C-NMR and DEPT spectra of **1** confirmed the presence of sixteen carbons, including four methylene groups at δ_C 120.8 (C-10), 25.9 (C-6), 69.7 (C-7), 62.7 (C-6'), ten methine groups at δ_C 99.7 (C-1), 153.9 (C-3), 28.4 (C-5), 133.3 (C-8), 43.8 (C-9) *Glc*: 99.7 (C-1'), 74.7 (C-2'), 77.9 (C-3'), 71.5 (C-4'), 78.4 (C-5') and two quaternary carbons at δ_C 106.0 (C-4), 168.5 (C-11). Other signals at δ_C 99.7 (C-1), 153.9 (C-3), and 106.0 (C-4) suggested an iridoid with a double bond at C-3/C-4 and carbon C-1 connected with two oxygen atoms. The comparison of the spectral data with the previously published data [10] confirmed that **1** was sweroside (Fig. 1). This compound was found in the roots of DA [11]. Sweroside inhibited the growth of the glioblastoma U251 cell with an IC_{50} of 10 μM [12].

Compound **2** was obtained as a colorless oil with a predicted molecular formula of $C_{18}H_{24}O_{10}$ by ESI-MS spectral data (m/z 362.7 [M-2H$_2$O-H]$^-$). Its NMR data were very similar to those of 1, except for the presence of an acetyl group at *s* 2.08 (3H, s, CH_3) and δ_C 172.7 (-C=O), 20.7 (6'-$COOCH_3$). The position of the acetyl group was determined through the HMBC interaction between the proton H-6' (δ_H 4.42 and 4.25) and the carbonyl carbon (δ_C 172.7), as well as the downfield shift of the carbon C-6' in the structure of **2** (δ_C 64.6) compared to the corresponding carbon in the structure of **1** (δ_C 62.7). By comparing the spectra with the literature [13], the structure of **2** was identified as 6'-O-acetylsweroside. This compound was reported from this plant for the first time (Fig. 1).

Compound **3** was isolated as a white powder, and its molecular formula $C_{16}H_{18}O_9$ was determined using ESI-MS. The ^{1}H-NMR spectrum exhibited ABX spin system protons at δ_H 7.04 (1H, s, H-2'), 6.72 (1H, d, J = 7.8 Hz, H-5'), and 6.93 (1H, d, J = 7.8 Hz, H-6'), as well as two olefinic protons of a *trans* C-C double bond at δ_H 7.45 (1H, d, J = 15.6 Hz, H-7') and 6.22 (1H, d, J = 15.6 Hz, H-8'). These proton signals suggested that **3** was a derivative of *trans*-caffeic acid. Additionally, three protons bonded with oxygen were observed at δ_H 3.90 (1H, d, J = 2.4 Hz, H-3), 3.47 (1H, m, H-4), and 5.16 (1H, m, H-5), as well as a pair of methylene protons at δ_H 1.77-1.84 (2H, m, H-2). The ^{13}C-NMR spectrum showed the presence of sixteen carbons, including two carbonyl groups at δ_C 176.5 (C-7) and 166.4 (C-9'), two olefinic carbons, and six carbon atoms at δ_C 125.1 (C-1'), 114.7 (C-2'), 146.1 (C-3'), 149.2 (C-4'), 115.9 (C-5'), 121.2 (C-6'), 144.8 (C-7'), and 114.3 (C-8'), four carbons bonded with oxygen at δ_C 75.3 (C-1), 71.6 (C-3), 73.3 (C-4), and 71.5 (C-5), and two methylene carbons at δ_C 39.8 (C-2) and 38.1 (C-6). Based on the spectroscopic data and comparison to the literature [14], **3** was identified as 3-(3,4-dihydroxycinnamoyl)quinate, commonly known as chlorogenic acid (Fig. 1). This compound was found in the roots of DA [15]. Chlorogenic acid has shown IC_{50} of 1.2, 1.3 and 241.5 μM to the hepatitis B virus extracellular DNA replication, intracellular DNA replication, and surface antigen, respectively [16].

Compound **4** was isolated as a colorless oil with a predicted molecular formula of $C_{25}H_{24}O_{12}$ based on ESI-MS analysis. The 1D-NMR spectra of **4** showed characteristic signals of a derivative of chlorogenic acid. The ^{1}H-NMR spectrum revealed two pairs of *trans*-caffeoyl groups at δ_H 7.52 (1H, d, J = 15.6 Hz, H-7'), 6.18 (1H, d, J = 15.6 Hz, H-8'), 7.62 (1H, d, J = 15.6 Hz, H-7''), 6.31 (1H, d, J =15.6 Hz, H-8''). Additionally, signals of three protons bonded with oxygen were observed at δ_H4.37 (1H, m, H-3), 5.12 (1H, dd, J = 3.6, 7.8 Hz, H-4) and 5.57 (1H, m, H-5). The ^{13}C-NMR spectrum showed the presence of twenty five carbons, including three carbonyl carbons at δ_C 174.8 (C-7), 167.9 (C-9'), and 168.5 (C-9''), four olefinic carbons at δ_C 147.6 (C-7'), 114.6 (C-8'), 147.7 (C-7''), and 114.8 (C-8''), and twelve carbon atoms at δ_C 127.7 (C-1'), 115.2 (C-2'), 146.8 (C-3'), 149.7 (C-4'), 116.5 (C-5'), 123.1 (C-6'), 127.6 (C-1''), 115.2 (C-2''), 146.8 (C-3''), 149.8 (C-4''), 116.5 (C-5''), and 123.1 (C-6''), four carbon atoms bonded to oxygen at δ_C 75.8 (C-1), 69.1 (C-3), 75.2 (C-4), and 69.3 (C-5), and two methylene carbons at δ_C 38.5 (C-2) and 38.5 (C-6). The interaction in the HMBC spectrum between H-7' (δ_H 7.52) with carbon C-8' (δ_C 114.6), C-1' (δ_C 127.7) and C-9' (δ_C 167.9), H-7'' (δ_H 7.62) with C-8'' (δ_C 114.8), C-1'' (δ_C 127.6) and C-9''(δ_C 168.5). The analysis of the spectral data in comparison with previously published data [17],[18] confirmed that **4** was 4,5-di-*O*-caffeoylquinic acid (Fig. 1). This compound was found in the roots of DA [19] and is known for its various effects, including antioxidant, anti-inflammatory, antimicrobial, and antifungal [20].

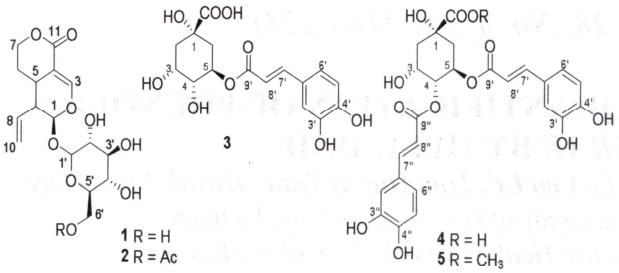

Fig. 1. Chemical structures of compounds **1-5**

Compound **5** was obtained as a yellow oil, and its spectral data exhibited similarities with those of **4**. However, an additional signal corresponding to one ester methyl group was observed in **5**, appearing at δ_H 3.74 (3H, s) and δ_C 53.1. The position of this group was confirmed through the HMBC interaction between the methyl proton at δ_H 3.74 and the carbonyl carbon at δ_C 175.2. After analyzing the data and comparing it to the previously published data [21], the structure of **5** was identified as methyl 4,5-di-*O*-caffeoylquinate (Fig. 1). This compound was found in the roots of DA and showed potent antioxidant effects [19].

4. Conclusions

Using various chromatographic techniques, five compounds were isolated from the ethyl acetate fraction of *Dipsacus asper* roots including two iridoids [sweroside (**1**) and 6'-*O*-acetylsweroside (**2**)], chlorogenic acid (**3**) and its derivatives [4,5-di-*O*-caffeoyl quinic acid (**4**) and methyl 4,5-di-*O*-caffeoylquinate (**5**)]. The structures of the isolated compounds were identified by analyzing their spectral data and comparing them with the previously published data. Compound **2** was isolated for the first time from this species.

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