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IRIDOID AND PHENOLIC COMPOUNDS FROM THE RHIZOMES OF *STACHYS SIEBOLDII*

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Summary

Iridoid and Phenolic Compounds from the Rhizomes of *Stachys sieboldii*

The rhizomes of *Stachys sieboldii* in Vietnam were investigated on the chemical constituents. Seven compounds were isolated and determined by NMR and MS analysis as palmitic acid, oleanolic acid, acteoside, isoacteoside, 8-*O*-acetyl harpagide, chrysoeriol 7-*O*-(2"-*O*-*β*-D-allopyranosyl)-*β*-D-glucopyranoside, and *trans*-cinnamic acid.

Keywords: *Stachys sieboldii, Iridoid, Oleanolic acid, Phenethyl glycoside, Flavone glycoside.*

1. Introduction

Stachys sieboldii Miq. (Lamiaceae) commonly called as Chinese artichoke is a perennial herbaceous plant originating from China, Japan, and France [1],[2]. Its rhizomes are used in Chinese traditional medicine for the treatment of inflammation, cold, pneumonia, and heart disease [3],[4]. Components isolated from *S. sieboldii* are iridoid and phenylethanoid glycosides. Acteoside is recognized in *S. sieboldii* as the component responsible for improving experimental autoimmune encephalomyelitis [5]. Harpagide and 8-*O*-acetyl harpagide are the principal iridoids in *S. sieboldii* with antibacterial, antiinflammatory, and antiviral activities [6]. However, the study on the chemical and biological activities of *S. sieboldii* rhizomes has not been reported in Vietnam. In this study, the chemical constituents of the *S. sieboldii* rhizomes of Vietnam were investigated. Seven compounds including palmitic acid, oleanolic acid, acteoside, isoacteoside, 8-*O*-acetyl harpagide, chrysoeriol 7-(2"-*O*-*β*-D-allopyranosyl)-*β*-D-glucopyranoside, *trans*-cinnamic acid were isolated and structurally determined.

2. Material and methods

2.1. Plant material

The rhizomes of *Stachys sieboldii* Miq. were collected by Mr. Pham Ngoc Khanh in Sa Pa province, Vietnam in March 2021. A voucher specimen of plant (NIMM-0019206) was conducted by Miss Nguyen Quynh Nga (Department of Medicinal Plant Resources - National Institute of Medicinal Materials (NIMM)).

2.2. General experimental procedures

Silica gel (Merck, Darmstadt, Germany, particle size 40 - 63 µm), D101 macroporous resin (Nankai Hecheng S&T, Tianjin, China), Sephadex® LH-20 (GE Healthcare, USA) were used for column chromatography. Thin-layer chromatography (TLC) was performed using pre-coated *silica gel* 60 F_{254} plates (Merck); spots were detected under UV 254 nm and/or by spraying with 10% H_2SO_4 reagent in ethanol, followed by heating at 120°C for 1 - 2 min. Compounds were isolated by a preparative HPLC Shimadzu LC20-AP system with Supelco Analytical Discovery ® HS C18 (250 × 21.2 mm; 10 µm) column. ^{1}H- and ^{13}C-NMR spectra were measured in $CDCl_3$ and DMSO-d_6 on a Bruker 600 NMR spectrometer (600 MHz for ^{1}H-NMR and 150 MHz for ^{13}C-NMR). Chemical shifts were expressed in δ (ppm) and tetramethylsilane (TMS) was used as an internal standard. Electrospray Ionization Mass Spectrometry (ESI-MS) spectra were recorded on an Agilent 1260 Series Single Quadrupole LC/MS system (Agilent, USA).

2.3. Extraction and isolation

The dried rhizomes (4.28 kg) of S. *sieboldii* were extracted three times with 70% aqueous ethanol under reflux. After filtration, solvents were removed under reduced pressure to obtain an ethanol extract. This extract was suspended in water and fractionated with dichloromethane. Solvents were evaporated *in vacuo* to obtain the corresponding dichloromethane (STD) and aqueous (ST) extracts. The dichloromethane extract (9.21 g) was subjected to *silica gel* column chromatography using gradient solvent system of dichloromethane-methanol (100/0-0/100) to give 8 subfractions D1-D8. Subfraction D1 (1.7 g) was separated by *silica gel* CC employing *n*-hexane-ethyl acetate (100:1, 50:1, v/v) to afford compound **1** (45 mg). Subfraction D3 (1.33 g) was chromatographed on *silica gel* column with gradient elution of *n*-hexane-ethyl acetate (50:1, 30:1, v/v) to obtain 3 fractions (D1.1-D1.3). Fraction D1.2 was filtered and washed with MeOH to obtain compound **2** (83 mg). The aqueous extract (ST) was separated by CC on D101 macroporous resin washing with water and eluting with 10%, 30%, 50% and 70% aqueous EtOH systems to afford four fractions (ST10% (151 g), ST30% (263 g), ST50% (362 g) and ST70% (11 g)).

Fraction ST30% was separated on a D101 macroporous resin column washing with water and eluting with 10%, 30%, 50%, 70%, and 95% aqueous EtOH systems to afford four fractions (ST30.1-ST30.4). Fraction ST30.2 was fractionated by column chromatography on Sephadex LH-20 using MeOH to yield four subfractions (ST30.21-ST30.24). Subfraction ST30.22 (570 mg) was further purified by preparative HPLC with solvent systems of acetonitrile (ACN) and 0.01% TFA in water (0-10 min: 15% ACN, 10-15 min: 15-20% ACN, 15-40 min: 20% ACN, 40-45 min: 20-95% ACN, 45-50 min: 95% ACN, 55-65 min: 15% ACN), flow rate of 10 mL/min; compounds were detected at wavelengths of 254 nm and 350 nm to obtain compounds **3** (25 mg) and **4** (17 mg). Subfraction ST30.24 was fractionated by column chromatography on Sephadex LH-20 using MeOH to yield compound **5** (30 mg).

Fraction ST50% was separated on D101 macroporous resin column washing with water and eluting with 10%, 30%, 50%, 70%, and 95% aqueous EtOH systems to afford two fractions ST50.1 (40 g) and ST50.2 (20 g). Fraction ST50.2 was fractionated by column chromatography on Sephadex LH-20 using MeOH as an eluent to yield five subfractions (ST50.21→ST50.25). Subfraction ST50.21 (1.2 g) was further purified by preparative HPLC with solvent systems of acetonitrile and 0.01 % TFA in water (0-10 min: 15% ACN, 10-15 min: 15-20% ACN, 15-40 min: 20% ACN, 40-45 min: 20-95% ACN, 45-50 min: 95% ACN, 55-65 min: 15% ACN), flow rate of 10 mL/min and detection at wavelengths of 210 nm, 245 nm, and 360 nm to obtain compound **6** (72 mg). Subfraction ST50.24 was purified by column chromatography on Sephadex LH-20 using MeOH as an eluent to yield compound **7** (16 mg).

Palmitic acid (1): Colorless wax. ESI-MS: *m/z* 254.9 ($[M-H]^-$). ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm) 2.36 (2H, *t*, *J* = 7.5 Hz, H-2), 1.63 (2H, *quint*, *J* = 7.5 Hz, H-3), 1.26-1.31 (24H, *br s*, H-4 → H-15), 0.88 (3H, *t*, *J* = 7.2 Hz, H-16). ^{13}C-NMR (150 MHz, $CDCl_3$): δ (ppm) 178.5 (C-1), 33.8 (C-2), 22.7-29.7 (C-3→C-13, C-15), 31.9 (C-14), 14.1 (C-16).

Oleanolic acid (2): White amorphous solid. ESI-MS (*m/z*): 457.2 ($[M+H]^+$). ^{1}H-NMR (600 MHz, $CDCl_3$): δ (ppm) 3.21 (1H, *dd*, *J* = 11.0, 4.5 Hz, H-3), 5.28 (1H, *br t*, *J* = 3.0 Hz, H-12), 2.82 (1H, *dd*, *J* = 13.0, 3,5 Hz, H-18), 0.99 (3H, *s*, H-23), 0.92 (3H, *s*, H-24), 0.76 (3H, *s*, H-25), 0.93 (3H, *s*, H-26), 1.14 (3H, *s*, H-27), 0.78 (3H, *s*, H-29), 0.90 (3H, *s*, H-30). ^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) 38.4 (C-1), 27.2 (C-2),

79.1 (C-3), 38.8 (C-4), 55.3 (C-5), 18.3 (C-6), 33.1 (C-7), 39.3 (C-8), 47.7 (C-9), 37.1 (C-10), 23.4 (C-11), 122.6 (C-12), 143.6 (C-13), 41.6 (C-14), 27.7 (C-15), 23.1 (C-16), 46.5 (C-17), 41.1 (C-18), 45.9 (C-19), 30.7 (C-20), 33.8 (C-21), 32.5 (C-22), 28.1 (C-23), 15.5 (C-24), 15.3 (C-25), 17.1 (C-26), 25.9 (C-27), 182.9 (C-28), 32.7 (C-29), 23.6 (C-30).

Acteoside (3): Colorless amorphous powder. ESI-MS: *m/z* 647.3 ([M+Na]$^+$). ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 6.62 (1H, *d*, *J* = 2.1 Hz, H-2), 6.62 (1H, *d*, *J* = 8.1 Hz, H-5), 6.49 (1H, *dd*, *J* = 8.1, 2.1 Hz, H-6), 2.70 (2H, *m*, H-7), 3.87-3.59 (2H, *m*, H-8), 7.02 (1H, *d*, *J* = 2.4 Hz, H-2'), 6.62 (1H, *d*, *J* = 8.4 Hz, H-5'), 6.97 (1H, *dd*, *J* = 8.4, 2.4 Hz, H-6'), 7.45 (1H, *d*, *J* = 16.2 Hz, H-7'), 6.19 (1H, *d*, *J* = 16.2 Hz, H-8'), 4.35 (1H, *d*, *J* = 7.8 Hz, H-1''), 3.2 (1H, *m*, H-2''), 3.45 (1H, *m*, H-5''), 4.71 (1H, *t*, *J* = 9.6 Hz, H-4''), 3.21-3.31 (4H, *m*, H-6'', H-2''', H-3''', H-4'''), 5.03 (1H, *d*, *J* = 1.2 Hz, H-1'''), 3.45 (1H, *m*, H-5'''), 0.96 (3H, *d*, *J* = 6.0 Hz, H-6'''). ^{13}C-NMR (125 MHz, DMSO-d_6): δ (ppm) 129.1 (C-1), 115.7 (C-2), 144.9 (C-3), 145.5 (C-4), 116.3 (C-5), 119.5 (C-6), 35.0 (C-7), 70.5 (C-8), 125.5 (C-1'), 114.7 (C-2'), 143.5 (C-3'), 148.4 (C-4'), 115.4 (C-5'), 121.4 (C-6'), 145.5 (C-7'), 113.6 (C-8'), 165.7 (C-9'), 102.3 (C-1''), 74.5 (C-2''), 79.1 (C-3''), 69.1 (C-4''), 74.5 (C-5''), 60.7 (C-6''), 101.2 (C-1'''), 70.2 (C-2'''), 70.4 (C-3'''), 71.6 (C-4'''), 68.7 (C-5'''), 18.1 (C-6''').

Isoacteoside (4): White amorphous powder. ESI-MS: *m/z* 647.3 ([M+Na]$^+$). ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm): 6.57 (1H, *d*, *J* = 8.4 Hz, H-2), 6.62 (1H, *d*, *J* = 2.4 Hz, H-5), 6.46 (1H, *dd*, *J* = 8.4, 2.4 Hz, H-6), 3.82-3.57 (2H, m, H-8), 2.67 (2H, *m*, H-7), 7.05 (1H, *d*, *J* = 2.4 Hz, H-2'), 6.75 (1H, *d*, *J* = 8.4 Hz, H-5'), 6.95 (1H, *dd*, *J* = 8.4, 2.4 Hz, H-6'), 7.46 (1H, *d*, *J* = 15.6 Hz, H-7'), 6.28 (1H, *d*, *J* = 15.6 Hz, H-8'), 4.27 (1H, *d*, *J* = 7.8 Hz) , H-1''), 3.45 (1H, *m*, H-2''), 3.40 (1H, *m*, H-3''), 3.86-3.93 (1H, *m*, H-4''), 3.15 (1H, *m*, H-5''), 3.38 (1H, *dd*, *J* = 11.7, 1.5 Hz, H-6''a), 4.19 (1H, *dd*, *J* = 11.7, 6.0 Hz, H-6b''), 5.03 (1H, *s*, H-1'''), 3.5 (1H, *m*, H-2'''), 3.7 (1H, *m*, H-3'''), 3.2 (2H, *m*, H-4''', H-5'''), 1.08 (3H, *d*, *J* = 6.6 Hz, H-6'''). ^{13}C-NMR (125 MHz, DMSO-d_6): δ (ppm) 129.2 (C-1), 115.8 (C-2), 143.5 (C-3), 145.0 (C-4), 116.3 (C-5), 119.5 (C-6), 35.1 (C-7), 70.6 (C-8), 125.5 (C-1'), 113.8 (C-2'), 148.4 (C-3'), 145.2 (C-4'), 115.4 (C-5'), 121.4 (C-6'), 145.5 (C-7'), 114.9 (C-8'), 166.5 (C-9'), 102.6 (C-1''), 73.7 (C-2''), 80.9 (C-3''), 68.1 (C-4''), 74.1 (C-5''), 63.4 (C-6''), 100.6 (C-1'''), 70.6 (C-2'''), 70.3 (C-3'''), 72.1 (C-4'''), 68.5 (C-5'''), 17.8 (C-6''').

8-*O*-Acetyl harpagide (5): Colorless crystals. ESI-MS: *m/z* 428.9 ([M+Na]$^+$). ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 5.87 (1H, *d*, *J* = 1.2 Hz, H-1), 6.34 (1H, *d*, *J* = 6.6 Hz, H-3), 4.86 (*m*, H-4), 4.54 (1H, *br s*, 5-OH), 3.58 (1H, *d*, *J* = 2.4 Hz, H-6), 2.05 (1H, *d*, *J* = 14.7 Hz, H-7a), 1.93 (3H, *s*, H-12), 1.79 (1H, *dd*, *J* = 14.7, 4.5 Hz, H-7b, 2.64 (1H, *s*, H-9), 1.35 (3H, *s*, H-10), 4.39 (1H, *d*, *J* = 7.8 Hz, H-1'), 2.98 (1H, *t*, *J* = 7.8 Hz, H-2'), 5.05 (1H, *br s*, 2'-OH), 3.17 (1H, *m*, H-3'), 4.97 (1H, *br s*, 3'-OH), 3.02 (1H, *m*, H- 4'), 4.50 (1H, *br s*, 4'-OH), 3.12 (1H, *m*, H-5'), 3.69 (1H, *dd*, *J* = 11.0, 1.8 Hz, H-6'a). ^{13}C-NMR (125 MHz, DMSO-d_6): δ (ppm) 92.5 (C-1), 141.3 (C-3), 107.3 (C-4), 73.0 (C-5), 76.1 (C-6), 44.6 (C-7), 86.5 (C-8), 54.3 (C-9), 22.1 (C-10), 170.3 (C-11), 22.1 (C-12), 97.4 (C-1'), 75.6 (C-2'), 76.1 (C-3'), 70.1 (C-4'), 77.1 (C-5'), 61.2 (C-6').

Chrysoeriol 7-(2''-*O*-*β*-D-allopyranosyl)-*β*-D-glucopyranoside (6): Yellow amorphous powder. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 12.94 (1H, *s*, 5-OH), 6.94 (3H, *m*, H-3, H-6, H-5'), 6.48 (1H, *d*, *J* = 1.8 Hz, H-8), 7.58 (2H, *br s*, H-2', H-6'), 3.89 (3H, *s*, 3'-OMe), 10.0 (1H, *s*, 4'-OH), 5.16 (1H, *d*, *J* = 7.2 Hz, H-1''), 4.79 (1H, *d*, *J* = 7.8 Hz, H-1'''). ^{13}C-NMR (125 MHz, DMSO-d_6): δ (ppm) 164.2 (C-2), 103.4 (C-3), 182.1 (C-4), 161.1 (C-5), 99.7 (C-6), 162.9 (C-7), 95.6 (C-8), 156.8 (C-9), 105.4 (C-10), 121.4 (C-1'), 110.3 (C-2'), 150.9 (C-3'), 148.1 (C-4'), 115.8 (C-5'), 120.5 (C-6'), 98.9 (C-1''), 82.6 (C-2''), 75.7 (C-3''), 70.9 (C-4''), 77.1 (C-5''), 60.5 (C-6''), 102.5 (C-1'''), 69.3 (C-2'''), 72.0 (C-3'''), 67.0 (C-4'''), 74.8 (C-5'''), 61.0 (C-6'''), 56.0 (OCH_3).

***trans*-Cinnamic acid (7)**: White amorphous powder. ^{1}H-NMR (600 MHz, DMSO-d_6): δ (ppm) 6.52 (1H, *d*, *J* = 16.0 Hz, H-2), 7.56 (1H, *d*, *J* = 16.0 Hz, H-3), 7.67 (2H, *m*, H-2', 6'), 7.42 (3H, *m*, H-3', 4', 5'). ^{13}C-NMR (125 MHz, DMSO-d_6): δ (ppm) 170.9 (C-1), 119.7 (C-2), 143.4 (C-3), 134.3 (C-1'), 128.1 (C-2'), 128.8 (C-3'), 130.0 (C-4'), 128.8 (C-5'), 128.1 (C-6').

3. Results and discussion

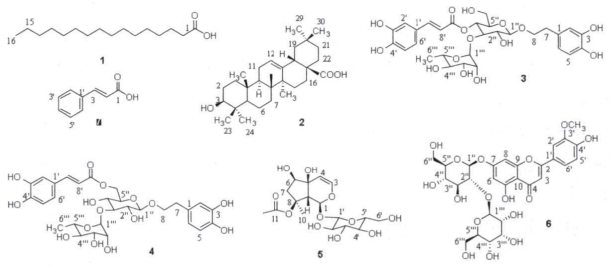

Fig. 1. Structures of compounds 1-7

Compound **1** was obtained as colorless wax. Its molecular formula $C_{16}H_{32}O_2$ was predicted by ESI-MS with a negative-ion pseudomolecular ion peak at *m/z* 254.9, $[M-H]^-$. The ^{1}H-NMR spectrum showed proton signals of the hydrocarbon chain at δ_H 2.36 (2H, *t*, *J* = 7.5 Hz, H-2), 1.63 (2H, *quint*, *J* = 7.5 Hz, H-3), 1.26-1.31 (24 H, *br s*, H-4 → H-15) and signals of a terminal methyl group at δ_H 0.88 (3H, *t*, *J* = 7.2 Hz, H-16). The ^{13}C-NMR spectrum included a carboxyl group at δ_C 178.5 (COOH) and the terminal methyl group at δ_C 14.1 (C-16). Compound **1** was identified as palmitic acid by comparing the spectral data with those in the literature [7].

Compound **2** was obtained as a white amorphous solid. The ^{1}H-NMR of compound **2** displayed seven tertiary methyl signals at δ_H 0.99, 0.92, 0.76, 0.93, 1.14, 0.78, 0.90 (each 3H, s), an olefinic proton at δ_H 5.28 and an oxygenated methine group at δ_H 3.21. The ^{13}C-NMR and DEPT spectra showed signals for thirty carbons, including seven methyl carbons (CH_3), ten methylene carbons (CH_2), six methine carbons (CH) and seven non-hydrogenated carbons (C). Signals of a carboxyl carbon and two olefinic carbons appeared at δ_C 182.9, 122.6 and 143.6, respectively. Based on the above spectral analysis, **2** was determined as an oleanane triterpene acid. The ESI-MS of **2** showed a peak at *m/z* 457 $[M+H]^+$ corresponding to the molecular formula $C_{30}H_{48}O_3$. By comparing its melting point and spectroscopic data with those reported in the literature [8], compound **2** was identified as oleanolic acid.

The ESI-MS of compound **3** showed a pseudomolecular ion peak at *m/z* 647.3 $[M+Na]^+$, corresponding to the molecular formula $C_{29}H_{36}O_{15}$. The ^{1}H-NMR spectrum of compound **3** showed three aromatic protons of an ABX system at δ_H 6.62 (1H, *d*, *J* = 2.1 Hz, H-2), 6.62 (1H, *d*, *J* = 8.1 Hz, H-5), and 6.49 (1H, *dd*, *J* = 8.1, 2.1 Hz, H-6), an oxymethylene at δ_H 3.87 (1H, *m*) and 3.59 (1H, *m*) H-8), a methylene at δ_H 2.70 (2H, *m*, H-7). The ^{13}C-NMR of compound **3** displayed 29 carbon signals, including 8 carbons of a phenethyl aglycon, 12 carbons of a glucopyranosyl and a rhamopyranosyl moieties, nine carbons of a caffeoyl group. The signals for the anomeric protons of the *β*-glucopyranosyl and *α*-rhamnopyranosyl moieties appeared at δ_C 102.3 (C-1") and 101.2 (C-1'"), respectively. The ^{1}H-NMR data of compound **3** showed the signals for the anomeric protons of the *β*-D-glucose moiety at δ_H 4.35 (1H, *d*, *J* = 7.8 Hz, H-1") and of the *α*-L-rhamnose moiety at δ_H 5.03 (1H, *d*, *J* = 1.2 Hz, H-1'"). The methyl signals of the rhamnose moiety appeared at δ_H 0.95 (*d*, *J* = 6.0 Hz) and δ_C 18.4. The HMBC correlation from H-1'" to C-3″ (δ_C 79.1) determined the location of the *α*-rhamnopyranosyl moiety at C-3″. The NMR spectra data of compound **3** agreed with those of acteoside in the literature [9],[10], so compound **3** was identified as acteoside.

Compound **4** was a white amorphous substance. Its molecular formula ($C_{29}H_{36}O_{15}$) was established by positive-ion mode ESI-MS. The molecular ion peak was observed at *m/z* 647.3 $[M+Na]^+$. The proton and carbon-13 signals of **4** shared similarities with those of **3**, except for the position of the caffeoyl group at C-6 of the glucosopyranosyl moiety. The NMR similarities of **3** and **4** in the NMR spectra were shown by proton signals of the 1,3,4-substituted benzene ring at δ_H 6.57 (1H, *d*, *J* = 8.4 Hz, H-2), 6.62 (1H, *d*, *J* = 2.4 Hz, H-5), and 6.46 (1H, *dd*, *J* = 7.8, 1.8 Hz, H-6), a methylene group at δ_H 2.67 (2H, *m*) and an oxymethylene group at δ_H 3.92 (1H, *m*), 3.70 (1H, *m*), whereas the B-ring protons were characterized by the ABX system at δ_H 7.05 (*d*, *J* = 2.4 Hz, H-2'), 6.75 (*d*, *J* = 7.8 Hz, H-5'), and 6.95 (*dd*, *J* = 8.4, 2.4 Hz, H-6'). The ^{13}C-NMR chemical shift of C-6" at δ_C 60.7 in **3** was downshifted to δ_C 63.4 in **4** suggesting the location of the caffeoyl group at C-6". The location of the *α*-rhamnopyranosyl moiety was determined at C-3″ by the HMBC correlation from H-1'" (δ_H 5.03, 1H, *s*) to C-3″ (δ_C 80.9). Based on the NMR data and comparison with the literature [9] compound **4** was identified as isoacteoside.

Compound **5** was isolated as colorless crystals. The ESI-MS spectrum of compound **5** revealed the pseudomolecular peak at *m/z* 428.9 $[M+Na]^+$ of a molecular formula of $C_{17}H_{26}O_{11}$ (406) with five degrees of unsaturation. The ^{1}H-NMR spectrum of compound **5** displayed signals of an anomeric proton at δ_H 4.39 (1H, *d*, *J* = 8.0 Hz), four oxymethine protons in the range of δ_H 2.98 - 3.17, and two oxymethylene protons at δ_H 3.58 (1H, *m*) and 3.69 (1H, *dd*, *J* = 11.0, 1.8 Hz), which revealed the presence of a *O*-glucose moiety. Two olefinic protons at δ_H 6.34 and 4.86 were correlated with a coupling constant of *J* = 6.6 Hz. Two singlet methyl groups were observed at δ_H 1.93 (*s*) and 1.35 (*s*). The ^{13}C-NMR of compound **5** displayed 17 carbon signals, including one ester carbonyl carbon (δ_C 170.3),

two olefinic carbons (δ_C 141.3 and 107.3), one acetal carbon (δ_C 92.5), two tertiary oxygenated carbons (δ_C 73.3 and 86.5), one sp^3 methylene carbon (δ_C 44.6), one oxymethine carbon (δ_C 76.1), a glucopyranosyl moiety (δ_C 97.4, 71.2, 76.1, 70.1, 77.1, 61.2), and two methyl groups (δ_C 22.1 and 22.1). The HMBC spectrum showed the correlation from the anomeric proton (H-1') to carbon C-1 (δ_C 92.5) which indicated that the sugar moiety was linked to C-1. Based on the NMR data and comparison with the literature [10], compound **5** was identified as 8-*O*-acetyl harpagide.

The ^{1}H-NMR of compound **6** showed an aglycon of chrysoeriol. The signal of the hydrogen-bonded H-5 appeared at δ_H 12.94 (1H, *s*, 5-OH), the flavone signals at δ_H 7.58 (*br s*, H-2', H-6'), 6.94 (3H, *m*, H-5', H-3, H-6), 6.48 (1H, *d*, J = 1.8 Hz, H-8). The methoxy group at C-3 was found at δ_H 3.89 (3H, s). The ^{13}C-NMR spectrum showed signals of chrysoeriol aglycon at δ_C 164.2 (C-2), 103.4 (C-3), 182.1 (C-4), 161.1 (C-5), 99.7 (C-6), 162.9 (C-7), 95.6 (C-8), 156.8 (C-9), 105.4 (C-10), 121.4 (C-1'), 110.3 (C-2'), 150.9 (C-3'), 148.1 (C-4'), 115.8 (C-5'), 120.5 (C-6'), 56.0 (OCH_3). Two hexose moieties were identified by comparison of the carbon-13 signals: a glucopyranosyl moiety resonanced at δ_C 98.9 (C-1''), 82.6 (C-2''), 75.7 (C-3''), 69.3 (C-4''), 77.1 (C-5''), 60.5 (C-6''), and an allopyranosyl moiety at δ_C 102.5 (C-1'''), 72.0 (C-2'''), 70.9 (C-3'''), 67.0 (C-4'''), 71.4 (C-5'''), 61.0 (C-6'''). Their anomeric proton signals appeared at δ_H 5.16 (1H, *d*, J = 7.2 Hz, H-1'') and 4.79 (1H, *d*, J = 7.8 Hz, H-1'''), respectively. Based on the NMR data and comparison with the compound reported in the literature, compound **6** was identified as chrysoeriol 7-(2''-*O*-*β*-D-allopyranosyl)-*β*-D-glucopyranoside [11],[12].

The ESI-MS of compound **7** showed a pseudomolecular ion peak at *m/z* 148.8 $[M+H]^+$ corresponding to the molecular formula $C_9H_8O_2$. The ^{1}H-NMR of compound **7** showed 4 proton signals at δ_H 6.52, 7.42, 7.56, and 7.67. The signals at δ_H 6.51 (1H, *d*, J = 16.0 Hz) and 7.56 ppm (1H, *d*, J = 16.0 Hz) determined a *trans*-double bond with the large coupling constant of 16.0 Hz. The ^{13}C-NMR of compound **7** showed 7 carbon signals at δ_C 119.7, 128.1, 128.8, 134.3, 143.4, and 170.9. The signal at δ_C 170.9 was of a carboxyl group. Two signals at δ_C 119.7 and 143.4 ppm were sp^2-hybridized carbon signals of the *trans*-double bond. The presence of a substituted benzene ring was proved by the four signals at δ_C 128.1, 128.8, 130.0, and 134.0. Each of the two signals at δ_C 128.1 and 128.8 arose from two magnetically equivalent carbon atoms with higher signal intensities than the remaining signals. By comparing spectroscopic data with those in the literatures [13], compound **7** was identified as *trans*-cinnamic acid.

4. Conclusion

From the rhizomes of *Stachys sieboldii* in Vietnam seven compounds (**1** – **7**) were isolated and determined as palmitic acid (**1**), oleanolic acid (**2**), acteoside (**3**), isoacteoside (**4**), 8-*O*-acetyl harpagide (**5**), chrysoeriol 7-*O*-(2''-*O*-*β*-D-allopyranosyl)-*β*-D-glucopyranoside (**6**), and *trans*-cinnamic acid (**7**). Oleanolic acid, acteoside, isoacteoside and 8-*O*-acetyl harpagide have been isolated from different plant species of the genus *Stachys*. The other compounds were isolated for the first time from *Stachys sieboldii*.

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ISOLATION AND PURIFICATION OF DARUTOSIDE FROM *SIEGESBECKIA ORIENTALIS* FOR ESTABLISHING A REFERENCE SUBSTANCE

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Summary

Isolation and Purification of Darutoside From *Siegesbeckia orientalis* for Establishing a Reference Substance

Siegesbeckia orientalis L. is an important medicinal herb that has been used traditionally in Vietnam, China for the treatment of back pain, knee pain, bone and joint pain, numb limbs, and boils. In this study, it is showed that *S. orientalis* collected in Thanh Hoa province, Vietnam contained significant content of darutoside, a reported constituent with various pharmacological activities. Darutoside was isolated and purified from the aerial parts of *S. orientalis* by using chromatography methods. The structure of the pure compound was identified by UV, IR, NMR, MS spectrometry. The establishment of a reference substance (RS) for darutoside was carried out according to ISO Guide 35 and WHO guideline. The purity of darutoside was determined as 95.16 ± 0.05% by HPLC. The results suggest that the isolated darutoside in this study could be used to be a RS for quality assessment of *S. orientalis* and related products.

Keywords: *Siegesbeckia orientalis, Darutoside, Reference substance.*

1. Introduction

The medicinal plant *Siegesbeckia orientalis* L. (family Asteraceae) has been traditionally used in Vietnam and China for the treatment of back pain, knee pain, bone and joint pain, numb limbs, and boils [1]. It has also widely been used in many pharmaceutical products in modern dosage forms [2].

Diterpenoids including *ent*-kauranes and *ent*-pimaranes have been reported as main chemical constituents in *S. orientalis* [3],[4]. Of which, kirenol and darutoside have been found to possess a variety of pharmacological activities such as topical anti-inflammatory and pain relieving, wound healing effects [5],[6]. Chinese [7] and Hongkong Pharmacopeias [8] regulate kirenol as the standard for the assay of *S. orientalis* by HPLC, meanwhile, Vietnamese Pharmacopoeia only tested for extractives in ethanol 96% [1]. Recent research showed that *S. orientalis* collected in Vietnam (Thanh Hoa, Nghe An...) contained not only kirenol but also a significant content of darutoside [2]. According to "WHO guidelines for selecting marker substances of herbal origin for quality control of herbal medicines", apart from meeting requirements for reference substance (RS), which isolated from herbs should be selected based on following priorities: (1) marker substances of constituents with known therapeutic activity; (2) marker substances of constituents with recognized pharmacological activities; (3) marker substances of characteristic constituents [9]. The previous reports indicated that darutoside is suitable to be selected as a marker substance for quality control of *S. orientalis* [2],[6]. This paper