Journal of Medicinal Materials, 2022, Vol. 27, No. 1 (pp. 3 - 9)

CHEMICAL CONSTITUENTS FROM BULBS OF *ALLIUM ASCALONICUM* L.

Vu Thi Diep[1], Nguyen Thi Thu[1], Nguyen Tra My[1], Hoang Dieu Huong[1], Nguyen Thi Hang[1], Ha Van Oanh[2], Nguyen Minh Khoi[1], Do Thi Ha[1,*], Pham Thi My Dung[3], Hoang Viet Dung[3]

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

[2]Hanoi University of Pharmacy, Vietnam; [3]Vietnam Military Medical University

*Corresponding author: hado.nimms@gmail.com

(Received January 14th, 2022)

Summary

Chemical Constituents from Bulbs of *Allium ascalonicum* L.

Nine compounds (**1** - **9**) were isolated from bulbs of *Allium ascalonicum*. Their chemical structures were identified using spectroscopic methods (NMR and MS) and comparison with literature data. These compounds are protocatechuic acid (**1**), *trans*-*N*-coumaroyltyramin (**2**), 5,7-dihydroxy-2-heptacosanyl-benzopyran-4-one (**3**), quercetin (**4**), quercetin 4'-glucopyranoside (**5**), isorhamnetin (**6**), isorhamnetin 4'-glucopyranoside (**7**), (22*R*,25*S*)-spirost-5-en-1β,3β-diol 1-*O*-β-D-galactopyranoside (**8**), and (22*R*,25*S*)-spirost-5-en-1β,3β-diol 1-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside] (**9**). This is the first report about the presence of compounds **2**, **3**, **8**, and **9** from *A. ascalonicum*.

Keywords: *Allium ascalonicum* L., *Flavonoid, Phenolic, Pyrimidine, Saponin*.

1. Introduction

Shallot (*Allium ascalonicum* L.), a member of the Liliaceae family, has been used as a spice, food, and folk medicine throughout history. It possesses a wide range of medicinal properties, including digestive stimulant, anti-inflammatory [1], antibacterial, antifungal [2], and anticancer effects [3], etc. In Vietnamese folk medicine, this plant has been used to treat typhoid, fever, colds, headaches, bloating and poisoning, blurred vision, deaf ears, and breast swelling [4]. There are many different varieties of *A. ascalonicum* L., which depend on where its cultivation is. Ly Son onions (Hanh Ly Son) are grown in Ly Son Island, Quang Ngai province, which has special soil and climate conditions that make Ly Son onion purple color and special flavor. Until now, not many studies on the chemical compositions and biological effects of Ly Son onion have been conducted. Previous studies indicated that *A. ascalonicum* contains essential oils [5], flavonoids [6],[7], triterpenoids [8], and saponins [6],[9]. Several studies on Ly Son onion have demonstrated that it exhibited antioxidant effects [10] and it improved behavior in autistic children [11]. The objective of the present study is to investigate the chemical composition of Ly Son onion.

2. Materials and methods

2.1. Plant material

Fresh bulbs of *Allium ascalonicum* L. were collected in Ly Son, Quang Ngai, Vietnam in September 2020 and were identified by MSc. Nguyen Van Hieu and MSc. Lai Viet Hung (Department of Medicinal Resource - National Institute of Medicinal Materials). The voucher specimen (DL-40221) was deposited at the Herbarium of NIMM.

2.2. General experimental procedures

NMR spectra were recorded on Bruker AM 500 or 600 FT-NMR spectrometer (Karlsruhe, Germany) using TMS as the internal standard. MS data were analyzed with an LC-MS/MS system (Shimadzu, Japan). *Silica gel* (Merck, 0.040 - 0.063 mm particle size), RP-C_{18} (Merck, 30 - 50 µm particle size) were used for column chromatography (CC). Thin-layer chromatography (TLC) was carried out with *silica gel* 60F_{254} (Merck, *silica gel*, 0.25 mm) and RP-18F_{254} (Merck, 0.25 mm) plates. Spots were detected with UV light 254 and 365 nm or by spraying with 10% sulfuric acid in 96% ethanol (EtOH) and then heating.

2.3. Extraction and isolation

The chopped bulbs (3.5 kg, dry weight) were macerated with 90% ethanol at room temperature 2 times x 24 h. After removal of the solvent, the ethanol extract (595.37 g) was suspended with water and successively separated with organic solvents to get *n*-hexane (25.01 g), ethyl acetate (36.4 g), and aqueous (450.1 g) extracts.

The *n*-hexane extract (23.0 g) was separated by *silica gel* CC and eluted by a gradient of *n*-hexane (Hx)-ethyl acetate (EtOAc) (100:0 - 0:100, v/v) to obtain 7 fractions (H1-H7).

Fraction H3 (2.36 g) was chromatographed on *silica gel* CC eluting with Hx-EtOAc (30:1, v/v) to yield compound **3** (20 mg).

The EtOAc extract (33.16 g) was fractionated by *silica gel* CC and eluted by a gradient of dichloromethane-methanol (DCM-MeOH, 100:1 to 2:1, v/v) to give 11 fractions (E1-E11). Fraction E4 (3.91 g) was chromatographed on *silica gel* CC with gradient solvents of DCM-MeOH (50:1, v/v) to DCM-MeOH (0:100, v/v) to yield compound **4** (20 mg). Fraction E6 (3.24 g) was chromatographed on *silica gel* CC eluting with DCM-acetone (40:1 - 0:100, v/v) to give 5 fractions (E6.1-E6.5). Fraction E6.1 (0.85 g) was washed using DCM-MeOH (80:1, v/v) to yield compound **6** (100 mg). Fraction E6.2 (0.29 g) was applied to RP-18 CC using MeOH-H_2O (1:1, v/v) to yield compounds **1** (20 mg) and **2** (20 mg). Fraction E8 (1.27 g) was purified by RP-18 CC and eluted with MeOH-H_2O (1:2, v/v) to obtain compound **7** (10 mg). Fraction E9 (2.81 g) was separated by RP-18 CC eluting with MeOH-H_2O (1:2, v/v) to give 7 fractions (E9.1-E9.7). Fraction E9.2 (0.63 g) was applied to *silica gel* CC and eluted with EtOAc-MeOH (20:1, v/v) to give compound **5** (8.6 mg). Compounds **8** (10 mg) and **9** (6.2 mg) were purified from fraction E9.6 (0.43 g) by *silica gel* CC eluting with EtOAc-MeOH-HCOOH (25:1:1, v/v/v).

Protocatechuic acid (**1**): White powder, **ESI-MS**: *m/z* 153.1 $[M-H]^-$; **^{1}H-NMR** (600 MHz, CD_3OD), δ_H (ppm): 7.46 (1H, d, J = 1.8 Hz, H-2), 6.82 (1H, d, J = 8.4 Hz, H-5), and 7.45 (1H, dd, J = 1.8, 8.4 Hz, H-6); **^{13}C-NMR** (150 MHz, CD_3OD), δ_C (ppm): 123.9 (C-1), 115.8 (C-2), 146.1 (C-3), 151.5 (C-4), 117.7 (C-5), 123.9 (C-6), and 170.2 (C-7).

Trans-N-coumaroyltyramin (**2**): White powder, **ESI-MS**: *m/z* 284.0 $[M+H]^+$; **^{1}H-NMR** (600 MHz, CD_3OD), δ_H (ppm): 7.41 (2H, d, J = 8.4 Hz, H-2, H-6), 6.81 (2H, d, J = 9.0 Hz, H-3, H-5), 6.40 (1H, d, J = 15.6 Hz, H-7), 7.46 (1H, d, J = 15.6 Hz, H-8), 7.07 (2H, d, J = 9.0 Hz, H-2', H-6'), 6.73 (2H, d, J = 8.4 Hz, H-3', H-5'), 2.77 (2H, t, J = 7.2 Hz, H-7'), and 3.47 (2H, t, J = 7.2 Hz, H-8'); **^{13}C-NMR** (150 MHz, CD_3OD), δ_C (ppm): 127.8 (C-1), 130.5 (C-2, C-6), 116.3 (C-3, C-5), 160.5 (C-4), 141.8 (C-7), 118.5 (C-8), 169.3 (C-9), 131.3 (C-1'), 130.7 (C-2', C-6'), 116.7 (C-3', C-5'), 156.9 (C-4'), 35.8 (C-7'), and 42.5 (C-8').

5,7-Dihydroxy-2-heptacosanyl-benzopyran-4-one (**3**): White powder, **ESI-MS**: *m/z* 555.4 $[M-H]^-$; **^{1}H-NMR** (600 MHz, $CDCl_3$): 6.03 (1H, s, H-3), 6.27 (1H, s, H-6), 6.33 (1H, s, H-8), 12.71 (1H, s, 5-OH), 2.56 (2H, t, J = 7.8 Hz, H-1'), 1.69 (2H, m, H-2'), 1.25 - 1.37 (m, H-3' - H-25'), 1.25 (2H, m, H-26'), and 0.87 (3H, t, J = 6.6 Hz, H-27'); **^{13}C-NMR** (150 MHz, $CDCl_3$): 170.8 (C-2), 107.9 (C-3), 182.6 (C-4), 162.3 (C-5), 99.3 (C-6), 162.4 (C-7), 94.1 (C-8), 158.3 (C-9), 105.3 (C-10), 34.2 (C-1'), 26.8 (C-2'), 28.9 - 31.9 (C-3' - C-25'), 22.7 (C-26'), and 14.1 (C-27').

Quercetin (**4**): Pale yellow powder, **ESI-MS**: *m/z* 301.0 $[M-H]^-$; **^{1}H-NMR** (600 MHz, $CDCl_3$): 6.18 (1H, d, J = 2.4 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 = 2.4, 8.4 Hz, H-6'), 12.48 (1H, s, 5-OH), 10.77 (1H, s, 7-OH), 9.34 (2H, s, 3',4'-OH), and 9.56 (1H, s, 3-OH); **^{13}C-NMR** (150 MHz, $CDCl_3$): 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'), and 119.9 (C-6').

Quercetin 4'-glucopyranoside (**5**): Pale yellow powder, **ESI-MS**: *m/z* 463.0 $[M-H]^-$; **^{1}H-NMR** (600 MHz, DMSO-d_6), δ_H (ppm): 6.19 (1H, d, J = 1.8 Hz, H-6), 6.44 (1H, d, J = 1.8 Hz, H-8), 7.69 (1H, d, J = 1.8 Hz, H-2'), 7.25 (1H, d, J = 9.0 Hz, H-5'), 7.62 (1H, dd, J = 1.8, 9.0 Hz, H-6'), 12.40 (1H, s, 5-OH), *Glc:* 4.84 (1H, d, J = 7.2 Hz, H-1"), 3.47 (2H, m, H-2", H-3"), 3.19 (2H, m, H-4", H-5"), and 3.73 (2H, m, H-6"); **^{13}C-NMR** (125 MHz, DMSO-d_6), δ_C (ppm): 146.8 (C-2), 136.4 (C-3), 176.0 (C-4), 160.7 (C-5), 98.3 (C-6), 164.1 (C-7), 93.5 (C-8), 156.2 (C-9), 103.1 (C-10), 125.1 (C-1'), 115.1 (C-2'), 146.4 (C-3'), 145.9 (C-4'), 115.9 (C-5'), 119.5 (C-6'), *Glc*: 101.4 (C-1"), 73.3 (C-2"), 76.0 (C-3"), 69.8 (C-4"), 77.3 (C-5"), and 60.7 (C-6").

Isorhamnetin (**6**): Pale yellow powder, **ESI-MS**: *m/z* 315.1 $[M-H]^-$; **^{1}H-NMR** (600 MHz, DMSO-d_6), δ_H (ppm): 6.19 (1H, d, J = 1.8 Hz, H-6), 6.47 (1H, d, J = 2.4 Hz, H-8), 7.75 (1H, d, J = 1.8 Hz, H-2'), 6.94 (1H, d, J = 8.4 Hz, H-5'), 7.69 (1H, dd, J = 2.4, 8.4 Hz, H-6'), 3.84 (3H, s, 3'-OCH_3), and 12.46 (1H, s, 5-OH); **^{13}C-NMR** (125 MHz, DMSO-d_6), δ_C (ppm): 146.6 (C-2), 135.6 (C-3), 175.9 (C-4), 160.6 (C-5), 98.2 (C-6), 163.9 (C-7), 93.6 (C-8), 156.1 (C-9), 103.0 (C-10), 121.9 (C-1'), 115.5 (C-2'), 147.3 (C-3'),

148.8 (C-4'), 111.7 (C-5'), 121.7 (C-6'), and 55.8 (3'-OCH_3).

Isorhamnetin 4'-glucopyranoside (**7**): Pale yellow powder, **ESI-MS**: *m/z* 479.3 $[M+H]^+$; **^{1}H-NMR** (500 MHz, DMSO-d_6), δ_H (ppm): 6.50 (1H, d, *J* = 2.0 Hz, H-8), 6.20 (1H, d, *J* = 2.5 Hz, H-6), 7.79 (1H, d, *J* = 2.0 Hz, H-2'), 7.24 (1H, d, *J* = 8.5 Hz, H-5'), 7.75 (1H, dd, *J* = 2.0, 8.5 Hz, H-6'), 3.85 (3H, s, 3'-OCH_3), 12.41 (1H, s, 5-OH), *Glc*: 5.03 (1H, d, *J* = 7.5 Hz, H-1"), 3.46 (2H, m, H-2", H-3"), 3.20 (2H, m, H-4", H-5"), and 3.69 (2H, m, H-6"); **^{13}C-NMR** (125 MHz, DMSO-d_6), δ_C (ppm): 145.9 (C-2), 136.5 (C-3), 176.1 (C-4), 160.7 (C-5), 98.3 (C-6), 164.1 (C-7), 93.7 (C-8), 156.3 (C-9), 103.1 (C-10), 124.6 (C-1'), 115.0 (C-2'), 148.6 (C-3'), 148.0 (C-4'), 111.8 (C-5'), 121.2 (C-6'), 55.9 (3'-OCH_3), *Glc*: 99.7 (C-1"), 76.9 (C-2"), 69.7 (C-3"), 73.2 (C-4"), 77.1 (C-5"), and 60.6 (C-6").

(22*R*,25*S*)-Spirost-5-en-1β,3β-diol 1-*O*-β-D-galactopyranoside (**8**): White powder, **ESI-MS**: *m/z* 615.2 $[M+Na]^+$; **^{1}H-NMR** (600 MHz, CD_3OD), δ_H (ppm): 3.51 (1H, m, H-1), 3.41 (1H, m, H-3), 5.57 (1H, d, *J* = 6.0 Hz, H-6), 4.41 (1H, m, H-16), 0.83 (3H, s, H-18), 1.12 (3H, s, H-19), 1.01 (3H, d, *J* = 7.2 Hz, H-21), 3.31 (1H, m, H-26), 3.95 (1H, dd, *J* = 2.4, 10.8 Hz, H-26), 1.10 (3H, d, *J* = 7.2 Hz, H-27), *Gal*: 4.29 (1H, d, *J* = 7.2 Hz, H-1'), 3.50 (1H, m, H-2'), 3.48 (1H, m, H-3'), 3.87 (1H, d, *J* = 2.4 Hz, H-4'), 3.45 (1H, m, H-5'), 3.72 (1H, dd, *J* = 6.0, 11.4 Hz, H-6'), and 3.77 (1H, dd, *J* = 6.0, 11.4 Hz, H-6'); **^{13}C-NMR** (150 MHz, CD_3OD), δ_C (ppm): 84.0 (C-1), 37.3 (C-2), 69.1 (C-3), 43.5 (C-4), 139.8 (C-5), 125.9 (C-6), 32.7 (C-7), 34.1 (C-8), 51.4 (C-9), 43.5 (C-10), 24.6 (C-11), 41.3 (C-12), 41.1 (C-13), 58.0 (C-14), 32.9 (C-15), 82.4 (C-16), 63.8 (C-17), 17.1 (C-18), 14.9 (C-19), 43.5 (C-20), 14.9 (C-21), 111.1 (C-22), 27.0 (C-23), 26.8 (C-24), 28.5 (C-25), 66.1 (C-26), 16.4 (C-27), *Gal*: 102.0 (C-1'), 72.6 (C-2'), 75.1 (C-3'), 70.2 (C-4'), 76.3 (C-5'), and 62.5 (C-6').

(22*R*,25*S*)-Spirost-5-en-1β,3β-diol 1-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside] (**9**): White powder, **ESI-MS**: *m/z* 731.3 $[M+Na]^+$; **^{1}H-NMR** (600 MHz, CD_3OD), δ_H (ppm): 3.38 (1H, m, H-1), 3.36 (1H, m, H-3), 5.58 (1H, d, *J* = 6.0 Hz, H-6), 4.41 (1H, m, H-16), 0.83 (3H, s, H-18), 1.12 (3H, s, H-19), 1.01 (3H, d, *J* = 6.6 Hz, H-21), 3.28 (1H, brd, *J* = 10.8 Hz, H-26), 3.95 (1H, dd, *J* = 2.4, 10.8 Hz, H-26), 1.10 (3H, d, *J* = 7.2 Hz, H-27), *Ara*: 4.28 (1H, d, *J* = 7.2 Hz, H-1'), 3.72 (1H, m, H-2'), 3.66 (1H, dd, *J* = 3.6, 9.0 Hz, H-3'), 3.76 (1H, m, H-4'), 3.50 (1H, dd, *J* = 1.2, 12.6 Hz, H-5'), 3.87 (1H, dd, *J* = 2.4, 12.6 Hz, H-5'), *Rham*: 5.31 (1H, d, *J* = 1.8 Hz, H-1"), 3.90 (1H, dd, *J* = 1.8, 3.0 Hz, H-2"), 3.70 (1H, m, H-3"), 3.42 (1H, m, H-4"), 4.10 (1H, m, H-5"), and 1.26 (3H, d, *J* = 6.6 Hz, H-6"); **^{13}C-NMR** (150 MHz, CD_3OD), δ_C (ppm): 84.6 (C-1), 37.3 (C-2), 69.2 (C-3), 43.5 (C-4), 139.6 (C-5), 126.0 (C-6), 32.7 (C-7), 34.1 (C-8), 51.5 (C-9), 43.5 (C-10), 24.8 (C-11), 41.3 (C-12), 41.1 (C-13), 58.0 (C-14), 32.9 (C-15), 82.4 (C-16), 63.8 (C-17), 17.1 (C-18), 15.3 (C-19), 43.5 (C-20), 14.8 (C-21), 111.1 (C-22), 27.0 (C-23), 26.8 (C-24), 28.5 (C-25), 66.1 (C-26), 16.4 (C-27), *Ara*: 101.0 (C-1'), 75.6 (C-2'), 76.1 (C-3'), 70.8 (C-4'), 67.4 (C-5'), *Rham*: 101.6 (C-1"), 72.4 (C-2"), 72.1 (C-3"), 74.2 (C-4"), 69.7 (C-5"), and 18.4 (C-6").

3. Results and discussion

Compound **1** was isolated as a white amorphous solid. The molecular formula of **2** was suggested as $C_7H_6O_4$ based on a *pseudo*-molecular peak at *m/z* 153.1 $[M-H]^-$ and NMR spectra. The ^{1}H-NMR spectrum of **1** appeared one ABX type signal pattern at δ_H 7.46 (1H, d, *J* = 1.8 Hz, H-2), 6.82 (1H, d, *J* = 8.4 Hz, H-5) and 7.45 (1H, dd, *J* = 1.8, 7.8 Hz, H-6). The signals of one carboxyl carbon at δ_C 170.2 (C-7), two aromatic carbons bonded with hydroxyl groups at δ_C 146.1 (C-3) and 151.5 (C-4), and 3 methine carbons at δ_C 115.8 (C-2), 117.7 (C-5), and 123.9 (C-6) in the ^{13}C-NMR spectrum suggested that **1** was a 1,3,4-trisubstituted benzene ring. From the above analysis and comparison with the literature [12], the structure of **1** was determined to be 3,4-dihydroxybenzoic acid or protocatechuic acid (Fig. 1).

Compound **2** was purified as a white solid. Its molecular formula was found to be $C_{17}H_{17}NO_3$ by the NMR data and a positive ESI-MS ion peak at *m/z* 284.0 $[M+H]^+$. The ^{1}H-NMR spectrum of **2** showed resonances for two sets of AA'BB' type signals at δ_H 7.41 (2H, d, *J* = 8.4 Hz, H-2, H-6), 6.81 (2H, d, *J* = 9.0 Hz, H-3, H-5) and 7.07 (2H, d, *J* = 9.0 Hz, H-2', H-6'), 6.73 (2H, d, *J* = 8,4 Hz, H-3', H-5'). In addition, the ^{1}H-NMR spectrum of **2** showed the proton signals of two methylene groups at δ_H 2.77 (2H, t, *J* = 7.2 Hz, H-7') and 3.47 (2H, t, *J* = 7.2 Hz, H-8') together with 2 olefinic protons of a *trans*-double bond at δ_H 6.40

(1H, d, J = 15.6 Hz, H-7) and 7.46 (1H, d, J = 15.6 Hz, H-8). These data indicated that **2** comprised one coumaroyl moiety and one tyramine moiety. The ^{13}C-NMR spectrum of **2** exhibited signals of the *p*-coumaroyl moiety [δ_C 127.8 (C-1), 130.5 (C-2, C-6), 116.3 (C-3, C-5), 160.5 (C-4), 141.8 (C-7), 118.5 (C-8), and 169.3 (C-9)] and tyramine moiety [δ_C 131.3 (C-1'), 130.7 (C-2', C-6'), 116.7 (C-3', C-5'), 156.9 (C-4'), 35.8 (C-7'), and 42.5 (C-8')]. Thus, **2** was elucidated as *trans-N*-coumaroyltyramine (Fig. 1) [13].

Compound **3** was obtained as a white powder. The ESI-MS spectrum of **3** showed a *pseudo*-molecular ion peak at *m/z* 555.4 [M-H]$^-$. Combining with NMR data, it indicated the molecular formula of **3** as $C_{36}H_{60}O_4$. The ^{1}H-NMR spectrum of **3** exhibited a pair of *meta*-coupled aromatic proton typical of H-6 (δ_H 6.27) and H-8 (δ_H 6.33) and one aromatic singlet attributable to H-3 (δ_H 6.03) commonly seen in a 5,7-dioxygenated flavone. However, no further aromatic signals were observed. The resonances for an alkyl chain at δ_H 2.56 (2H, t, J = 7.8 Hz, H-1'), 1.69 (2H, m, H-2'), 1.25 - 1.37 (46H, m, H-3' - H-25'), 1.25 (2H, m, H-26'), and 0.87 (3H, t, J = 6.6 Hz, H-27') suggested **3** was not a flavone but a 5,7-dihydroxybenzopyran-4-one substituted at C-2 with an alkyl group. The ^{13}C-NMR and DEPT spectra of **3** exhibited the signals of 9 carbons: a ketone group at δ_C 182.6 (C-4), 4 oxygen-bearing carbons at δ_C 170.8 (C-2), 162.3 (C-5), 162.4 (C-7), and 158.3 (C-9), 3 methine carbons at δ_C 107.9 (C-3), 99.3 (C-6), and 94.1 (C-8), a quaternary carbon at δ_C 105.3 (C-10), and signals of an alkyl chain at δ_C 14.1 - 34.2. In the HMBC spectrum, the H-1' proton (δ_H 2.56) showed a correlation with the carbonyl carbon at δ_C 170.8 (C-2) which confirmed the attachment of the side chain at C-2. Based on these analyses and comparison of the data with the literature [14], **3** was elucidated as 5,7-dihydroxy-2-heptacosanyl-benzopyran-4-one (Fig. 1)

Compound **4** was obtained as a pale-yellow powder. The ESI-MS spectrum of **4** gave a *pseudo*-molecular ion peak at *m/z* 301.0 [M-H]- suggested that the molecular formula of **4** was $C_{15}H_{10}O_7$. The ^{1}H-NMR spectrum of **4** showed signals of two doublet protons of the *meta*-substituted A ring [δ_H 6.18 (1H, d, J = 2.4 Hz, H-6) and 6.40 (1H, d, J = 1.8 Hz, H-8), an aromatic ABX system of the B ring [δ_H 7.67 (1H, d, J = 2.4 Hz, H-2'), 6.87 (1H, d, J = 8.4 Hz, H-5'), and 7.53 (1H, dd, J = 2.4, 8.4 Hz, H-6'), and 5 proton signals of hydroxy groups at δ_H 9.56 (1H, s, 3-OH), 12.48 (1H, s, 5-OH), 10.77 (1H, s, 7-OH), and 9.34 (2H, s, 3',4'-OH). The ^{13}C-NMR spectrum of **4** showed signals of 15 carbons, including one ketone carbon at δ_C 175.8 (C-4), seven carbons bonded to oxygen at δ_C 146.8 (C-2), 135.7 (C-3), 160.7 (C-5), 163.9 (C-7), 156.1 (C-9), 145.0 (C-3'), 147.7 (C-4'), 5 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 2 quaternary carbons at δ_C 103.0 (C-10) and 121.9 (C-1'). Therefore the structure of **4** was determined to be 3,3',4',5,7-pentahydroxyflavone or quercetin (Fig. 1) [15].

Compound **5** was isolated as a pale-yellow powder. Its molecular formula of **5** was found to be $C_{21}H_{20}O_{12}$ by the NMR data and a positive ESI-MS ion peak at *m/z* 463.0 [M-H]$^-$. The NMR spectra of **5** were similar to those of **4**, except for the addition of one anomeric proton signal at δ_H 4.84 (1H, d, J = 7.2 Hz, H-1") as well as the presence of 6 carbon signals at δ_C 101.4 (C-1"), 73.3 (C-2"), 76.0 (C-3"), 69.8 (C-4"), 77.3 (C-5"), and 60.7 (C-6") of a sugar moiety. The position of the sugar moiety was determined at C-4′ by the HMBC correlation between the anomeric proton (δ_H 4.89)/H-2' (δ_H 7.69)/H-6' (δ_H 7.62) and C- 4′ (δ_C 145.9). From the above spectral data and comparison with the literature [16], the structure of **5** was determined to be quercetin-4'-*O*-glucopyranoside (Fig. 1).

Compound **6** was obtained as a pale-yellow powder. The ESI-MS spectrum which showed a molecular ion peak at *m/z* 315.1 [M-H]$^-$ suggested that the molecular formula of **6** was $C_{16}H_{12}O_7$. The NMR spectral data of **6** was also similar to those of **4** except for the presence of a methoxy group at δ_H 3.84 (s) and δ_C 55.8 ppm. The position of the methoxy group at C-3' was determined by HMBC correlations between the methoxy protons (δ_H 3.84)/H-2′ (δ_H 7.75)/H-5′ (δ_H 6.94) and C-3′ (δ_C 146.6). From the above data and comparison with reference [17], the structure of **6** was identified as isorhamnetin (Fig. 1).

Compound **7** was obtained as a pale-yellow powder. The ESI-MS spectrum which showed a molecular ion peak at *m/z* 479.3 [M+H]$^+$ and NMR data suggested that the molecular formula of **7** was $C_{22}H_{22}O_{12}$. The NMR data of **7** were similar to those of **6**, except for the addition of a

β-D-glucopyranosyl unit. The position of the sugar unit at C-4′ was determined by the HMBC interactions between the anomeric proton (δ_H 5.03)/H-2′ (δ_H 7.79)/H-5′ (δ_H 7.24)/H-6′ (δ_H 7.75) and C-4′ (δ_C 148.0). The methoxy group at C-3′ was also identified through the HMBC interactions between the methoxy protons (δ_H 3.84)/H-2′ (δ_H 7.79)/H-5′ (δ_H 7.24) and C-3′ (δ_C 148.6). So, the structure of **7** was determined to be isorhamnetin-4'-*O*-glucopyranoside (Fig. 1) by its NMR data and comparison with the reported data [18].

Compound **8** was obtained as a white powder. Its molecular formula was suggested as $C_{33}H_{52}O_9$ based on the positive ion peak at *m/z* 615.2 $[M+Na]^+$ and NMR spectra. The 1D-NMR of **8** showed characteristic signals of a spirostane-type saponin with four methyl groups at δ_H 0.83 (3H, s, H-18), 1.12 (3H, s, H-19), 1.01 (3H, d, J = 7.2 Hz, H-21), and 1.10 (3H, d, J = 7.2 Hz, H-27), one olefinic proton at δ_H 5.57 (1H, d, J = 6.0 Hz, H-6), two oxymethylene protons at δ_H 3.31 (1H, m, H-26) and 3.95 (1H, dd, J = 2.4, 10.8 Hz, H-26). The ^{13}C-NMR and DEPT spectrum of **8** exhibited signals of 33 carbons, of which 27 carbons were assigned to the aglycone moiety and 6 carbons to the monosaccharide moiety. The positions of two hydroxyl groups at C-1 and C-3 were determined by HSQC and HMBC correlations between H-19 (δ_H 1.12) with C-1 (δ_C 84.1), C-5 (δ_C 139.6), C-9 (δ_C 51.5), and C-10 (δ_C 43.5) together with COSY correlations of the sequence H-1/H-2/H-3. The NOESY correlations between H-1 (δ_H 3.51) and H-9 (δ_H 1.30) and between H-3 (δ_H 3.41) and H-9 (δ_H 1.30), suggesting both configurations of H-1 and H-3 to be α. The 25*S* configuration was confirmed by the difference of chemical shifts between H_a-26 and H_b-26 (Δ = 0.64 > 0.48) [19]. Considering these data and the reported literature [20], the aglycone of **8** was identified as 25(*S*)-spirost-5-en-1β,3β-diol. On the other hand, one anomeric proton signal at δ_H 4.29 (1H, d, J = 7.2 Hz, H-1') together with the proton signals of the hydroxymethine and hydroxymethylene groups at δ_H 3.50 (1H, m, H-2'), 3.48 (1H, m, H-3'), 3.87 (1H, d, J = 2.4 Hz, H-4'), 3.45 (1H, m, H-5'), 3.72 (1H, dd, J = 6.0, 11.4 Hz, H-6'), 3.77 (1H, dd, J = 6.0, 11.4 Hz, H-6') in the ^{1}H-NMR spectrum of **8** as well as signals of six carbons in the ^{13}C-NMR spectrum [δ_C 102.0 (C-1'), 72.6 (C-2'), 75.1 (C-3'), 70.2 (C-4'), 76.3 (C-5'), and 62.5 (C-6')], identified the sugar moiety as galactopyranosyl. The position of the galactopyranosyl at C-1 was determined by the HMBC correlation between H-1' (δ_H 4.29) and C-1 (δ_C 84.0). Based on the comparison of its NMR data with those reported [20], it can be concluded that **8** was (22*R*,25*S*)-spirost-5-en-1β,3β-diol 1-*O*-β-D-galactopyranoside (Fig. 1).

Compound **9** was also obtained as a white powder. The molecular formula of **9** was suggested as $C_{38}H_{60}O_{12}$ based on a *pseudo*-molecular peak at *m/z* 731.3 $[M+Na]^+$ and NMR spectra. The 1D-NMR spectral data of **9** were similar to those of **8** except for the presence of an α-L-arabinopyranosyl unit [δ_H 4.28 (1H, d, J = 7.2 Hz, H-1'), 3.72 (1H, m, H-2'), 3.66 (1H, dd, J = 3.6, 9.0 Hz, H-3'), 3.76 (1H, m, H-4'), 3.50 (1H, dd, J = 1.2, 12.6 Hz, H-5'), and 3.87 (1H, dd, J = 2.4, 12.6 Hz, H-5') and δ_C 101.0 (C-1'), 75.6 (C-2'), 76.1 (C-3'), 70.8 (C-4'), and 67.4 (C-5')] and α-L-rhamnopyranosyl moiety [δ_H 5.31 (1H, d, J = 1.8 Hz, H-1"), 3.90 (1H, dd, J = 1.8, 3.0 Hz, H-2"), 3.70 (1H, m, H-3"), 3.42 (1H, m, H-4"), 4.10 (1H, m, H-5"), and 1.26 (3H, d, J = 6.6 Hz, H-6") and δ_C 101.6 (C-1"), 72.4 (C-2"), 72.1 (C-3"), 74.2 (C-4"), 69.7 (C-5"), and 18.4 (C-6")]. The coupling constant (J = 7.2 Hz) of the arabinopyranosyl moiety suggested an α-anomer [21]. The α anomeric configuration of the rhamnopyranosyl moiety was confirmed by the small coupling constant (J = 1.8 Hz). The location of the sugar chain was identified by the correlations in COSY and HMBC spectra. In the HMBC spectrum, the correlation of H-1′ (δ_H 4.28) with C-1 (δ_C 84.6) along with a set of ^{1}H-^{1}H COSY cross-peaks H-1′/H-2′/H-3′/H-4′/H-5′ determined the *O*-glycosidic bond of this sugar unit to C-1 of the aglycone. The position of the rhamnose moiety at C-2′ was determined by the correlations of H-1" (δ_H 5.31) and C-2' (δ_C 75.6) in the HMBC spectrum and H-1″/H-2"/H-3"/H-4"/H-5"/H-6" system in the ^{1}H-^{1}H COSY spectrum. The location of the arabinose unit was identified at C-3′ *via* the HMBC correlations between H-1‴ (δ_H 4.88) and C-3' (δ_C 88.3) along with a set of ^{1}H-^{1}H COSY cross-peaks H-1‴/H-2‴/H-3‴/H-4‴/H-5‴/H-6‴. Comparing with the literature [22], it can be concluded that **9** was (22*R*,25*S*)-spirost-5-en-1β,3β-diol 1-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside] (Fig. 1).

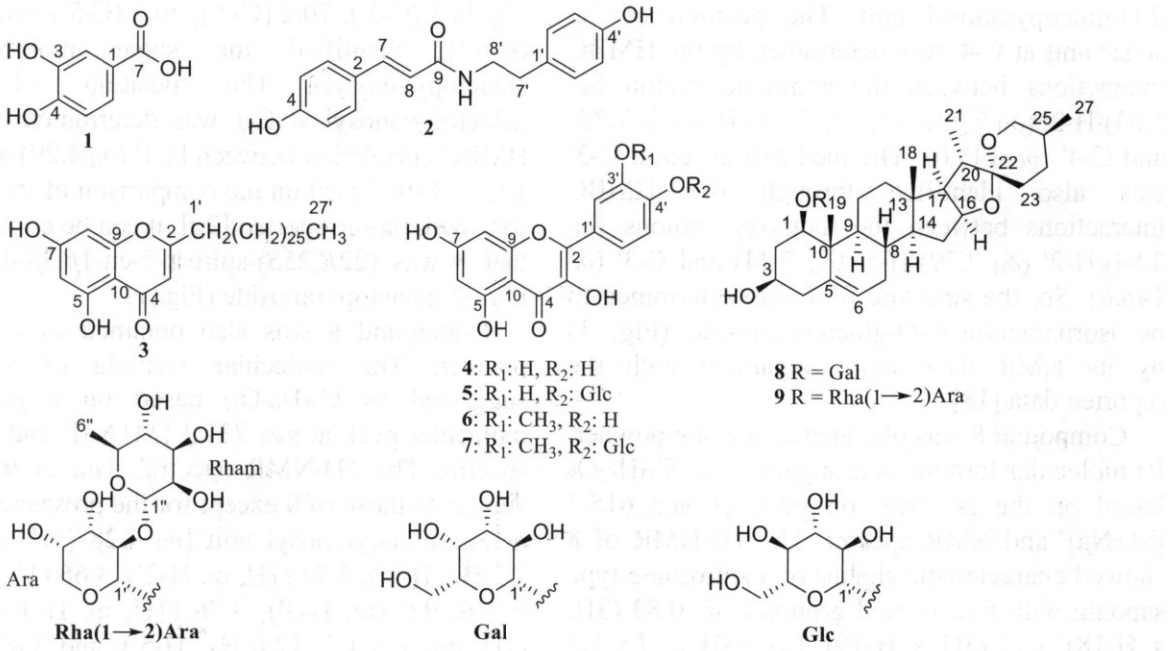

Fig. 1. Structures of compounds (**1-9**)

This is the first study on the chemical constituents of Ly Son onion, a typical onion variety of Ly Son Island, Quang Ngai province. As a result, nine compounds were isolated from bulbs of Ly Son onion, in which compound **3** was isolated from the *n*-hexane extract and 4 flavonoids (**4-7**), 2 saponins (**8** and **9**), protocatechuic acid (**1**), *trans-N*-coumaroyltyramine (**2**) were isolated from the ethyl acetate extract. Previous studies also indicated the presence of flavonoids and saponins in Ly Son onion [6],[9], however, compounds **2**, **3**, **8**, and **9** were reported for the first time from this plant. The results show that Ly Son onion possessed rich flavonoids, with a large content of isorhamnetin, quercetin, and their derivatives.

4. Conclusion

From 90% EtOH extract of the bulbs of Ly Son onion, nine compounds were isolated, including protocatechuic acid (**1**), *trans-N*-coumaroyltyramine (**2**), 5,7-dihydroxy-2-heptacosanyl-benzopyran-4-one (**3**), quercetin (**4**), quercetin 4'-glucopyranoside (**5**), isorhamnetin (**6**), isorhamnetin 4'-glucopyranoside (**7**), (22*R*,25*S*)-spirost-5-ene-1β,3β-diol 1-*O*-β-D-galactopyranoside (**8**), and (22*R*,25*S*)-spirost-5-en-1β,3β-diol 1-*O*-[α-L-rhamnopyranosyl-(1→2)-α-L-arabinopyranoside] (**9**). Among them, compounds **2**, **3**, **8**, and **9** were isolated for the first time from *A. ascalonicum* L..

Acknowledgments: *This research is financially supported by the National Institute of Medicinal Materials 2020 - 2021:* **Study on the chemical composition of Ly Son onion (*Allium ascalonicum* L.).**

References

1. Mohammadi-Motlagh H. R., Mostafaie A., Mansouri K. (2011), Anticancer and anti-inflammatory activities of shallot (*Allium ascalonicum*) extract, *Archives of Medical Science*. 7(1), 38. **2.** Mahmoudabadi A. Z., Nasery M. G. (2009), Anti fungal activity of shallot, *Allium ascalonicum* Linn.(Liliaceae), *in vitro*, *Journal of Medicinal Plants Research*. 3(5), 450-453. **3.** Mohammadi-Motlagh H. R., Mostafaie A., Mansouri K. (2011), Anticancer and anti-inflammatory activities of shallot (*Allium ascalonicum*) extract, *Archives of Medical Science: AMS*. 7(1), 38. **4.** National Institute of Medicinal Materials (2006), *The Medicinal Plants and Animals in Vietnam*, vol 1, Science and Technology Publishing, 902-903. **5.** Ehsani A., Mahmoudi R. (2012), Phytochemical properties and hygienic effects of *Allium ascalonicum* and *Pimpinella anisum* essential oils in Iranian white brined cheese, *Journal of Essential Oil Bearing Plants*. 15(6), 1013-1020. **6.** Fattorusso E., Iorizzi M., Lanzotti V., Taglialatela-Scafati O. (2002), Chemical composition of shallot (*Allium ascalonicum* Hort.), *Journal of Agricultural and Food Chemistry*. 50(20), 5686-5690. **7.** Pobłocka-Olech L., Głód D., Żebrowska M. E., Sznitowska M., Krauze-Baranowska M. (2016), TLC determination of flavonoids from different cultivars of *Allium cepa* and *Allium ascalonicum*, *Acta Pharmaceutica*. 66(4), 543-554. **8.** Mai N. T., Dung D. T., Nga T. T., Xuan V. T., Doan VV., Tai B. H., Nhiem N. X., Yen P. H., Kiem P. V., Seo Y., Namkung W. (2020), Triterpenoid glycosides from the rhizomes of *Allium ascalonicum* and their anoctamin-1 inhibitory activity, *Natural Product Research*. 35(22), 4338-4336. **9.** Kang L. P., Liu Z. J., Zhang L., Tan D. W., Zhao Y., Zhao Y., Chen H. B., Ma B. P. (2007), New furostanol saponins from *Allium ascalonicum* L., *Magnetic Resonance in Chemistry*. 45(9), 725-733. **10.** Huynh Nguyen Duy Bao, Tran Thi Loan and Pham Thi Hien

(2017), Effect of ultrasonic-assisted extraction conditions on antioxidant activity of *Allium ascalonicum* extract, *Journal of Fisheries Science and Technology* (1), 91-98. **11.** Pham Thi Nguyet Hang, Tran Nguyen Hong, Nguyen Van Hiep, Phi Thi Xuyen, Dinh Thi Minh, Nguyen Huu Son, Do Thi Ha, Pham Thanh Huyen, Nguyen Minh Khoi, Nguyen Trong Tue, Nguyen Thi Lap (2020), Screening study on the effect of some medicinal extracts on an experimental autistic *Drosophila melanogaster* model, *Journal of Medicinal Materials-Hanoi*. 25(2), 67-74. **12.** Gutzeit D., Wray V., Winterhalter P., Jerz G. (2007), Preparative isolation and purification of flavonoids and protocatechuic acid from sea buckthorn juice concentrate (*Hippophaë rhamnoides* L. ssp. *rhamnodes*) by high-speed counter-current chromatography, *Chromatographia*. 65(1), 1-7. **13.** Al-Taweel A. M., Perveen S., El-Shafae A. M., Fawzy G. A., Malik A., Afza N., Iqbal L., Latif M. (2012), Bioactive phenolic amides from *Celtis africana*, *Molecules*. 17(3), 2675-2682. **14.** Sultana N., Armstrong J. A., Waterman P. G. (1999), Benzopyran derivatives from the aerial parts of *Eriostemon rhomboideus*, *Phytochemistry*. 52(5), 895-900. **15.** Zhang Y., Wang D., Yang L., Zhou D., Zhang J. (2014), Purification and characterization of flavonoids from the leaves of *Zanthoxylum bungeanum* and correlation between their structure and antioxidant activity, *PloS One*. 9(8), e105725. **16.** Fossen T., Pedersen A. T., Andersen Ø. M. (1998), Flavonoids from red onion (*Allium cepa*), *Phytochemistry* 47(2), 281-285. **17.** Cao X., Wei Y., Ito Y. (2008), Preparative isolation of isorhamnetin from *Stigma maydis* using high speed countercurrent chromatography, *Journal of Liquid Chromatography & Related Technologies*. 32(2), 273-280. **18.** Park Y. K., Lee C. Y. (1996), Identification of isorhamnetin 4'-glucoside in onions, *Journal of Agricultural and Food Chemistry*. 44(1), 34-36. **19.** Agrawal P. K. (2004), Dependence of ^{1}H NMR chemical shifts of geminal protons of glycosyloxy methylene (H2-26) on the orientation of the 27-methyl group of furostane-type steroidal saponins, *Magnetic Resonance in Chemistry*. 42(11), 990-993. **20.** Chen N., Zhang J., Liu J., Yu B. (2010), Highly efficient and regio-selective glucosylation of 25(*S*)-ruscogenin by *Gliocladium deliquescens* NRRL1086, *Chinese Journal of Chemistry*. 28(3), 439-442. **21.** Wang X., Wang M., Xu M., Wang Y., Tang H., Sun X. (2014), Cytotoxic oleanane-type triterpenoid saponins from the rhizomes of *Anemone rivularis* var. *flore-minore*, *Molecules*. 19(2), 2121-2134. **22.** Liu Y., Qiu P., Wang M., Lu Y., He H., Tang H., Zhang B. L. (2021), New steroidal saponins isolated from the rhizomes of *Paris mairei*, *Molecules*. 26(21), 6366.

Journal of Medicinal Materials, 2022, Vol. 27, No. 1 (pp. 9 - 14)

CHEMICAL CONSTITUENTS FROM THE ETHYL ACETATE EXTRACT OF *PAEDERIA SCANDENS* (LOUR.) MERR. COLLECTED IN VIETNAM

Tran Thanh Ha [1,*], Nguyen Thi Ha[1], Nguyen Tuan Hiep[1], Le Thi Huyen[2]

[1]*National Institute of Medicinal Materials, Hanoi, Vietnam;*

[2]*University of Natural Sciences - Vietnam National University, Hanoi*

*Corresponding author: thanhha.tran889@gmail.com

(Received December 21th, 2021)

Summary

Chemical Constituents from Ethyl Acetate Extract of *Paederia scandens* (Lour.) Merr. Collected in Vietnam

Phytochemical study on the ethyl acetate extract of the rhizomes of Paederia scandens (Lour.) Merr. resulted in the isolation of nine compounds: anthraquinone (**1**), 3,3′-dimethoxyellagic acid 4′-O-α-L-rhamnopyranoside (**2**), 3′,4′,7-trihydroxyflavanone (**3**), betulinic acid (**4**), kaempferol (**5**), linarin (**6**), paederoside (**7**), quercetin 3-O-α-L-rhamnosyl-(1→6)-β-D-glucopyranosyl-(1→3)-β-D-glucopyranoside (**8**), and kaempferol 3-O-β-D-glucopyranoside (**9**) on the basis of experimental spectral data. Compounds **1**, **2**, **3**, **6**, and **8** were reported from P. scandens for the first time.

Keywords: *Paederia scandens* (Lour.) Merr., *Rubiaceae quercetin glycoside.*

1. Introduction

Paederia scandens (Lour.) Merrill belonging to Rubiaceae, is used to treat aches, jaundice, dysentery, and dyspepsia as a folk medicine in the southern region of China, Vietnam, India, and Japan [1]. The roots, leaves, bark, and fruits of this plant have been used for the treatment of toothache, chest pain, inflammation, jaundice, and dysentery [2]. In 1969, Inouye et al. isolated four iridoid glycosides from this species for the first time [3]. From then on, more than 50 compounds have been obtained from *P. scandens*. Among these, iridoid glycosides, flavonoids, and volatile oils are the major constituents [4], which were mainly responsible for the anti-nociceptive, anti-inflammatory, and antitumor activities of *P. scandens* [5],[6],[7].

Quang et al. reported the isolation of anthraquinones and iridoid glycosides from the leaves of *P. scandens* in Vietnam as 1,3-dihydroxy-2,4-dimethoxy-9,10-anthraquinone, 2-hydroxy-1,4-dimethoxy-9,10-anthraquinone, 1-methoxy-2-methoxymethyl-3-hydroxy-9,10-anthraquinone, 1-hydroxy-2-hydroxymethyl-9,10-anthraquinone, paederoside, asperuloside, paederosidic acid, asperulosidic acid, and geniposide [8],[9]. This paper reports the isolation and structural elucidation of five known compounds from this plant, collected in Dong Nai province, Vietnam.

2. Experimental

2.1. Plant material

The rhizomes of *Paederia scandens* (Lour.) Merr. were collected in Dong Nai province,