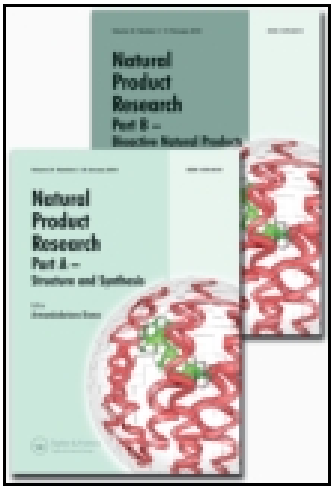

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Main constituents from the seeds of Vietnamese Cnidium monnieri and cytotoxic activity

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SHORT COMMUNICATION

Main constituents from the seeds of Vietnamese *Cnidium monnieri* and cytotoxic activity

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From the ethyl acetate extract of the seeds of Vietnamese *Cnidium monnieri* L., three coumarins, osthole (**1**), xanthotoxin (**2**), imperatorin (**3**) and a sterol, daucosterol (**4**) have been purified. Their structures were elucidated by spectroscopic analysis. Furthermore, 8-(3-hidroxy-3-methylbutyl)-7-methoxycoumarin (**5**) was synthesised from osthole (**1**) with a good yield (80%). In addition, compound **1** and its synthesis product (**5**) show moderate and non-selective cytotoxic activities against four cancer cells, KB (a human epidermal carcinoma), MCF7 (human breast carcinoma), SK-LU-1 (human lung carcinoma) and HepG2 (hepatocellular carcinoma).

Keywords: *Cnidium monnieri*; cytotoxicity; cancer cell

1. Introduction

Cnidium monnieri L. (Apiaceae) is a medicinal plant widely grown in regions around Vietnam, China, Japan, Russia, etc. It has been used in folk medicine for the treatment of several diseases such as male sexual dysfunction and impotence, allergy, osteoporosis, asthma and AIDS (Do, 2001; Vo, 1999). The chemical investigation of the seeds of this plant revealed that it contains volatile components such as pinene, camphene, osthole (Do, 2001; Vo, 1999), and several sesquiterpenes with hepatoprotective activity such as torilin and torilolone (Oh et al., 2002). The major compounds obtained from this seeds are coumarins, osthole, imperatorin, bergapten, isopimpinellin, and xanthotoxin (Yang, Wang, Chen, & Wang, 2003) which have various biological activities. For instance, both osthole and imperatorin caused apoptotic bodies, DNA fragmentation and enhanced PARP degradation in HL-60 cells (Yang et al., 2003). In addition, osthole could increase androgen, gonadotropin and nitric oxide synthase activity (Yuan, Xie, Li, & Zhou, 2004). In this article, we report the chemical constituents of the seeds of Vietnamese *C. monnieri* L. with the isolation of four compounds osthole, xanthotoxin, imperatorin and daucosterol together with the cytotoxic properties of osthole and its synthesised derivative against four cancer cells.

2. Results and discussion

The ethyl acetate extract of *C. monnieri* L. was subjected to silica gel column chromatography to yield four compounds (**1**–**4**) (Figure 1) as described in Section 3.

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Table 1. The cytotoxic activities of compounds **1** and **5**.

Compound \ Cell	IC_{50} ($\mu g\,mL^{-1}$)			
	KB	MCF7	SK-LU-1	HepG2
1	62.42	62.17	>128	21.58
5	87.43	100.8	110.27	35.91

Compound **1** was obtained as white crystal, m.p. 83.5°C. Its IR spectrum exhibits the absorption of a α,β-unsaturated lactone (1719 cm^{-1}). Then, compound **1** is determined to be osthole by interpreting its NMR and MS spectra as well as comparing then with those given in the published paper (Okamoto, Kobayashi, & Yoshida, 2007). Similarly, other compounds are determined as xanthotoxin (**2**) (Huong et al., 1999), imperatorin (**3**) (Ngwendson et al., 2003) and daucosterol (**4**) (Han, Li, Zhang, Zheng, & Qin, 2006). These coumarins (**1**–**3**) have been also reported from Chinese *C. monnieri* L. and they could be isolated by a high-speed counter-current chromatography method (Renmin, Lei, Ailing, & Lingyi, 2004).

Osthole (**1**) has been converted into its derivatives by several reactions, for instance, oxidation with chromium trioxide, and some of its derivatives showed valuable immunomodulatory and anti-inflammatory activities (Zimecki et al., 2009). In this case, we obtained a large amount of osthole (**1**) which allowed us to make its derivative, compound **5**, by one pot reaction with a good yield (80%). The structure of **5** was suggested by the presence of a hydroxyl absorption band at 3412 cm^{-1} in its IR spectrum. In addition, the signal for an olefinic proton (H-2′) disappeared, but the signals for an aliphatic protons H-2′ (2.89 ppm) are observed in its ^{1}H-NMR spectrum, proven that compound **5** is formed by the addition of a water molecule to a double bond (C2′-C3′) of osthole (**1**). Then, two compounds **1** and **5** were tested for their cytotoxic activities against cancer cells, KB, MCF7, SK-LU-1 and HepG2. The results are illustrated in Table 1. Accordingly, both of them inhibit the growth of cancer cells, especially compounds **1** and **5** strongly suppress HepG2 cell with the IC_{50} values 21.58 and 35.91 $\mu g\,mL^{-1}$, respectively. In addition, the activity against SK-LU-1 cancer cell of compound **5** sufficiently increases when losing one double bond in a side chain of osthole (**1**).

3. Experimental

3.1. *General procedure*

Melting points were determined on a Gallenkamp apparatus. IR spectra were measured on a Shimadzu 8101 M FT-IR. Mass spectra were recorded on MS-Engine 5989-HP. The ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Advance 500 MHz. Column chromatography was carried out on silica gel 60 (0.2–0.5 mm, 0.04–0.063 mm, Merck). TLC was performed on silica gel plates (Kiesegel 60 F254, Merck). The spots of TLC were detected under UV 254 nm and by spraying with 10% H_2SO_4 in methanol, followed by heating at 120°C.

3.2. *Plant material*

Seeds of *C. monnieri* L. was collected in Haiduong, Vietnam in September 2009 and identified by Dr Ninh Khac Ban, Institute of Ecology and Biological Resource, Vietnam

Academy of Sciences and Technology. Voucher specimen (no. PHD20090318) has been deposited at Faculty of Chemistry, Hanoi University of Education, Vietnam.

3.3. *Extraction and isolation*

Dried seeds of *C. monnieri* L. (2 kg) were extracted with ethanol : water (4 : 1). The total extract was concentrated by rotary evaporator to give 130 g residue which was partitioned between *n*-hexane, EtOAc and MeOH. The EtOAc layer was collected and solvent evaporated to yield a residue (50 g) which was later chromatographed on a silica gel column (gradient of hexane–EtOAc) to give compounds **1** (25 mg), **2** (120 mg), **3** (84 mg) and **4** (25 mg).

Compound 1: Colourless needles; m.p. 83–84°C; IR (KBr) ν_{max}: 3038, 3017, 2916, 2852, 1720, 1610, 1562, 1432, 1278, 1248 and 828 cm^{-1}; EI-MS *m/z* 245 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$): δ 7.61 (1H, dd, $J = 2.0$, 9.5 Hz, H-4), 7.29 (1H, dd, $J = 1.5$, 7.0 Hz, H-5), 6.83 (1H, dd, $J = 1.5$, 8.5 Hz, H-6), 6.22 (1H, dd, $J = 3.5$, 9.5 Hz, H-3), 5.22 (1H, tt, $J = 1.5$, 6.0 Hz, H-2′). 3.53 (2H, d, $J = 7.0$ Hz, H-1′), 3.92 (3H, s, 7-OMe), 1.84 (3H, s, H-4′), 1.66 (3H, s, H-5′) and ^{13}C-NMR ($CDCl_3$): δ 161.3 (C-2), 160.2 (C-7), 152.8 (C-9), 143.7 (C-4), 132.6 (C-3′), 126.2 (C-5), 121.1 (C-2′), 117.9 (C-8), 112.9 (C-3, C-6), 107.3 (C-10), 56.0 (7-OMe), 25.7 (C-4′), 21.9 (C-1′) and 17.9 (C-5′).

Compound 2: Colourless needles; m.p. 147–148°C; IR (KBr) ν_{max}: 3142, 2973, 1717, 1625, 1585, 1455, 1131 and 981 cm^{-1}; EI-MS *m/z* 246 $[M]^+$; ^{1}H-NMR (DMSO): δ 8.18 (1H, d, $J = 9.5$ Hz, H-4), 8.08 (1H, d, $J = 2.5$ Hz, H-2′), 7.36 (1H, d, $J = 2.5$ Hz, H-3′), 6.33 (1H, d, $J = 10$ Hz, H-3), 4.17 (3H, s, 8-OMe), 4.04 (3H, s, 5-OMe) and ^{13}C-NMR (DMSO): δ 159.5 (C-2), 149.4 (C-7), 146.2 (C-2′), 144.2 (C-5), 143.1 (C-9), 139.6 (C-4), 127.2 (C-8), 114.2 (C-6), 112.5 (C-3), 106.8 (C-10), 105.6 (C-3′), 61.2 (5-OMe) and 60.8 (8-OMe).

Compound 3: Powder, m.p. 95–96°C; IR (KBr) ν_{max}: 3111, 2981, 1717, 1628, 1584, 1400, 1148 and 881 cm^{-1}; EI-MS *m/z* 270 $[M]^+$; ^{1}H-NMR (DMSO): δ 8.12 (1H, d, $J = 2.5$ Hz, H-2′), 8.10 (1H, d, $J = 9.5$ Hz, H-4), 7.66 (1H, s, H-5), 7.07 (1H, d, $J = 2.5$ Hz, H-3′), 6.42 (1H, d, $J = 9.5$ Hz, H-3), 5.50 (1H, tt, $J = 1.5$, 7.5 Hz, H-2″), 4.90 (1H, d, $J = 7.0$ Hz, H-1″), 1.64 (3H, s, H-5″), 1.63 (3H, s, H-4″). and ^{13}C-NMR (DMSO): δ 159.7 (C-2), 147.7 (C-7, C-2′), 145.2 (C-9), 145.0 (C-4), 138.9 (C-3″), 130.5 (C-8), 125.6 (C-6), 119.6 (C-2″), 116.0 (C-10), 114.1 (C-3), 114.0 (C-5), 106.9 (C-3′), 69.3 (C-1″), 25.4 (C-4″), 17.7 (C-5″).

Compound 4: Colourless needles; m.p. 280–281°C; ^{13}C-NMR ($CDCl_3$ and CD_3OD): δ 140.1 (C-5), 121.8 (C-6), 100.8 (C-1′), 78.9 (C-3), 76.2 (C-3′), 75.6 (C-5′), 73.3 (C-2′), 69.9 (C-4′), 61.5 (C-6′), 56.6 (C-17), 56.5 (C-14), 51.0 (C-9), 45.6 (C-24), 42.1 (C-13), 40.2 (C-4), 39.4 (C-12), 38.4 (C-1), 37.0 (C-10), 36.9 (C-20), 36.4 (C-11), 33.7 (C-22), 31.6 (C-7 and C-8), 29.3 (C-2, C-25), 28.6 (C-16), 25.8 (C-23), 24.0 (C-15), 23.9 (C-28), 20.7 (C-26), 19.4 (C-19), 18.9 (C-21), 18.6 (C-27), 11.8 (C-18) and 11.5 (C-29); IR (KBr) ν_{max}: 3423, 2939, 2875, 1622 and 1467 cm^{-1}.

Synthesis of 5: (8-(3-hydroxy-3-methylbutyl)-7-methoxycoumarin): Osthole (**1**, 0.25 g) was dissolved in acetic acid (7 mL) in a flask and then the flask was cooled in ice water. After that, 98% H_2SO_4 (1.5 mL) was added and stirred in 1 h, at 5–10°C. Finally, ice water (50 mL) was added to the reaction mixture to give a colourless crystal that was recrystallised several times in EtOH to yield **5** (0.21 g) with a good yield (80%).

Colourless needles (ethanol), m.p. 101–102°C. IR (KBr): 3411, 2979, 2852, 1698, 1608, 1565, 1253, 1086 and 841 cm^{-1}. FT-ICR-MS: *m/z* 285.1103 $[M + Na]^+$ (Calcd for $C_{15}H_{18}O_4Na$: 285.1102). ^{1}H-NMR (500 MHz, acetone-d_6): 7.86 (1H, d, $J = 9.5$ Hz, H-4), 7.48 (1H, d, $J = 9$ Hz, H-5), 7.02 (1H, d, $J = 8.5$ Hz, H-6), 6.19 (1H, d, $J = 9.5$ Hz,

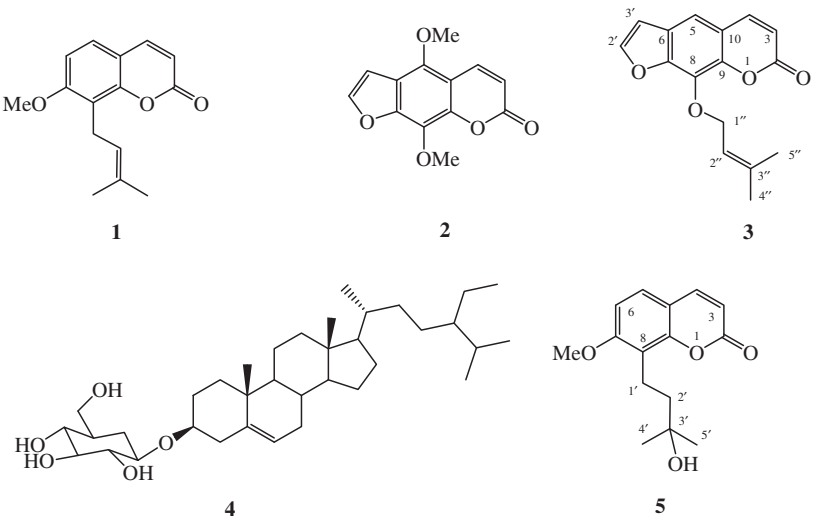

Figure 1. Structures of **1**–**5**.

H-3), 3.95 (3H, s, 7-OMe), 2.89 (2H, m, H-2′), 1.65 (2H, m, H-1′) and 1.27 (3H, s, H-4′ and H-5′).

3.4. *Cytotoxicity assay*

Compounds **1** and **5** were tested against KB, MCF7, SK-LU-1 and HepG2 from American Type Culture Collection according to the method described by Scudiero et al. (1988). Cell lines were cultured in RPMI 1640 medium supplemented with 10% foetal bovine serum (FBS) in standard condition, sterile with 5% CO_2, 37°C, 98% humidity and harvested at log phase for assays. In this assay, 200 µL volumes of cells at the concentration of 3×10^4 cells mL^{-1} were inoculated into a 96-well plate in RPMI 1640 medium. Compounds **1** and **5** were applied at final concentrations 128, 32, 8, 2 and 0.5 µg mL^{-1} and cultures incubated for 3 days at 37°C with 5% CO_2. Then, 50 µL of MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, prepared at 1 mgmL^{-1} in FBS, was added to microculture wells. After 4 h of incubation, 250 µL of the supernatant was removed from each well and 100 µL of DMSO added and thoroughly mixed. Absorbance was measured at 540 nm in a Genios TECAN spectrophotometer. IC_{50} value was calculated based on the percentage of growth inhibition $(OD_{control} - OD_{sample})/OD_{control.}$

4. Conclusions

Phytochemical studies on the EtOAc extract of the seeds of Vietnamese *C. monnieri* L. has led to the isolation and structural elucidation of three coumarins, osthole (**1**), xanthotoxin (**2**), imperatorin (**3**) and a sterol, daucosterol (**4**). Osthole (**1**) and its derivative (**5**) possess moderate cytotoxic activities against four cancer cells, KB, MCF7, SK-LU-1 and HepG2 (Figure 1).

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