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Phytochemical constituents and cytotoxic activity of *Physalis angulata* L. growing in Vietnam

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ABSTRACT

A comprehensive phytochemical investigation of the dichloromethane-soluble fraction of *Physalis angulata* led to the isolation of eight compounds (**1–8**) including two new withanolides named physagulin P and Q (compounds **5** and **8**). Their chemical structures were established using two-dimensional nuclear magnetic resonance (spectroscopy. The cytotoxic activity of these metabolites was investigated against A549, HeLa, and PANC-1 cancer cell lines. Among them, compound **1** showed significant cytotoxic activity against A549 cells with a half-maximal inhibitory concentration (IC_{50}) value of 8.27 μM, while compound **7** showed the most potent activity against PANC-1 cells with an IC_{50} value of 3.18 μM.

1. Introduction

Physalis angulata L. (Solanaceae) is a branching annual shrub widely distributed throughout Vietnam. The four *Physalis* genus include *P. angulata*, *P. alkekengi*, *P. peruviana*, and *P. minima* (Võ, 2012). Most species have been used for a long time in the ethno-medical folk traditions of Asian and American populations to treat different illnesses such as malaria, asthma, hepatitis, dermatitis, liver disorders; and as anti-mycobacterial, anti-cancer, anti-leukemic, antipyretic, and immune-modulatory agents (Chiang et al., 1992a, 1992b; Lin et al., 1992; Pietro et al., 2000). These species are a rich natural resource of withanolides with many pharmacological effects (Nagafuji et al., 2004; Soares et al., 2006; Magalhães et al., 2006; He et al., 2007; Damu et al., 2007). For example, withangulatin A, an active withanolide isolated from *P. angulata*, exhibits anti-tumour and trypanocidal activities, and affects T lymphocyte function in mice by inhibiting COX-2 expression via the MAPKs and NF-kappaB nuclear translocation signalling pathways (Sun et al., 2010). Physalins have dose-related antinociceptive effects in writhing and formalin tests, and inhibit inflammatory parameters such as hyperalgesia, edema, and local production of TNF-α following induction with complete Freund's adjuvant (Lima Mda et al., 2014). Physalin F, a *seco*-steroid, displays immunosuppressive activity in peripheral blood mononuclear cells from patients with HTLV1-associated myelopathy (Pinto et al., 2016). The present work reports the isolation and identification of two new withanolides (**5** and **8**) along with six known compounds as well as their cytotoxicity properties against adenocarcinomic human alveolar basal epithelial (A549), epitheloid cervix carcinoma (HeLa), and human pancreas (PANC-1) cancer cell lines.

2. Results and discussion

The whole plant of *P. angulata* was air dried, ground to a powder, and extracted with methanol at room temperature using ultrasonic radiation. The obtained alcohol extract was partitioned into dichloromethane, ethyl acetate, and aqueous fractions. As previously reported, most withanolide compounds are present in chloroform fractions. So in our study, this solvent was selected for the isolation of active constituents. Chromatographic purification of the dichloromethane-soluble fraction led to the isolation of eight compounds

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(**1–8**). The known compounds were identified as physagulin J (**1**), withaminimine (**2**), physagulin N (**3**), physagulin K (**4**), physagulin L (**6**), and physagulin M (**7**) (Supporting Information) by comparison of their NMR spectroscopic data to those previously reported (Ding et al., 2014; Zhang and Tong, 2016; Maldonado et al., 2015; Gao et al., 2015).

Compound **5** was obtained as a white amorphous powder. The molecular formula of **5** was assigned as $C_{30}H_{40}O_7$ (from the positive-mode high-resolution electrospray ionisation mass spectrometry (HR-ESI-MS) quasi molecular ion at *m/z* 535.2639 $[M + Na]^+$ (calculated for $C_{30}H_{40}O_7Na$, 535.2666). The ^1H NMR spectrum of **5** exhibited five methyl single signals at δ 1.22 (3H, s, H-18), 1.44 (3H, s, H-19), 1.85 (3H, s, H-27), 1.98 (3H, s, H-28), and 1.85 (3H, s, H-30), five methylenes including a lactone ring methylene (δ 2.09 [1H, br d, J = 16.5 Hz, H-23a] and 2.52 [br t, J = 16.5 Hz, H-23b]), and six sp^3 methines including three oxymethines at δ 4.58 (1H, t, J = 3.0 Hz, H-6), 4.92 (1H, d, J = 4.5 Hz, H-15), and 4.44 (1H, dt, J = 3.5, 13.0 Hz, H-22). The signals at δ 5.99 (1H, d, J = 9.5 Hz, H-2), 7.10 (1H, dd, J = 6.0, 9.5 Hz, H-3), and 6.25 (1H, d, J = 6.0 Hz, H-4) indicated the presence of three olefinic protons. The ^{13}C and DEPT NMR spectra presented 30 signals that could be assigned to a physagulin derivative bearing a withanolide skeleton with a lactone ring in the side chain (He et al., 2007; Maldonado et al., 2015). Among them, the signals at δ 74.4 (C-6), 81.3 (C-15), and 80.2 (C-22) were assigned to three oxygenated methines, while the other four sp^3 methine carbons were observed at δ 38.1 (C-8), 46.5 (C-9), 53.3 (C-17), and 39.0 (C-20). In addition, other featured signals at δ 160.3 (C-5), 55.5 (C-10), and 47.3 (C-13) were assigned to three quaternary carbons, and the signal at δ 85.7 was attributed to an oxygenated carbon (C-14). The ketone carbon signal at δ 208.1 (C-1), an oxygenated quaternary carbon at δ 169.3 (C-26). Two signals at δ 152.7 and 122.5 corresponded to the tetrasubstituted carbons C-24 and C-25 of a lactone ring, respectively. Interestingly, an acetyl group present in this compound was identified by the carbon and proton signals at δ_C 171.2 (C-27), δ_C 21.3 (C-28), and δ_H 1.85 (3H, s, H-28). The proton (^1H) and carbon-13 (^{13}C) NMR and correlation spectroscopy (COSY) data of **5** indicate that this compound is quite similar to physagulin J (Zhang and Tong, 2016) by sharing the same substitution pattern at rings B, C, D, and E, except for the absence of one hydroxyl group of **5** at the C-5 position of ring A (Figs. 1 and 2). The coupling constant J = 6.0 Hz of the olefinic proton signals at δ 7.10 (H-3) and 6.25 (H-4) confirmed the location of a double bond between C-3 and C-4, and C-4 and C-5. This difference was again confirmed by the heteronuclear multiple bond correlation (HMBC) spectrum, in which the signal at δ 6.25 (H-4) was correlated with δ 126.4 (C-2), 142.6 (C-3), 160.3 (C-5), and 55.6 (C-10) (Fig. 2). From these data, the two C=C bonds were located at C−2 and C−4 of ring A, which is similar to that of physagulins L and M isolated from the same plant (He et al., 2007; Maldonado et al., 2015). Interestingly, there was no nuclear Overhauser effect spectroscopy (NOESY) cross-peak between proton δ 4.58 (1H, t, J = 3.0 Hz, H-6) and the methyl group at δ 1.44 (3H, s, H-19), which suggests that they are not in the same orientation; this implies that the hydroxyl group at C-6 of **1** is in the *β*-orientation (Supporting Information). Accordingly, the chemical structure of **1** was determined to

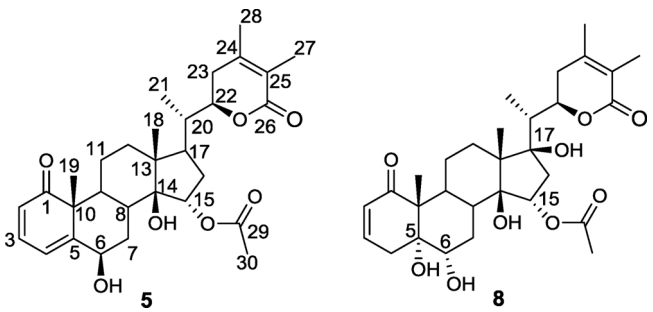

Fig. 1. Chemical structures of isolated compounds **5** and **8**.

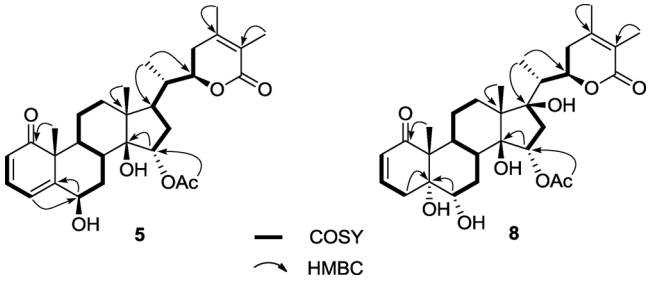

Fig. 2. Key COSY and HMBC correlations of compounds **5** and **8**.

be (6β,14β,15α,22R)-22,26-epoxy-6,14-dihydroxyergosta-2,24-diene-1,26-dione,15-yl acetate, and has been given the name physagulin P.

Compound **8** was obtained as a white amorphous powder. Its molecular formula was established as $C_{30}H_{42}O_9$ from the positive-mode HR-ESI-MS quasi molecular ion at *m/z* 569.2718 $[M + Na]^+$ (calculated for $C_{30}H_{42}O_9Na$, 569.2721). The ^1H-NMR spectrum showed six methyl, six methylene, two methine, and three oxymethine signals. Two olefinic proton signals appeared at δ 6.01 (1H, dd, J = 2.0, 10.0 Hz, H-2) and 6.88 (1H, ddd, J = 2.5, 5.0, 10.0 Hz, H-3). Analyses of the ^{13}C distortionless enhancement by polarisation transfer (DEPT) NMR spectrum of **8** revealed signals for 30 carbons, which were divided into nine non-protonated carbons including three carbonyls, nine methines including four carbons of double bonds, six methylenes, and six methyl carbon signals. Two carbon signals corresponding to an acetyl group were also evident (Table 1 and Fig. 1). The assignments of the ^1H and ^{13}C NMR data of **8** were completed using heteronuclear single quantum coherence (HSQC) and HMBC spectroscopies (Fig. 2). These NMR data were in good agreement with those reported for physagulin K (**4**) (Zhang and Tong, 2016; Maldonado et al., 2015). However, in **8**, NOESY cross-peaks between methyl groups 18-Me/19-Me and proton H-6, and between proton H-6 and proton H-15, were observed. This suggests that the withanolide skeleton had the *α*-orientation of hydroxyl group at C-6 (Supporting Information). In addition, the chemical shift of the β-orientated proton was upfield at δ 4.31 (dd, J = 5.0 and 12.0 Hz), which was quite different from the chemical shift of the same position in compound **4** at δ 3.61 (d, 3.5 Hz) (He et al., 2007). Based on the above evidence, **8** was elucidated as 5α,6α,14β,15α,17β,22R)-22,26-epoxy-5,6,14,17-tetrahydroxy-1,26-dioxoergosta-2,24-dien-15-yl acetate, and was given the name physagulin Q.

To investigate anti-cancer effects, the isolated withanolides (**1–8**) were tested for their inhibitory activities against human cancer cells as A-549, HeLa, and PANC-1; etoposide was used as the positive control. Compounds **5–7** showed significant inhibitory activities toward the A549 cancer cell line with IC_{50} values ranging from 11.8 to 21.5 μM (Table 2); compounds **3**, **4,** and **8** displayed weak inhibitory activities. Interestingly, compound **1** had the most potent inhibitory effect against A549 with an IC_{50} value of 8.27 μM. All compounds were inactive against HeLa cancer cells. Concerning the PANC-1 cytotoxic effect, compound **7** exhibited very potent inhibitory activity with an IC_{50} value of 3.18 μM, followed by **6** with an IC_{50} value of 6.30 μM. Compounds **5** and **2** showed significant cytotoxic activity with IC_{50} values of 20.2 and 34.0 μM, respectively. The remaining compounds showed no effects.

3. Materials and methods

3.1. Plants material

The whole plant of *P. angulata* was collected at Thai Binh province, Vietnam, in August 2015. Its scientific name was identified by Dr. Tran Thi Phuong Anh, Vietnam National Museum of Nature. A voucher specimen (TB14.2015) was deposited at the Herbarium of Mientrung Institute for Scientific Research, Vietnam Academy of Science and Technology.