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Original Research

Peruranolides A–D, four new withanolides with potential antibacterial and cytotoxic activity from *Physalis peruviana* L.

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Abstract

Background: Many drugs for anti-tumour have been developed, nevertheless, seeking new anticancer drug is the focus of ongoing investigation. Withanolides have been reported to possess potent antiproliferative activity. Literature findings revealed that a diversity of withanolides were obtained from *Physalis peruviana*, however, the antitumor activity of these bioactive compounds is still unclear. **Methods**: The EtOAc fraction of *P. peruviana* were decolorized on Middle Chromatogram Isolated (MCI) Gel column, repeatedly subjected to column chromatography (CC) over sephadex LH-20, preparative High Performance Liquid Chromatography (HPLC) and silica gel to afford compounds. Their chemical structures of the new isolates were elucidated through analyzing spectroscopic and HRESIMS data. All these obtained metabolites were appraised for their potential antiproliferative activity against the human breast cancer cell line MCF-7 by MTT assay, and *in vitro* antibacterial activity of the isolated compounds (**1**–**7**) were evaluated against *E. coli*, *B. cereus* and *S. aureus*. **Results**: Four new withanolides, including one withaphysalin-type withanolide (peruranolide A, **1**), two 13,14-seco-withaphysalins (peruranolides B–C, **2**–**3**), as well as one normal withanolide (peruranolide D, **4**), were purified and separated from *P. peruviana* L.. Compound **5** was discovered to exhibit potent cytotoxic effect with an IC_{50} value of 3.51 μM. *In vitro* antibacterial activities, compounds **1**–**7** had no obvious inhibitory activity against *E. coli*, but had moderate inhibitory activities against *B. cereus* and *S. aureus*. **Conclusions**: Our findings might offer valuable clues for the utilization of withanolides as lead compounds for antineoplastic or antibacterial drug development.

Keywords: withanolides; *Physalis*; structure elucidation; antiproliferative activity; antibacterial activity

1. Introduction

In the past decade, considerable attention has been paid to tumour, which was the primary leading cause of premature death (age between 30 and 69 years) [1]. At present, many drugs for anti-tumour have been developed, such as alkylating agents, anti-metabolic drugs and anti-tumor antibiotics; nevertheless, seeking new anticancer drug is the focus of ongoing investigation.

Withanolides, the natural steroids mainly distributed in Solanaceae, are a group of ergostane compounds with 28 carbons, in which C-23/C-26, or C-22/C-26 properly oxidized resulting in the formation of a δ- or γ-lactone ring [2]. To date, withanolides have been reported to possess potent antiproliferative activity [3,4]. For instance, 4β-Hydroxywithanolide E, physagulide P, irinans A–B, isolated from the genus of *Physalis* (Solanaceae), were exploited to be effective against the cancer cell lines of liver, lung, and breast [5–9].

The genus *Physalis*, containing approximately 120 species around the world, distributed mostly in tropical and temperate regions of America. *Physalis peruviana* L., as a traditional folk medicine, has been extensively used for a variety of therapeutic purposes [10]. For example, *P. peruviana* has been exploited as heat-clearing and detoxifying, antiphlogistic, diuretic, applied in Sore throat, swollen gums, pemphigus, eczema, etc. [11]. Literature findings revealed that a diversity of physalins, C28 steroidal lactones as well as withanolides were obtained from *P. peruviana* [12,13], however, the antitumor activity of these bioactive compounds is still unclear [14,15]. This indicated the strong possibility that withanolides obtained from *P. peruviana* have a tendency to anticancer.

Herein, the detailed isolation and structural characterization of these four novel withanolides along with three known ones from the title plant, as well as their antiproliferative toward the human breast cancer cell line MCF-7 and *in vitro* antibacterial activity against *E. coli*, *B. cereus* and *S. aureus* were presented.

2. Materials and methods

2.1 General experimental procedures

The materials and instruments for the purification and for the spectroscopic measurements of the compounds from the title plant are detailed in the Supporting Information.

2.2 Plant material

P. peruviana (whole plant) were collected in Maoming City of Guangdong province, China, in September 2019. The plant material was authenticated by Dr. Jiewei Wu from Guangzhou University of Chinese Medicine, and a voucher specimen (No. 20190901) was deposited at the Laboratory of New Drug Lead Compound, Guangzhou University of Chinese Medicine.

2.3 Extraction and isolation

10 kilograms of air-dried powder *P. peruviana* were extracted with EtOH/H_2O (3 × 20 L, 95:5, v/v, three times, room temperature) to obtain a crude extract, which was then extracted with ethyl acetate (EtOAc). The EtOAc fraction (177.95 g) was decolorized on MCI gel column with EtOH/H_2O (30/70, 50/50, 70/30, v/v) to divide into three fractions (A, B, C). Frs. A and B were repeatedly subjected to column chromatography (CC) over sephadex LH-20, preparative HPLC and silica gel to afford compounds **1** (10.3 mg), **5** (14.2 mg), **6** (11.1 mg) and **7** (18.3 mg). Similarly, Fr. C (30.32 g) was applied to CC over silica gel and eluted with CH_2Cl_2/MeOH (40/1, 20/1, 10/1, 1/1, 0/100, v/v) to give three subfractions (Frs. C1-3). Fr. C2 was subjected to sephadex LH-20, and then preparative HPLC (H_2O/CH_3CN, 40/60, V/V) to afford three subfractions (C2A-C). Fr. C2B was then re-subjected by silica gel CC with the solvent system (CH_2Cl_2/MeOH, 40/1, 20/1, 10/1, v/v) to afford nine subfractions (C2B1-9). Fr. C2B9 was further subjected to preparative HPLC with MeCN/H_2O (5/95-95/5, v/v, 18 mL/min, 0–90 min) to yield **2** (10.3 mg). Three subfractions (C3A-C) were afforded by CC over sephadex LH-20 (MeOH) from Fr. C3. Fr. C3A was further subjected to silica gel with a step gradient of CH_2Cl_2/MeOH solvent system (50/1, 25/1, 10/1, v/v) to afford subfractions C3A1-C3A5. Fr. C3A5 was further purified by preparative HPLC (H_2O/CH_3CN, 5/95-60/40, v/v, 18 mL/min, 0–100 min) to afford **3** (11.5 mg) and **4** (13.7 mg).

peruranolide A (1). Yellow amorphous powder; $[\alpha]_D^{28}$ +34 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3384, 2945, 1687, 1654, 1570, 1014, 952 cm^{-1}; ^1H NMR (400 MHz, CD_3OD) and ^{13}C NMR (101 MHz, CD_3OD) data (see Tables 1,2,3); HR-ESI-MS: *m/z* 502.2797 $[M + NH_4]^+$ (calcd. 502.2799 for C_{28} $H_{40}O_7N^+$).

peruranolide B (2). Yellow amorphous solid; $[\alpha]_D^{28}$ + 16 (*c* 0.10, MeOH); IR (KBr) ν_{max} 3419, 2506, 1697, 1563, 1465, 1438, 1017, 977 cm^{-1}; ^1H NMR and ^{13}C NMR data (CD_3OD, Tables 1,2,3); HR-ESI-MS: *m/z* 501.2493 $[M-H]^-$ (calcd. 501.2494 for $C_{28}H_{37}O_8^-$).

peruranolide C (3). White amorphous powder; $[\alpha]_D^{28}$ + 48 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3480, 2922, 1771, 1715, 1662, 1269, 1033, 1018 cm^{-1}; ^1H NMR and ^{13}C NMR data (DMSO-d_6, see Tables 1,2,3); HR-ESI-MS: *m/z* 516.2601 $[M + NH_4]^+$ (calcd. 516.2592 for C_{28} H_{38} O_8N^+).

peruranolide D (4). Yellow amorphous powder; $[\alpha]_D^{28}$ + 32 (*c* 0.1, MeOH); IR (KBr) ν_{max} 3417, 2922, 1689, 1381, 1242, 1131, 1025 cm^{-1}; ^1H NMR and ^{13}C NMR data (Tables 1,2,3); HR-ESI-MS: *m/z* 562.3003 $[M + NH_4]^+$ (calcd. 562.3011 for $C_{30}H_{44}O_9N^+$).

2.4 Cytotoxicity assay

The human breast cancer cell line (MCF-7) was purchased from the Kunming Institute of Zoology. Cells were supplemented with streptomycin, 10% fetal bovine serum and penicillin (Gibco, USA) in DMEM medium. Cytotoxic assays were proceeded using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method followed by the reported protocol [16]. In the experiment, the compounds were prepared into stock solution with dimethyl sulfoxide (DMSO), and then an appropriate amount of secondary mother solution was prepared into 100 μM with culture medium, which was then diluted by doubling. Cells were seeded in 96-well microplates at a density of 1 × 10^4 cells/well and then treated with compounds for 24 h at 3.13, 6.25, 12.5, 25, 50 and 100 μM. The volume of different concentrations of compounds added to the corresponding wells was 100 μL. Doxorubicin was chosen as the positive control. The final concentration of DMSO in the culture medium was <0.05% [17]. After the addition of 20 μL of the MTT solution (5 mg/mL) to each well, the plate was incubated for 4 h under the same conditions to stain live cells. The supernatants were removed and the crystals were dissolved in 150 μL of DMSO. The absorption was measured at 490 nm.

Inhibitory ratio (%) = [OD (Control) – OD (Sample)] / [OD (Control) – OD (Blank)] × 100.

The cytotoxic activity of each compound was calculated and expressed as the concentration of compound that achieved 50% inhibition (IC_{50}) of the cells.

2.5 Antibacterial assay in vitro

In vitro antibacterial activity of the isolated compounds (**1–7**) were studied against three bacteria strains using broth microdilution technique. The bacteria tested were purchased from Microbial Culture Preservation Center, Guangdong Institute of Microbiology. These include *Escherichia coli* (*E. coli* ATCC8739), *Bacillus cereus* (*B. cereus* CMCC63302), *Staphylococcus aureus* (*S. aureus* CMCC26003). Minimum inhibitory concentration (MIC) of the compounds were carried out following the procedure described by the reported protocol [18]. Briefly, stock solutions were prepared with DMSO at a certain concentration. In the 96-well plate, add 100 μL of the mixture of diluted bacteria solution and citrate indicator to the first and eighth rows of Wells. An appropriate amount of solution sample and MH liquid medium (200 μL in total) were added to the first row of well plates. After evenly mixing the solution, move 100 μL of the solution to the corresponding wells in the second row and dilute successively to the eighth row.

Table 1. [1]**H NMR data of Compounds 1–4 (400 MHz).**

Position	**1**[1]	**2**[1]
2	6.00 (d, J = 9.5)	2.50 (m) 1.27 (m)
3	7.10 (dd, J = 9.5, 6.0)	1.93 (overlapped)
4	6.24 (d, J = 6.0)	1.93 (overlapped)
6	4.57 (br s)	3.29 (m)
7	α: 1.38–1.44 (overlapped) β: 2.33–2.40 (overlapped)	α: 1.80 (overlapped) β: 2.35 (overlapped)
8	2.41 (overlapped)	1.73 (m)
9	1.20 (m)	1.48 (m)
11	2.06–2.11 (overlapped) 1.57–1.64 (overlapped)	1.36–1.29 (m)
12	2.06–2.11 (overlapped) 1.38–1.44 (overlapped)	2.82 (m) 2.32 (m)
14	1.41 (overlapped)	1.79 (overlapped) 1.60–1.64 (m)
15	4.48 (m)	1.83 (overlapped)
16	2.44–2.49 (overlapped) 2.04–2.10 (overlapped)	1.94 (overlapped)
17	2.20 (m)	5.26 (s)
18	5.30 (s)	1.10 (s)
19	1.43 (s)	
21	1.30 (s)	1.29 (s)
22	4.61 (m)	4.42 (m)
23	2.37–2.46 (overlapped)	2.89 (m) 2.29 (m)
27	1.86 (s)	1.87 (s)
28	1.98 (s)	2.02 (s)

[1] CD_3OD was used as solvent.
[2] DMSO-d_6 was used as solvent.
[3] $CDCl_3$ was used as solvent.

Table 2. [1]**H NMR data of Compounds 1–4 (400 MHz).**

Position	**3**[2]	**4**[3]
2	6.00 (d, J = 9.6)	α: 2.52–2.53 (m) β: 2.53–2.61 (m)
3	7.09 (dd, J = 9.7, 6.0)	4.27–4.31 (m)
4	6.20 (d, J = 6.0)	4.41 (d, J = 6.4)
6	4.46 (br s)	4.08 (dd, J = 7.7, 2.5)
7	α: 1.49–1.56 (m) β: 1.82–1.88 (overlapped)	α: 1.22–1.33 (m) β: 2.31–2.35(m)
8	3.40 (m)	1.98–2.03 (m)
9	2.03 (overlapped)	2.45–2.50 (m)
11	2.26–2.18 (overlapped) 1.99–2.06 (overlapped)	1.34–1.40 (m) 1.40–1.48 (m)
12	2.10–2.14 (m) 1.82–1.89 (m)	1.48–1.56 (m) 1.68–1.76 (m)
15	2.31–2.39 (overlapped) 2.42–2.55 (overlapped)	5.30 (d, J = 2.8)
16	1.83–1.88 (overlapped) 1.41–1.48 (m)	5.59 (d, J = 2.7)
17	2.24 (m)	
18		1.11 (s)
19	1.38 (s)	1.19 (s)
20		2.40–2.43 (m)
21	1.38 (s)	1.06 (s)
22	4.51 (m)	4.25 (dd, J = 8.2, 4.8)
23	2.31–2.39 (m) 2.42–2.55 (m)	2.36–2.38 (m) 2.27–2.31 (m)
27	1.76 (s)	1.81 (s)
28	1.93 (s)	1.95 (s)
15-OAc		2.06 (s)

[1] CD_3OD was used as solvent.
[2] DMSO-d_6 was used as solvent.
[3] $CDCl_3$ was used as solvent.

Each of these solutions was then serially diluted (8 times) in 200 μL of nutrient broth in a 96 well plates to the desired concentrations (100, 50, 25, 12.5, 6.25,3.16,1.56 and 0.78 μg/mL). Finally, the 96-well plates were incubated in a 37 ℃ constant temperature incubator for 18 h, and the color changes of the bacteria liquid were observed. Vancomycin was chosen as the positive control. All equipment and culture media were sterilised before use.

3. Results and discussion

Compound **1** was isolated as a yellow amorphous powder with a molecular formula $C_{28}H_{36}O_7$ established by an HRESIMS ion at *m/z* 502.2797 $[M + NH_4]^+$ (calcd. for 502.2799), requiring 11 degrees of unsaturation. In the ^{1}H NMR experiment, the ring A was confirmed to have a dienone system by three olefinic protons at δ_H 6.00 (d, J = 9.5 Hz), δ_H 7.10 (dd, J = 9.5, 6.0 Hz) and δ_H 6.24 (d, J = 6.0 Hz) attached to H-2, H-3 and H-4, respectively [19]. The existence of two hydroxyl groups was evidenced by two downfield signals at δ_H 4.57 and δ_H 4.48. Their positions were established to be at C-6 and C-15, respectively, as inferred by the correlations from δ_H 4.57 to C-4 (δ_C 118.6), and from δ_H 4.48 to C-14 (δ_C 61.0) and C-16 (δ_C 37.8) in the HMBC spectrum. Interpretation of its ^{1}H and ^{13}C NMR data suggested the chemical structure of **1** closely resembles that of physaminimin E [20], differing by the substituent pattern of C-18. Interestingly, it was found that the chemical shifts of C-18 could occur downfield at ≥108 ppm when the hydroxyl group attached at C-18 was etherified [19,20]. In our case, a naked hydroxy linked at C-18 was determined by its upfield chemical shifts occurring at 103.1 ppm. The configurations of chiral carbons in **1** were determined to be the same as those in physaminimin E by ROESY experiment and biogenetic considerations. For instance, the observed ROESY correlations from Me-19 to H-8, from H-8 to H-15, from H-15 to H-16β, and from Me-21 to H-18 supported the pro-

Table 3. ^{13}C NMR data of Compounds 1–4 (101 MHz).

Position	**1**[1]	**2**[1]	**3**[2]	**4**[3]
1	208.1	215.2	204.3	211.3
2	126.6	35.7	125.7	41.4
3	143.0	19.0	140.7	72.8
4	118.6	29.2	116.9	77.5
5	160.4	65.5	158.0	77.4
6	74.5	62.2	71.4	77.9
7	41.7	32.2	30.1	25.5
8	33.1	45.0	44.4	36.4
9	51.5	44.2	44.4	37.4
10	55.5	55.5	53.8	55.5
11	24.6	24.7	21.0	21.5
12	37.5	30.7	26.7	39.2
13	60.8	83.8	75.8	52.2
14	61.0	101.0	215.8	82.7
15	74.7	29.1	38.8	83.3
16	37.8	16.7	20.7	121.8
17	54.0	56.4	59.7	162.2
18	103.1	104.9	178.8	16.6
19	19.4	12.5	22.6	15.5
20	85.6	84.8	83.7	35.8
21	20.4	26.5	18.9	18.5
22	83.6	80.6	75.8	80.5
23	32.8	37.8	30.1	34.0
24	151.9	153.3	150.2	150.7
25	122.3	121.5	119.8	121.8
26	168.7	168.7	164.1	168.3
27	12.4	12.3	12.1	12.7
28	20.4	20.5	20.2	20.7
15-OAc				21.5
15-OAc				170.6

[1] CD_3OD was used as solvent.

[2] DMSO-d_6 was used as solvent.

[3] $CDCl_3$ was used as solvent.

Table 4. The cytotoxicity data of Compounds 1–7 against MCF-7.

Compounds	$IC_{50} \pm SD$ (μM)	Compounds	$IC_{50} \pm SD$ (μM)
1	>100	**5**	3.51 ± 0.013
2	>100	**6**	36.89 ± 1.78
3	>100	**7**	48.64 ± 0.07
4	>100	**DOX**[1]	0.90 ± 0.03

[1]: doxorubicin.

posed α-orientation of HO-15 and β-orientation of HO-18. While the β-orientation of HO-6 was inferred by its small coupling constant (br s) [21,22]. Consequently, compound **1** was established as 18,20-epoxy-6β,15α,18β-trihydroxy-1-oxowitha-2,4,24-trien-26,22-olide, name as peruranolide A.

Table 5. *In vitro* antibacterial activities of Compounds 1–7 (μg/mL).

Compounds	Strains		
	E. coli	*B. cereus*	*S. aureus*
1	100	12.5	25
2	100	25	50
3	100	25	50
4	100	25	50
5	100	25	50
6	100	25	50
7	100	25	50
Van[1]	1.56	1.56	0.78

[1]: Vancomycin.

Compound **2** was isolated as a yellow amorphous powder and the molecular formula of $C_{28}H_{38}O_8$ was assigned by the $[M\text{-}H]^-$ at *m/z* 501.2493 (calcd. for 501.2494) in HR-ESI-MS, implying an unsaturation equivalence of ten. Two characteristic signals appeared at δ_C 101.0 and δ_C 104.9 in the ^{13}C NMR spectrum, indicated a 13,14-seco-withaphysalin skeleton [21,23]. A thorough interpretation of the NMR data distinctly suggested the structure of **2** to be similar to that of the known compound 2,3-dihydro-withaphysalin C [24], with the key differences in the ring B attributing to some signals from C-5 to C-6. The cross-peaks of H-6/H-7/H-8/H-9 in the ^{1}H–^{1}H COSY spectrum, together with the HMBC correlations from H-6 (δ_H 3.3) to C-7 (δ_C 32.2), and from H_3-19 to C-5 and C-10 (δ_C 56.4), demonstrated a 5,6-epoxide moiety occurring in ring B. Based on the cross-peaks of H-18 (δ_H 5.26) and H-21 (δ_H 1.29) in the ROESY experiment, the β-orientation of the hydroxyl group at C-18 was established. Besides, the observed ROESY correlations of H-2α/H-6 indicated the β-orientation of 5,6-epoxide moiety. Consequently, **2** was identified as (14α,18β)-13,14:18,20-diepoxy-14,18-dihydroxy-1-oxo-13,14-secowitha-24-dien-26,22-olide, and named as peruranolide B.

Compound **3** with the molecular formula of $C_{28}H_{34}O_8$, was obtained as a white amorphous powder. The ^{13}C NMR and DEPT spectra analyzed with the HSQC spectrum exhibited 28 carbon resonances attributing to four methyls, six methylenes, eight methines, and ten quaternary carbons. From these signals, four characteristic resonances including two ketone carboxyls (δ_C 204.3, 215.8) and two ester carboxyls (δ_C 178.8, 164.1) could be clearly identified, suggesting the same skeleton as minisecolide C [24]. The olefinic signal at δ_H 7.09 (1H, dd, J = 9.7, 6.0 Hz), showing ^{1}H–^{1}H COSY correlations to δ_H 6.00 (1H, d, J = 9.6 Hz) and δ_H 6.20 (1H, d, J = 6.0 Hz) and HMBC correlation with C-1, were indicative of a dienone fragment in ring A [19]. A hydroxyl group was deduced to be positioned at C-6 from the evidence

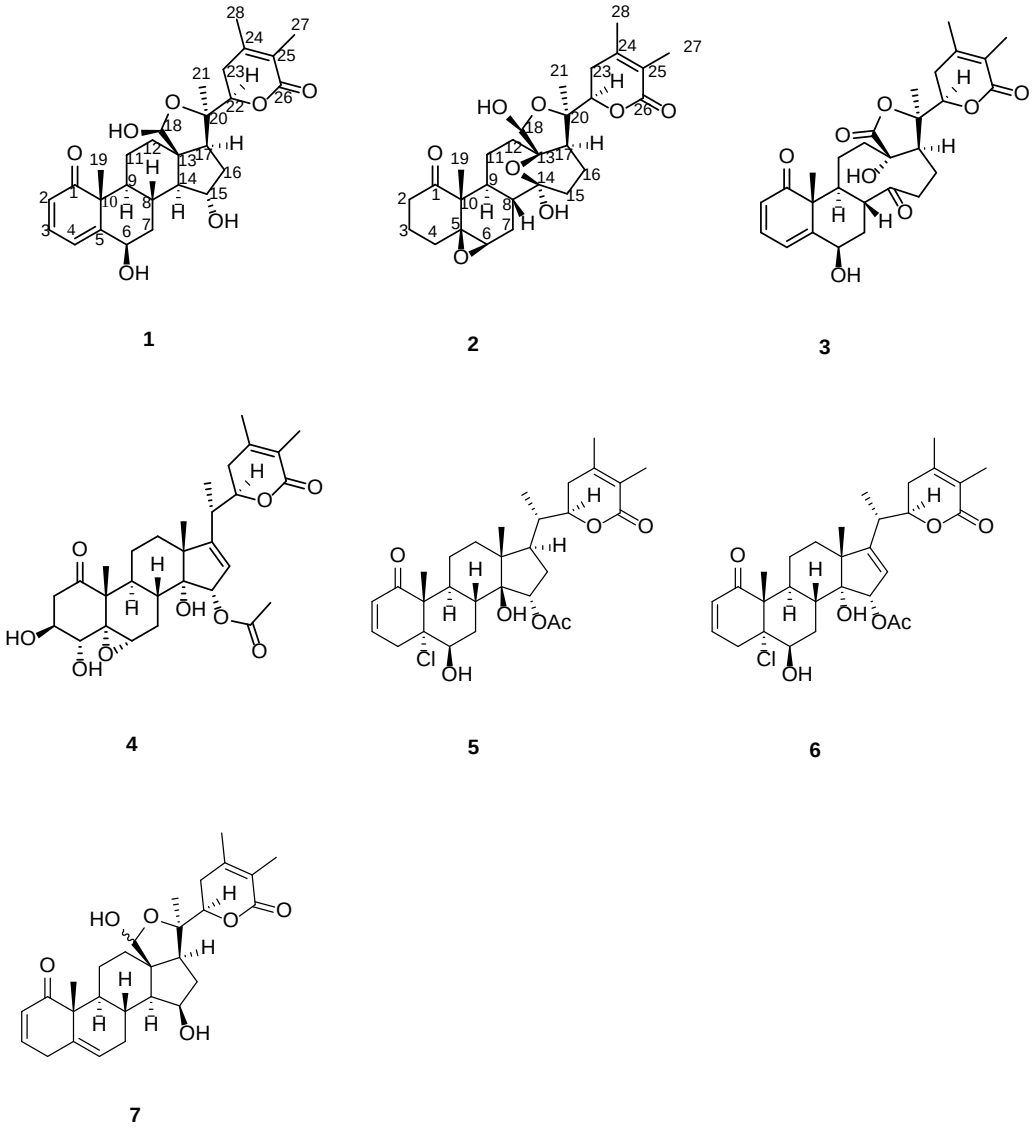

Fig. 1. The structures of Compounds 1–7.

of the HMBC correlations from H-6 (δ_H 4.46) to C-4 (δ_C 116.9) and C-10 (δ_C 53.8). Similarly, the β-orientation of HO-6 could be inferred by the small coupling constant of H-6 (br s) [21,22], and further confirmed by the ROESY correlation between HO-6 and Me-19. As described in the previous studies, in the case of 13,14-seco-withaphysalins, HO-13α tend to form H-bonds with α, β-unsaturated ketone moieties, thus contributing to the stabilization of this skeleton, while HO-13β can make cyclization prone to the formation of 13,14-epoxy units and results in the structural instability [21]. Comparison between NMR data of **3** with the known analogues based on biogenetic considerations [21,22], the remaining chiral carbons in **3** remained the same configurations as those in 13,14-seco-withaphysalins. Thus, **3** was verified as (6β,13α)-6,13-dihydroxy-1,14-dioxo-13,14-secowitha-2,4,24-trien-18,20:26,22-diolide, and named as peruranolide C.

Compound **4** possessed a molecular formula of $C_{30}H_{40}O_9$, was purified as a yellow amorphous powder. The ^1H and ^{13}C NMR data of **4** were discovered to be very similar to those of physaminimin F [20], except for the resonances arising from C-5 and C-6. The HMBC correlations from H-3 at δ_H 4.29 (1H, m) to C-1 at δ_C 211.2 and C-5 at δ_C 77.4, from H-4 at δ_H 4.41 (1H, d, J = 6.4 Hz) to C-2 at δ_C 41.4 and C-10 at δ_C 55.5, from H-6 at δ_H 4.08 (1H, dd, J = 2.5, 7.7 Hz) to C-8 at δ_C 36.4 and C-10 at δ_C 55.5, from H-19 at δ_H 1.19 (3H, s) to C-5 at δ_C 77.4 and C-9 at δ_C 37.4 confirmed the positions of four oxygen substituents at C-3, C-4, C-5, and C-6. Additionally, on the basis of molecular formula and the degree of unsaturation, an epoxy moiety placed at C-5 and C-6 was proposed. In general, the R-O-6 in withanolide-type compounds adopted β-orientation, and thus the H-6 resonance with a small coupling constant and then showed as a broad singlet. In the case of **4**, the H-6 pro-

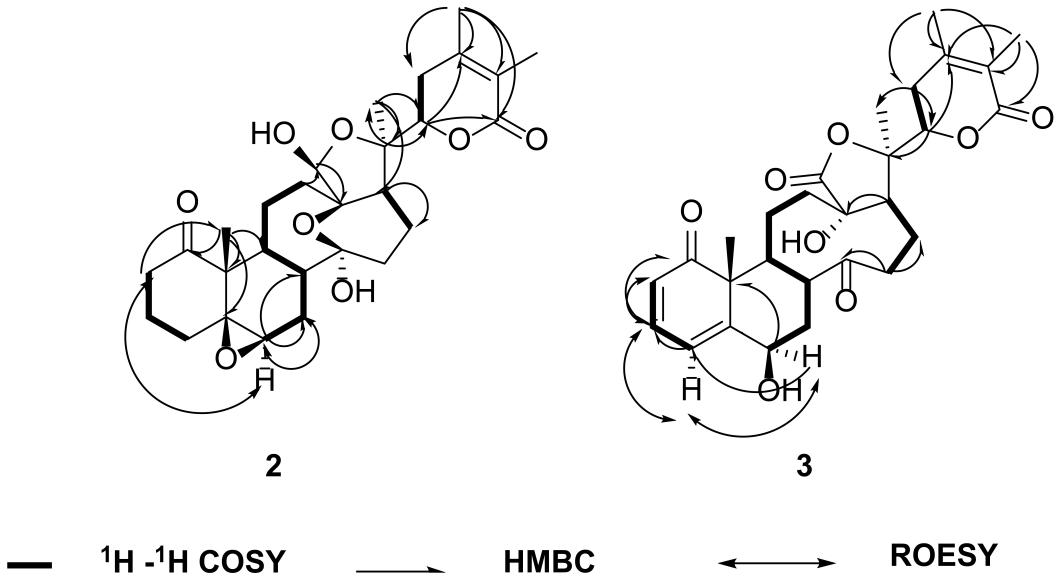

Fig. 2. Key HMBC, NOE correlations and 1H-1H COSY of Compounds 2–3.

ton appeared as a double doublet with J values of 2.5 and 7.7 Hz, suggesting R-O-6 being α-oriented. This deduction was confirmed by the observed NOESY correlation of H-6/H-8. The correlation from H-6 to H-4 was also observed in the NOESY experiment, indicating the α-orientation HO-4. Similarly, the H-4 signal showed as a doublet with a large J value of 6.4 Hz, implying an axial position of H-3 and therefore HO-3 was β-oriented. Thus, the structure of **4** was established as 15α-acetoxy-5α,6α-epoxy-3β,4α,14α-trihydroxy-1-oxo-witha-16,24-dienolide, named as peruranolide D.

In addition to the four new Compounds **1**–**4**, three known analogues including (20*S*,22*R*)-15α-acetoxy-5α-chloro-6β,14β-dihydroxy-1-oxowitha-2,24-dienolide (**5**) [25], physagulin B (**6**) [26] and Withaphysalin U (**7**) was also purified and identified from *P. peruviana* [27]. The structures of Compounds **1–7** are shown as Fig. 1. Key HMBC, NOE correlations and ^{1}H-^{1}H COSY of Compounds **2–3** are shown as Fig. 2.

In the previous investigation, the plants from *Physalis* were verified to be the major sources for the exploitation of new antitumor drugs [28–30]. All isolated metabolites were therefore appraised for their cytotoxicity against the human breast cancer cell line MCF-7 by MTT method. As shown in Table 4, compound **5** exhibited potent inhibitory activity with an IC_{50} value of 3.51 μM, comparable to that of the positive control doxorubicin at 0.90 μM. On the other hand, compounds **6**–**7** showed moderate with IC_{50} values at 36.89 and 48.64 μM, respectively. Additionally, *in vitro* antibacterial activities of the compounds **1**–**7** were tested. MIC results of the compounds were shown in the Table 5. As a result, compounds **1**–**7** had moderate inhibitory activities against *B. cereus* and *S. aureus*. The MIC of **1** were 12.5 and 25 μg/mL, and the others were 25 and 50 μg/mL, respectively. However, they had no obvious inhibitory activity against *E. coli* with MIC of 100 μg/mL, compared with 1.56 μg/mL of vancomycin.

4. Conclusions

In summary, four novel withanolide-type compounds (**1**–**4**), together with three known analogues (**5**–**7**), were obtained from *P. peruviana* L.. Compounds **2**–**3** possess a 13,14-seco-withaphysalin skeleton, while others were two withaphysalin-type withanolides (**1**, **7**) and three normal withanolides (**4**–**6**). The cytotoxic activities of these isolated compounds were evaluated against MCF-7. Compound **5** exhibited potent activity with an IC_{50} value of 3.51 μM and compounds **5**–**7** showed moderate inhibitory effect. *In vitro* antibacterial activities, compounds **1**–**7** had no obvious inhibitory activity against *E. coli*, but had moderate inhibitory activities against *B. cereus* and *S. aureus*. Overall, our findings might offer valuable clues for the utilization of withanolides as lead compounds for antineoplastic or antibacterial drug development.

Abbreviations

B. cereus, *Bacillus cereus*; CC, column chromatography; *E. coli*, *Escherichia coli*; EtOAc, ethyl acetate; EtOH, ethyl alcohol; DMSO, dimethyl sulfoxide; MIC, Minimum inhibitory concentration; MTT, (4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; *P. peruviana*, *Physalis peruviana*; *S. aureus*, *Staphylococcus aureus*.

Author contributions

JWW, JY, JZW and QRL designed the research study. QRL and HJL performed the research. BLL, ZYA, YWF, WJZ, XL and JYC provided help and advice on the re-

search. JWW and QRL analyzed the data. QRL and HJL wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

The authors declare no conflict of interest.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at https://www.imrpress.com/journal/FBL/27/3/10.31083/j.fbl2703098.

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