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Short communication

Chemical constituents from ethanol extract of *Polyalthia rumphii* branches and their cytotoxicity evaluation

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ABSTRACT

Twelve known compounds, including eight alkaloids, three lignans and one gossypol derivative, were isolated from the branches of *Polyalthia rumphii* (Blume ex Hensch.) Merr., Annonaceae. The chemical structures were determined by spectroscopic methods and comparison with literature data. All the isolates were evaluated the cytotoxicity against three human cancer cell lines: Hela, MCF-7 and A549, the results showed that partial of isolates displayed weak cytotoxicities with the IC_{50} values ranging from 25 to 40 μg/ml.

Introduction

Polyalthia rumphii (Blume ex Hensch.) Merr., Annonaceae, distributes widely in tropic and subtropic area (Editorial Board of FOC, 2011). Chinese used many species of this genus as traditional medicinal plants to treat intractable diseases (Yuan et al., 2011). Especially Li ethnic minority, located in Hainan Island, used the extracts of *P. rumphii* for prevention of fever, hypertension and inhibition of cancer cells (Yuan et al., 2011; Machana et al., 2012). Previous phytochemical research reported that alkaloids and lignans, isolated from *P. rumphii*, were with anticancer activities (Wang et al., 2012a,b, 2013). As part of our ongoing purpose of screening the bioactive constituents from *P. rumphii*, this present investigation described the isolation, structure identification, and all isolates were evaluated the cytotoxic activities against three human cancer cell lines by MTT assay. By chromatographic methods, twelve know compounds were isolated, the chemical structures of them were mainly determined by physical and spectroscopic methods including $[\alpha]_D$, NMR and MS as well as by comparing with literature data.

Materials and methods

General

NMR spectra were recorded on a Bruker AV-400, deuterated reagents were $CDCl_3$ (TMS as internal standard) and CD_3OD (residual solvent as internal standard). ESI-MS was measured on a Bruker amaZon SL mass spectrometer, and HR-ESI-TOF-MS were measured on an AB Sciex TripleTOF 5600$^+$ high-resolution mass spectrometer. Preparative HPLC were performed on a Waters 2545-2767-2489 HPLC system. The single-crystal was diffracted on an X-Ray Diffractometer of Bruker Smart Apex-II CCD.

Plant material

Fresh branches of *P. rumphii* (Blume ex Hensch.) Merr., Annonaceae, were collected in September 2013, from Bawang Mountain of Hainan Island, China, and authenticated by Prof. Qiongxin Zhong (Hainan Normal University). The voucher specimen (No. 201309XHAL) was deposited at our key laboratory.

Extraction and isolation

The branches of *P. rumphii* were air-dried at room temperature, and then reduced to coarse powder by crushing machine. This coarse powder (6.78 kg) was extracted in July 2014, with 95% EtOH

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(15 l × 3) at room temperature, and the solvents were evaporated under reduced pressure to get crude EtOH gum, which was continuously suspended in 0.1% H_2SO_4 (4 l). The liposoluble components were removed by PE (petroleum ether) from acidic solution, and the acidic aqueous solution was extracted by EtOAc (4 l × 3) to get part I (94.8 g), the remaining aqueous layer was alkalinized (pH 9–10) by ammonia and extracted with $CHCl_3$ (4 l × 3) to get part II (48.1 g).

Part I was fractioned on a silica gel column (200–300 mesh, Qingdao Haiyang Chem. Co., Ltd.) using gradient PE-EtOAc solvent system to afford forty fractions (XMZ-1 to XMZ-40). XMZ-10 (0.3 g) was isolated by silica gel column (400 mesh, Qingdao Haiyang Chem. Co., Ltd.) with gradient PE-EtOAc solvent system, then followed by HPLC on an Agilent C-8 column (Agilent Zorbax Eclipse XDB C-8, 250 × 9.4 mm, 5 μm, MeOH:H_2O 80:20, 4 ml/min) to provide **7** (44.4 mg). XMZ-12 (4.2 g) was purified by repeated silica gel column (400 mesh) with gradient light petroleum and EtOAc to give **3** (5.9 mg). XMZ-30 (0.7 g) was similarly purified by repeated silica gel column and followed by HPLC on Agilent C-8 column (MeOH:H_2O 80:20, 4 ml/min) to give **8** (37.9 mg). Combined fractions (1.6 g) of XMZ-22 to XMZ-29 was introduced to a silica gel column (400 mesh) and eluted by gradient PE-EtOAc system (3:1 to pure EtOAc) to give **9** (23.2 mg). **10** (95.7 mg), **11** (40 mg) and **12** (8.9 mg) were isolated and purified from XMZ-33 (2.5 g) by repeated silica gel column (400 mesh) with gradient $CHCl_3$-CH_3OH system. XMZ-35 (3.1 g) was separated by HPLC on YMC-Pack ODS-A column (250 × 20 mm, 5 μm, MeOH:H_2O 35:65, 10 ml/min) to obtain **1** (4.4 mg). XMZ36 was re-chromatographed on a silica gel column (gradient PE-EtOAc 10:1 to 2:8), followed by HPLC on YMC-Pack ODS-A column (MeOH:H_2O 2:8, 10 ml/min) to obtain **6** (12 mg).

Part II was chromatographed on a silica gel column (200–300 mesh) using PE-EtOAc gradient (50:1 to 1:5) to afford 31 fractions (SWJ-1 to SWJ-31). SWJ-12 (4.2 g) was subjected to a silica gel column (400 mesh) with gradient PE-EtOAc solvent system (5:1 to 1:4) to yield **2** (9.6 mg), **4** (9.2 mg) and **5** (8.7 mg).

Cytotoxicity assay

The cytotoxic activities of all compounds were evaluated by MTT method to against three human cancer cell lines: Hela, MCF-7 and A549, using Doxorubicin as positive control. Cells were cultured in DMEM medium, supplemented with 10% fetal bovine serum in 5% CO_2 at 37 °C. Cells (100 μl) were distributed evenly into 96-well microplates, containing 5×10^3 cells per well, and incubated for 12 h. Designated wells were treated with DMSO solutions of tested compound at various concentrations (0.0625, 0.32, 1.6, 8 and 40 μg/ml) in triplicate. After 48 h incubation, MTT was added to each well to incubate an additional 4 h. After treatment, the absorbance of each well was read at 490 nm by an enzyme-labeled detector (BioTek ELx800). IC_{50} values to different cell lines were determined by the dose–response curve (Yu et al., 2016).

Statistical analysis

Statistical significance was determined with the standard deviation generally less than 10%, and all values were carried out using the *t*-test (SPSS 12.0).

Results and discussion

In present phytochemical investigation on searching for bioactive agents from this plant, the extracting method was optimized to afford two parts, part I was from acidic solution of EtOH extracts, part II was from basic solution. Chromatographic separation of these two parts, twelve compounds were obtained, containing nine isolates from part I, and three isolates from part II.

The chemical structures were identified mainly by spectroscopic analysis to be (−)-8-oxodiscretamine (**1**) (Shono et al., 2016), *N*-formylannonaine (**2**) (Yusoff et al., 2014), isooncine (**3**) (Wu et al., 1990), isoursuline (**4**) (Chen et al., 2000), cleistopholine (**5**) (Wang et al., 2011), northalifoline (**6**) (Lee et al., 2010), *N*-2-phenylethylcinnamamide (**7**) (Gu et al., 2013), *N*-*trans*-cinnamoyltyramine (**8**) (Wang et al., 2012a,b), binaphthalene-2-phenol-3-aldehyde (**9**) (Wu et al., 1989), arborone (**10**) (Tulake et al., 2012), syringaresinol (**11**) (Mo and Mai, 2012), balanophonin (**12**) (Wang et al., 2010), including eight alkaloids (**1–8**), three lignans (**10–12**) and one gossypol derivative (**9**).

It should be noted that all isolates were reported from this plant for the first time. Among them, binaphthalene-2-phenol-3-aldehyde (**9**) was firstly isolated from the natural resources, and its complete ^{1}H NMR and ^{13}C NMR were assigned by 2D NMR. *N*-formylannonaine (**2**) exists as a mixture of two conformational isomers (*Z*/*E* = 1/2.5, calculated by the integration of ^{1}H NMR) in deuterated methanol. A single crystal of isoursuline (**4**) was firstly recrystalized, and the crystal structure had been determined by single-crystal X-ray diffraction method (Niu et al., 2015). The X-ray data were deposited in the Cambridge Crystallographic Data Centre with number of CCDC-1494107.

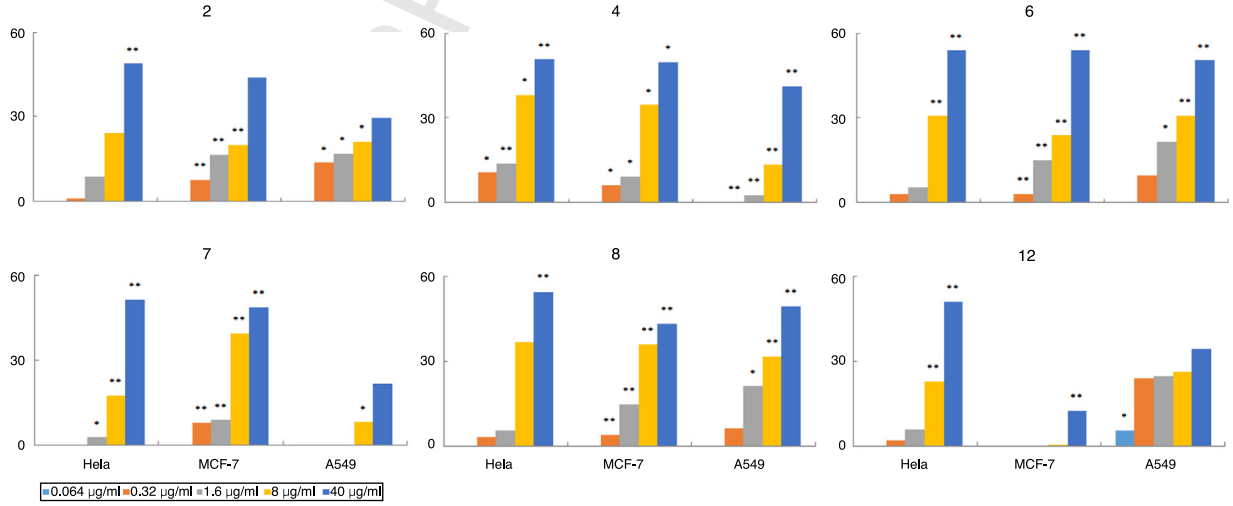

Fig. 1. The inhibition ratio (IR, %) of six cytotoxical compounds against three cancer cell lines at various concentrations. IR values were calculated using the formula: IR (%) = [OD (control group) − OD (administration group)]/OD (control group) × 100%. *$p < 0.05$, **$p < 0.01$.

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