Anticancer activities of constituents from the stem of *Polyalthia rumphii*

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Abstract: Nine known compounds were firstly isolated from the chloroform extract of *Polyalthia rumphii* stem by anticancer activity guidance, and the chemical structures were identified by using spectroscopic and physico-chemical analysis. Cytotoxic evaluation against four cancer cell lines was performed on all these compounds, which showed that K562 could be significantly inhibited by partial compounds with IC_{50} values at the range from 40 to 60μg/mL.

Keywords: *Polyalthia rumphii*, anticancer activities, constituents.

INTRODUCTION

People using medicinal plants for treating disease is as old as ancient times, about 60 percent of anticancer drugs are structural modifications and derivatives from plant sources (David *et al*., 2003), e.g., vinblastine from *Catharanthus roseus* (L.) G. Don, taxol from *Taxus brevifolia* and Camptothecin from *Camptotheca acuminata* Decne. It's noted that the management of cancer is still not up to mark and there are always needs to search new drugs for the treating cancer. In our phytochemical investigation on the tropic plants in Hainan Island, P.R.China, to search new substances with anticancer activities, the chloroform extract of *Polyalthia rumphii* (Bl. ex Hensch.) Merr. stem showed significant inhibition to four human cancer cell lines.

P. rumphii, belonging to genus *Polyalthia* (family Annonaceae), is an evergreen tree cultivated on southern China and also widely distributed in the tropic and subtropic regions in the world. It's reported that phytochemical research on *Polyalthia* species had led to the isolation of many constituents with cytotoxic activities: Clerodane diterpenoid from the bark of *P. longifolia* (Chang *et al*., 2006), styryl-lactones from the leaves and twigs of *P. crassa* (Tuchinda *et al*., 2006), acetogenin and alkaloids from the seeds and stems of *P. plagioneura* (Zheng *et al*., 1994; Liu *et al.*, 2010).

This present paper is about the study of isolation and anticancer activities of the compounds from the stem of *P. rumphii*: Vanillin (**1**), Syringaldehyde (**2**), β-sitosterol (**3**), Bis(2-ethylheptyl) phthalate (**4**), Dibutyl phthalate (**5**), diisobutyl phthalate (**6**), Epiyangambin (**7**), Diayangambin (**8**) and N-trans-feruloyltyramine (**9**).

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MATEARIALS AND METHODS

Plant material

The stems of *P. plagioneura* was collected from BaWangling mountain in Hainan Province, P.R.China in Sept. 2009, and identified as *Polyalthia plagioneura* by Vice-Professor Qiongxin Zhong from the College of Life Science in Hainan Normal University. A voucher specimen has been preserved in the Key Laboratory of Tropical Medicinal Plant Chemistry of Ministry of Education, Hainan Normal University.

General experimental procedures

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AV-400 in $CDCl_3$, using TMS as an internal standard. Melting points were determined at XT4 Microscope type (uncorrected). IR spectra were recorded on a Nicolet Nexus 670 FT-IR spectrophotometer. Silica gel (200-300 mesh, Qingdao Marine Chemical Factory, China). TLC was performed on precoated silica-gel plates (GF254), and spots were visualized under a UV lamp (254 and 365nm) before spraying with 5% H_2SO_4 in EtOH and/or 5% $FeCl_3$ solution, followed by heating.

Extraction and isolation

Air-dried and powdered stem of *P. rumphii* (9.7 kg) were extracted three times with 85% EtOH (3×20L) under reflux. The EtOH extract were evaporated under reduced pressure which then was suspended in 1L double distilled water and further partitioned with petroleum ether (3×5L), $CHCl_3$ (3×5L), EtOAc (3×5L) and *n*-BuOH (3×5L). And $CHCl_3$ extracts (94.8 g) with anticancer activity was applied to a silica gel column chromatography with a stepwise gradient of petroleum ether / EtOAc (100:0-0:100) to obtain 40 fractions. Compounds **1-4** were obtain from Fr. 8 by normal-phase medium-pressure liquid chromatography, eluted with increasing concentrations of EtOAc in petroleum ether and on the same way, the isomers mixture of **5** and **6** were from Fr. 16, compounds

7 and **8** were from Fr. 18 after recrystalization, and compound **9** was from Fr. 37.

MTT assay

BEL-7402 (Human Hepatocellular Carcinoma Cell Line, 7×10^4 cells/well), SPC-A-1 (Human Lung Cancer Cell Line, 2×10^4 cells/well), K562 (Human Myelogenous Leukaemia Cell Line, 1×10^4 cells/well) and SGC-7901 (Human Gastric Cancer Cell Line, 4×10^4 cells/well) cell lines were firstly incubated on 96-well plates for 12h, and treated with 100μL solution of various concentration samples for 44 h. After treatment, 20μL MTT solution was introduced to each incubated plate at a concentration of 5mg/mL and incubated for another 4h. The purple formazan crystal was dissolved in 200μL DMSO solvent and the optical density of each well sample was measured with ELISA Reader at 570 nm. The effect of samples on cell proliferation was measured as a percentage, relative to vehicle-treated control, by which the IC_{50} values were calculated.

Characterization of compounds [1-9]:

Vanillin (1)

Yellow oil (41mg); **^{1}H-NMR** (400 MHz, $CDCl_3$) δ_H (ppm): 7.42(*d*, *J*=1.6Hz, H-2), 7.04(*d*, *J*=8.4Hz, H-5), 7.42(*dd*, *J*=8.0, 2.0Hz, H-6), 9.82(*s*, H-1´), 3.97(*s*, H-3´), 6.28(*brs*, -OH).

Syringaldehyde(2)

Yellow oil (23mg); **^{1}H-NMR** (400 MHz, $CDCl_3$) δ_H (ppm): 7.15(*s*, H-2), 7.15(*s*, H-6), 9.82(*s*, H-1´), 3.97(*s*, H-3´), 3.97(*s*, H-5´), 6.07(*brs*, -OH).

***β*-sitosterol (3)**

Colorless needle crystal (90mg); **IR** ν_{max} KBr 3434, 2937, 2865, 1643, 1452, 1376, 1058 cm^{-1}; **TLC**: showed similar TLC *Rf* value with authentic agent.

Bis(2-ethylheptyl) phthalate (4)

Yellow oil (36mg); **IR** ν_{max} KBr 3080, 2961, 2874, 1729, 1600, 1580, 1467, 1380, 1286, 1123, 1074, 744cm^{-1}; **^{1}H-NMR** (400 MHz, $CDCl_3$) δ_H (ppm): 7.70(1H, *dd*, *J*=8.8,3.2Hz, H-3), 7.53(1H, *dd*, *J*=8.8,3.2Hz, H-4), 7.53(1H, *dd*, *J*=8.8,3.2Hz, H-6), 7.70(1H, *dd*, *J*=8.8,3.2Hz, H-7), 4.22(2H, H-1′), 1.69(1H, H-2′), 1.34(2H, H-3′), 1.32(2H, H-4′), 1.26(2H, H-5′), 1.31(2H, H-6′), 0.92(3H, H-7′), 1.42(2H, H-8′), 0.89(3H, H-9′) , 4.22(2H, H-1″), 1.69(1H, H-2″), 1.34(2H, H-3″), 1.32(2H, H-4″), 1.26(2H, H-5″), 1.31(2H, H-6″), 0.92(3H, H-7″), 1.42(2H, H-8″), 0.89(3H, H-9″); **^{13}C-NMR** (100 MHz, $CDCl_3$) δ_C (ppm): 167.65(C-1), 132.45(C-2), 128.74(C-3), 130.81(C-4), 130.81(C-5), 128.74(C-6), 132.45(C-7) ,167.65(C-8), 68.12(C-1′), 38.69(C-2′), 30.35(C-3′), 28.87(C-4′), 29.64(C-5′), 22.92(C-6′), 13.96(C-7′), 23.74(C-8′), 10.90 (C-9′) , 68.12(C-1″), 38.69(C-2″), 30.35(C-3″), 28.87(C-4″), 29.64(C-5″), 22.92(C-6″), 13.96(C-7″), 23.74(C-8″), 10.90 (C-9″).

Dibutyl phthalate (5) and diisobutyl phthalate (6).

yellow oil (32mg); **IR** ν_{max} KBr 2962, 1724 , 1513, 1461, 1409, 1261, 1099, 1026, 804cm^{-1}; **Dibutyl phthalate (5): ^{1}H-NMR** (400 MHz, $CDCl_3$) δ_H (ppm): 7.71(2H, dd, J = 3.6, 6.0 Hz ,H-3,6), 7.52(2H, dd, J = 3.6, 6.0 Hz,H-4,5), 4.30(4H, t, J = 6.4 Hz, H-1′, 1″), 1.71(4H, m, H-2′,2″), 1.43(4H, m H-3′, 3″), 0.95(6H, t, J = 7.6 Hz, H-4′,4″).

Diisobutyl phthalate (6): **^{1}H-NMR** (400 MHz, $CDCl_3$) δH (ppm): 7.71(2H, dd, J = 3.6, 6.0 Hz ,H-3,6), 7.52(2H, dd, J = 3.6, 6.0 Hz,H-4,5), 4.08 (4H, t, J = 6.4 Hz, H-1′, 1″), 2.03 (2H, sept, J=7Hz,H-2′,2″), 0.98 (12H, d, J=7Hz, H-3′, 4′, 3″,4″).

Epiyangambin (7)

Colorless needle crystal (120mg); **mp**: 113-115℃; **UV** λ_{max} ($CHCl_3$): 286nm; **IR** ν_{max} KBr 3030, 2964, 2930, 2850, 2834, 1600, 1590, 1509, 1458, 1450, 1377, 1342, 1234, 1133 cm^{-1}; **^{1}H-NMR** (400 MHz, $CDCl_3$) δ_H (ppm): 4.43 (1H,*d*, *J*=5.6Hz, H-1), 3.90 (2H,m, H-3α);3.36 (1H, m, H-3β), 3.38 (1H, m, H-3a), 4.85 (1H, *d*, *J*=4.8 Hz, H-4), 3.90(2H,*m*, H-6α);4.17(1H, *m*, H-6β), 2.91 (1H, *m*, H-6a), 6.60(*s*, H-2′), 6.60(*s*, H-6′), 6.60(*s*, H-2″), 6.60(*s*, H-6″), 3.87(*s*, 3′-OCH_3), 3.85(*s*, 4′-OCH_3), 3.87(*s*, 5′-OCH_3), 3.87(*s*, 3″-OCH_3), 3.85(*s*, 4″-OCH_3), 3.87(*s*, 5″-OCH_3); **^{13}C-NMR** (100 MHz, $CDCl_3$) δ_C (ppm): 87.74(C-1), 69.76(C-3), 49.98(C-3a), 82.12(C-4), 71.03(C-6), 54.47(C-6a), 136.75(C-1′), 102.90(C-2′), 153.37(C-3′), 137.54(C-4′), 153.37(C-5′), 102.90(C-6′), 133.97(C-1″), 102.54(C-2″), 153.18(C-3″), 136.90(C-4″), 153.18(C-5″), 102.54(C-6″), 56.11 (3′-OCH_3), 60.82 (4′-OCH_3), 56.11 (5′-OCH_3), 56.11 (3″-OCH_3), 60.78 (4″-OCH_3), 56.11 (5″-OCH_3).

Diayangambin (8)

Colorless needle crystal (34mg); **mp**: 144-145℃; **UV** λ_{max} ($CHCl_3$): 286nm; **IR** ν_{max} KBr 3080, 2996, 2863, 2842, 1938, 1592, 1508, 1458, 1420, 1345, 1229, 1128 cm^{-1}; **^{1}H-NMR** (400 MHz, $CDCl_3$) δ_H (ppm): 4.91 (1H,*d*, *J*=4.8 Hz, H-1), 3.73(2H,m, H-3α);3.58 (2H, m, H-3β), 3.20(2H, m, H-3a), 4.91 (1H,*d*, *J*=4.8 Hz, H-4), 3.73(2H, m, H-6α);3.58 (2H, m, H-6β), 3.20(2H, m, H-6a), 6.60(s, H-2′), 6.60(s, H-6′), 6.60(s, H-2″), 6.60(s, H-6″), 3.88(s, 3′-OCH_3), 3.85(s, 4′-OCH_3), 3.88(s, 5′-OCH_3), 3.88(s, 3″-OCH_3), 3.85(s, 4″-OCH_3), 3.88(s, 5″-OCH_3); **^{13}C-NMR** (100 MHz, $CDCl_3$) δ_C (ppm): 68.86 (C-1), 84.07 (C-3), 49.44 (C-3a), 68.86 (C-4), 84.07 (C-6), 49.44 (C-6a), 134.56 (C-1′), 103.22 (C-2′), 153.22 (C-3′), 137.08 (C-4′), 153.22 (C-5′), 103.22 (C-6′), 134.56 (C-1″), 103.22 (C-2″), 153.22 (C-3″), 137.08 (C-4″), 153.22 (C-5″), 103.22 (C-6″), 56.2 (3′-OCH_3), 60.8 (4′-OCH_3), 56.2 (5′-OCH_3), 56.2 (3″-OCH_3), 60.8 (4″-OCH_3), 56.2 (5″-OCH_3).

N-*trans*-feruloyltyramine (9)

White amorphous solid (230mg); **^{1}H-NMR** (400 MHz, $CDCl_3$) δ_H (ppm): 7.43(1H, *d*, *J*=15.6Hz, H-2), 7.06(1H, *d*,

Table: Anticancer activities of comp. 1-9

Comp.	Cell lines /IC_{50} (μg/mL)			
	SPC-A-1	BEL-7402	SGC-7901	K562
1	-	-	-	-
2	-	-	-	-
3	-	-	-	-
4	-	-	-	47
5+6	122	122	-	55
7	-	313	-	42
8	-	294	-	-
9	108	-	-	-

"-": inactive.

J=1.6Hz, H-1), 7.03 (2H, *d*, *J*=8.2Hz, H-2′,8′), 7.00(1H,*dd*, *J*=8.4,1.6Hz, H-9), 6.78(1H, *d*, *J*=8.0Hz, H-8), 6.71 (2H, *d*, *J*=8.4 Hz, H-5′,7′), 6.40 (1H, *d*, *J*=15.6Hz, H-3), 3.84 (1H, *s*, H-10), 3.45(1H, *t*, *J*=7.2 Hz, H-1′), 2.73(1H, *t*, *J*=7.6 Hz, H-2′); **^{13}C-NMR** (100 MHz, $CDCl_3$) δ_C (ppm): 169.1(C-1), 156.8(C-6′), 149.7(C-7), 147.2(C-6), 142.0(C-3), 131.3(C-3′), 130.7(C-4′), 128.2(C-4), 123.2(C-9), 118.8(C-2), 116.5(C-8), 116.3(C-5′), 111.6(C-5), 56.4(C-10), 42.5(C-1′), 35.8(C-2′).

RESULTS

Structure identification

The $CHCl_3$ extracts with anticancer activities from the stems of *P. rumphii* led to the isolation of comp. **1-9**, and all chemical structures were identified by comparison with their literature spectrum data (SDBS No.726HSP-00-126; SDBS No. 5097HSP-06- 221; Wang *et al.*, 2008; Li *et al*., 2008; Shi *et al.*, 2005; Ahmed *et al.*, 2005; Kim *et al*., 2005). Comp. **5** and **6** were obtained as an oily mixture of isomers, also reported from the leaves of *Macaranga hemsleyana* Pax & Hoffm (Wang *et al.*, 2009). The ratio of **5** to **6** in the mixture was determined by calculating the protons integration of oxygenated methylene groups to be 2.6:1.

^{1}H-NMR and ^{13}C-NMR signals distribution of comp. **8** suggested an identical carbon skeleton to that of **7** with symmetrical structure, whose carbon skeleton was determined by HMBC spectrum to be furofuran lignans, and suggesting that **8** was the stereoisomer to **7**. By comparing the literatural ^{1}H-NMR data with diayangambin reported from *Artemisia absinthium* without ^{13}C-NMR data, **8** was determined to be diayangambin (Greger *et al*., 1980). Thus we reported the ^{13}C-NMR data of **8** in $CDCl_3$ for the first time.

Anticancer activity

All these isolates were evaluated against four human cancer cell lines, and 50-percent inhibitory concentrations (IC_{50} values) were calculated (μg/mL) in table. From the activity screening, it was demonstrated that **4** to **7** exhibited moderate IC_{50} values at the range of 40 to 60 μg/mL to against K562, **5** to **9** partially exhibited unremarkable activities with the IC_{50} values above 100 μg/mL to against SPC-A-1 and BEL-7402, and **1** to **3** showed inactivity to all these cancer cell lines.

DISCUSSION

In the investigation on screening for new therapeutic agents in China tropical plants, it's found that the extracts from the stems of *P. rumphii* showed remarkably cytotoxic activity on four selected immortal cell lines. On the basis of this discovery, we firstly studied on the phytochemical isolation from the chroloform extracts of stems, and the biological activities were evaluated. Nine konwn compounds were obtained: Vanillin (**1**), Syringaldehyde (**2**), β-sitosterol (**3**), Bis(2-ethylheptyl) phthalate (**4**), Dibutyl phthalate (**5**), diisobutyl phthalate (**6**), Epiyangambin (**7**), Diayangambin (**8**) and N-trans-feruloyltyramine (**9**). The cytotoxic evaluation indicated that the cototoxic compounds with moderate activity were **4-9**, which partially against four selected immortal cell lines. It's regretted that there was no compound against SGC-7901 cancer cell line in this chroloform extracts of *P. rumphii*.

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