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Full Length Research Paper

In vitro screening of five Hainan plants of *Polyalthia* (Annonaceae) against human cancer cell lines with MTT assay

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To screen the anticancer potential of the extracts of five Hainan plants of *Polyalthia*, which are also China Li minority ethnic medicines. The ethanol roots extracts of *Polyalthia laui* and *Polyalthia rumphii* inhibited proliferation of the selected four tumor cell lines at dose of 20 and 200 µg/ml demonstrating potential antitumor activities. Furthermore, the chloroform fraction and petroleum ether fractions of *P. rumphii* and *P. laui* exhibited cytotoxic effects against the four tumor cell lines and the IC_{50} values ranged from 0.98 ± 0.3 µg/ml to 58.0 ± 3.5 µg/ml.

Key words: *Polyalthia*, anticancer activity, cell lines, extracts.

INTRODUCTION

Polyalthia is a genus of plant in family Annonaceae and about 17 species have been identified in China. Previous phytochemical investigations on this genus have isolated of various types of bioactive compounds such as alkaloids (Prachayasittikul et al., 2009), terpenes (Lee et al., 2009), lactone (Faizi et al., 2003) and other compounds. Experimental pharmacology researches in animal models or on cell lines have confirmed that the extracts and/or isolated purified compounds derived from this genus possess many beneficially biological effects, including cytotoxic activity [(Prachayasittikul et al., 2009; Lee et al., 2009; Sashidhara et al., 2009; Verma et al., 2008; Prayong et al., 2008; Tuchinda et al., 2006; Chang et al., 2006; Lamidi et al., 2005; Ravikumar et al., 2009), antimicrobial property (Prachayasittikul et al., 2009; Misra et al., 2010; Sashidhara et al., 2009; Faizi et al., 2008), anti-AIDS effects (Saepou et al., 2009), anti-inflammatory and analgesic activity (Chang et al., 2006; Shih et al., 2010; Tanna et al., 2009; Nicolis et al., 2009; Malairajan et al., 2008; Chang et al., 2008; Malairajan et al., 2006). Hainan, the second largest island administered by the People's Republic of China, has a tropical moist monsoonal climate, which is suitable to the existence of plants belonging to genus *Polyalthia*. There are five indigenous species of *Polyalthia*, that is, *Polyalthia cerasoides (Roxb.)* Benth. and Hook. f. ex Bedd, *Polyalthia consanguinea* Merr, *Polyalthia laui* Merr, *Polyalthia nemoralis* A. et DC, and *Polyalthia rumphii*, which are used as folk medicine by China Li ethnic minority to prevent and/or combat some disease such as fever and hypertension.

To the best of our knowledge, only a few works have been done on the phytochemical studies of the five plants (mainly *P. cerasoides*) and the bioactive potential also remain largely unknown up to now. Zafra-Polo et al. (1996) showed that polyalthidin, a newly isolated compound from *P. cerasoides*, had potent biological activity as an inhibitor of the mammalian mitochondrial respiratory chain (Zafra-Polo et al., 1996). Kanokmedhakul et al. (2007) isolated nine compounds from *P. cerasoides* showing antimalarial activity and antimycobacterial

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Abbreviations: SPC-A-1, Human lung cancer cell line; **BEL-7402,** human hepatocellular carcinoma cell line; **SGC-7901,** human gastric cancer cell line; **K562,** human myelogenous leukaemia cell line; **MTT,** 3-(4, 5-dimethyl-2- thiazolyl)-2, 5-diphenyl-2H-tetrazoliumbromide; **DMSO,** dimethyl sulphoxide; **OD,** optical density.

activity (Kanokmedhakul et al., 2007). Ravikumar et al. (2009) reported that the alcohol extracts of *P. cerasoides* had significant reactive oxygen species (ROS) scavenging activity and moderate cytotoxicity against L929 cell lines (mouse fibroblast cells) but without genotoxic effects (Ravikumar et al., 2008).Screening for new therapeutic agents in plants implies the screening of extracts for the presence of novel compounds and an investigation of their biological activities. The aim of this study is to screen the cytotoxic potential of the alcohol extracts of the various parts (that is, leaves, roots and stems) of the five plants by typical MTT assay against four human cancer cell lines. From these studies *in vitro* we find that the extracts from roots of *P. rumphii* show remarkably potent cytotoxic activity on the four selected immortal cell lines. On the basis of this discovery, subsequent phytochemical isolation using different solvents had been done on this plant and the biological activities also screened.

MATERIALS AND METHODS

Preparation of crude extracts

The stems, leaves and roots of *P. cerasoides*, *P. consanguinea*, *P. laui*, *P. nemoralis*, and *P. rumphii* were collected in Hainan Province, and identified by Professor Qiong-xin Zhong, Hainan Normal University. Voucher specimens were deposited at the Key Lab of Tropical Medicinal Plant Chemistry of Hainan Province, Haikou, China. The air-dried and powdered stems, leaves and roots of these five plants (each 200 g) were refluxed with 80% aqueous ethanol (3 x 2000 ml) for 3 x 3 h. The ethanol extract was concentrated under reduced pressure at 40°C to an aqueous residue, and then freeze-dried in vacuum to powders for further testing.

Cells and chemicals

The human cancer cell lines used for assay, that is, SPC-A-1 (Human Lung Cancer Cell Line), BEL-7402 (Human Hepatocellular Carcinoma Cell Line), SGC-7901 (Human Gastric Cancer Cell Line), K562 (Human Myelogenous Leukaemia Cell Line), were purchased from Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. MTT was purchased from Sigma Chemical Co. (USA) and dimethyl sulphoxide (DMSO) from Merck Co. (USA). RPMI-1640 was obtained from Gibco Co. Ltd. (USA) and neonatal bovine serum (NBS) from Lanzhou Minhai Bioengineering Co., Ltd. (China).

Antitumor activity assay

The tested samples (20 mg per extract) were dissolved in 0.5 ml of DMSO, and diluted with 0.5 ml of phosphate buffered saline (PBS). Then the solutions were serially diluted with PBS until the final concentration in the 96-well microtitre plate were as follows, 200, 20 and 2 µg/ml.

The human cancer cells were cultured in RPMI-1640 (pH 7.4) with 10% NBS, 100 IU/ml penicillin and 100 IU/ml streptomycin in a humidified atmosphere containing 5% CO_2 at 37°C until confluent. The cells were incubated in a 96-well microtitre plate with 180 µl/well at a proper density (that is, 2×10^4, 7×10^4, 3×10^4 and 1×10^4 cells per micro liter medium for SPC-A-1, BEL-7402, GSC-7901 and K562, respectively).

The samples of different concentrations of various extracts, 20 µl per well, were added to the medium after 8 to 12 h seeding. Control groups received the same amount of DMSO. 5-FU (10 µg/ml) was used as a positive control. The microtitre plates were incubated for 44 h in a humidified atmosphere of 5% CO_2 at 37°C. Then 50 µl of MTT solution (1 mg/ml MTT in PBS) was added. Growth of tumor cells was assessed by the ability of living cells to reduce the yellow dye MTT to a blue formazan product (Nakamura et al., 2006). After incubation for 4 h at 37°C, the MTT reagent was removed before adding 150 µl DMSO to each well and gently shaken. The optical density (OD) of the formazan solution was measured at 570 nm by an enzyme-labeled detector (EI x 800, BioTek Instruments, Inc.). Each experiment was performed using 6 replicate wells for each extract concentration. The inhibition ratio of the immortal cell proliferation was calculated according to the following equation: IR (%) = [OD (control group) – OD (administration group)]/OD (control group) x 100%.

Statistical analysis

All statistical tests aiming at evaluating the significance were determined by analysis of variance (one-way analysis of variance) using the SPSS V.13.0 software package (SPSS Inc., Chicago, USA). All results are expressed as the mean ± standard deviation.

RESULTS AND DISCUSSION

All the tested ethanol extracts exhibited very different cytotoxicity against the four selected human cell lines. As shown in Figure 1, the roots extracts of *P. laui* and *P. rumphii* inhibited the proliferation of the tested cell lines at intermediate and high dose level, that is, 20 and 200 µg/ml, respectively. Furthermore, the ethanol extracts of leaves and stems derived from *P. rumphii* also showed inhibitory effects on the cell lines at high dose level. The ethanol extracts of *P. cerasoides*, *P. consanguinea*, and *P. nemoralis* only showed slight inhibition of the proliferation of the tested cell lines even at the highest concentration, that is 200 µg/ml, and inhibition effects were not detected at the lower concentrations. The IC_{50} values were shown in Table 1. From this table we could find that the IC_{50} values of *P. cerasoides*, *P. consanguinea*, and *P. nemoralis* extracts were almost above 90 µg/ml, and some even larger than 200 µg/ml. However, the IC_{50} values of the roots ethanol extracts of *P. rumphii* were less than 10 µg/ml except for the IC_{50} value against the SGC-7901 cell lines (that is 32.9 ± 3.4 µg/ml) demonstrating potent cytotoxic activities. The roots extracts of *P. laui* inhibited the proliferation of BEL-7402 and SGC-7901 with the IC_{50} values at 13.81 ± 4.6 and 6.74 ± 1.9 µg/ml, respectively. From the above results we might concluded that the ethanol extracts of *P. laui* and *P. rumphii*, especially roots extracts, had potent cytotoxic effects. Therefore, additional work was required to these two plants to determine the effects of the different organic solvent fractions in order to find the active constituents. As shown in Table 2, the ethyl acetate, chloroform and petroleum ether fractions of *P. laui* showed different

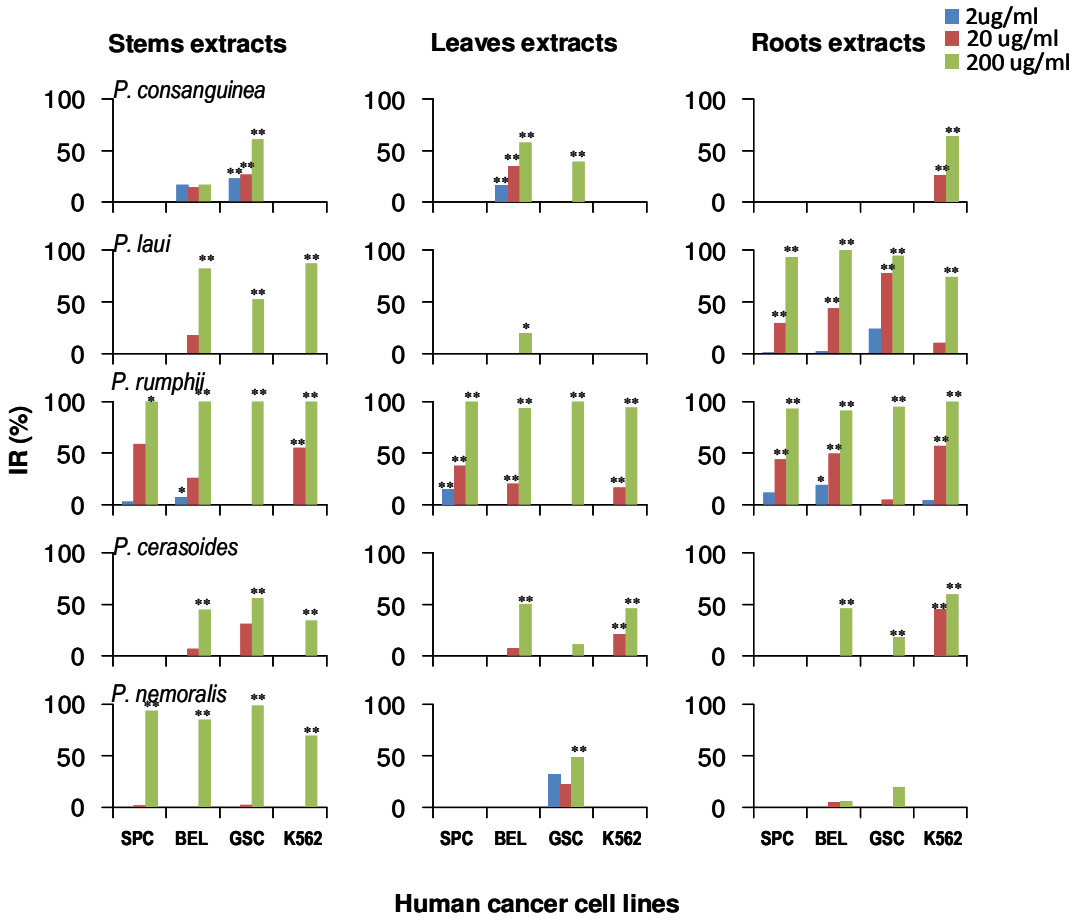

Figure 1. *In vitro* cytotoxicity of the ethanol extracts at three different concentrations (that is, 2, 20 and 200 µg/mL) of various parts of the five tested plants derived from the genus *Polyalthia* against four human cancer cell lines using MTT assay. The inhibition ratio (IR, %) was used to assessed the cytotoxic activities compared to the negative control groups, which was calculated according to the equation: IR (%) = [OD (control group) – OD (administration group)]/OD (control group) x 100%. Asterisks indicate significant differences from negative group cells (* $P < 0.05$; ** $P < 0.01$).

Table 1. The IC50 (µg/ml) of the tested ethanol extracts against four human immortal cell lines using MTT assay.

Species	Cell lines	IC_{50} (µg/mL)		
		Stems extracts	Leaves extracts	Roots extracts
P. consanguinea	SPC-A-1	-[a]	-	-
	BEL-7402	>200	95.0 ± 2.4b	-
	SGC-7901	102 ± 5.6	>200	-
	K562	-	-	92.7 ± 4.7
P. laui	SPC-A-1	-	-	44.3 ± 2.1
	BEL-7402	51.1 ± 7.2	>200	13.81 ± 4.6
	SGC-7901	>200	-	6.74 ± 1.9
	K562	195 ± 20.3	-	94.7 ± 4.5
P. rumphii	SPC-A-1	11.7 ± 2.7	10.7 ± 3.5	9.16 ± 2.5
	BEL-7402	13.2 ± 4.6	56.4 ± 3.3	7.27 ± 1.1
	SGC-7901	59.9 ± 6.1	59.9 ± 4.5	32.9 ± 3.4
	K562	24.5 ± 3.2	57.0 ± 4.3	5.68 ± 7.7

Table 1. Continued.

P. cerasoides	SPC-A-1	-	-	-
	BEL-7402	>200	141 ± 31.0	>200
	SGC-7901	98.5 ± 19.8	>200	>200
	K562	>200	>200	85.2 ± 23.4
P. nemoralis	SPC-A-1	82.1 ± 4.6	-	-
	BEL-7402	>200	-	>200
	SGC-7901	58 ± 9.9	>200	>200
	K562	>200	-	-

a. The symbol (-) represents the values of IC50 which cannot be calculated according the MTT assay results. b. Values are averages and standard deviations for 3 independent experiments.

Table 2. The IC50 (µg/ml) of the tested organic solvents fractions against four human immortal cell lines using MTT assay.

Extract fraction		**Cell lines**	**IC_{50}(µg/mL)**
Root of *P. laui*	Acetate fraction	SPC-A-1	-[a]
		BEL-7402	189 ± 31.1b
		SGC-7901	46.6 ± 9.8
		K562	4.86 ± 2.1
	Chloroform fraction	SPC-A-1	8.65 ± 2.3
		BEL-7402	58.0 ± 3.5
		SGC-7901	10.1 ± 1.9
		K562	9.68 ± 1.1
	Petroleum ether fraction	SPC-A-1	55.9 ± 3.4
		BEL-7402	26.1 ± 4.2
		SGC-7901	34.7 ± 7.9
		K562	0.98 ± 0.3
	n-butanol fraction	SPC-A-1	-
		BEL-7402	-
		SGC-7901	-
		K562	-
Root of *P. rumphii*	Acetate fraction	SPC-A-1	-
		BEL-7402	-
		SGC-7901	>200
		K562	>200
	Chloroform fraction	SPC-A-1	32.1 ± 3.3
		BEL-7402	35.0 ± 4.5
		SGC-7901	26.2 ± 5.1
		K562	42.3 ± 4.6
	Petroleum ether fraction	SPC-A-1	34.0 ± 7.8
		BEL-7402	28.4 ± 2.9
		SGC-7901	35.5 ± 6.9
		K562	26.1 ± 5.3

a. the symbol (-) denotes the values of IC50 which cannot be calculated according to the MTT assay results, b. Values are averages and standard deviations for 3 independent experiments.

inhibitory effects. The chloroform fraction exhibited the inhibitory activity against SPC-A-1 with an IC_{50} value at 8.65 ± 2.3 µg/ml. The petroleum ether fraction showed most potent inhibitory effects against the K562 cell line and its IC_{50} value was 0.98 ± 0.3 µg/ml. However, the chloroform fraction and petroleum ether fractions of *P. rumphii* exhibited weaker cytotoxic effects against the human cell lines and the IC_{50} values ranged from 26 to 42 µg/ml. Furthermore, the active constituents both *P. laui* and *P. rumphii* concentrated in the chloroform and petroleum ether fractions indicating the potential active compounds having lipophilic properties. Comparison between the ethanol extracts and the three different fractions of *P. laui*, we found that some results were inconsistent, e.g. the inhibitory effects against K562 cell lines.

The possible explanation for these discrepancies could be that the complex multiple effect of the extracts or the fractions. In addition, the inhibitory activities of *P. rumphii* ethanol extracts were relatively potent as aforementioned, but the results of the chloroform and petroleum fractions were different. Although the weaker cytotoxic effects of the chloroform fraction derived from *P. rumphii*, we had isolated six purified compounds (that is, Ax-5-RP1, Ax-8-1, Ax-8-3, Ax-8-3, Ax-8-8, and Ax-8-9). Ax-5-RP1 and Ax-8-1 showed potent cytotoxic activities against K562 cell lines with an IC_{50} value at 9.55 and 9.31 µM, respectively. This work will be issued after further research.

In summary, the results presented here suggested that the roots extracts of *P. laui* and *P. rumphii* inhibited the human cell lines to some extent. The presence of purified compound(s) might contribute to the anticancer effects.

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