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Full Length Research Paper

Cytotoxic activities of some selected medicinal plants of the genus *Euphorbia*

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The present study was undertaken to evaluate the cytotoxic activities of extracts of *Euphorbia* genus plants, *Euphorbia fischeriana, Euphorbia tirucalli, Euphorbia humifusa and Euphorbia antiquorum*. The plant selection was based on ethnobotanic information and interviews with local healers. Cytotoxic activities were screened by an *in vitro* assay system of growth inhibition against two human cancer cell lines, human hepatocyte carcinoma cell (BEL-7402) and human lung cancer cell (A-549). Extracts which exhibited IC_{50} value less than 30 µg/ml were considered active. The $CHCl_3$ extracts from *E. tirucalli* and *E. antiquorum* exhibited strong cytotoxic effect on the two cell lines. The $CHCl_3$ and EtOAc extracts from *E. fischeriana* showed significant cytotoxic activity on the A-549 cell. This study revealed that the $CHCl_3$ extracts from *E. tirucalli* and *E. antiquorum,* the $CHCl_3$ and EtOAc extracts from *E. fischeriana* may have a great potential to be exploited for the search of anticancer drug. It is necessary for chemical characterization of the active principles and more extensive biological evaluations.

Key words: Cytotoxic activity, Euphorbia, human cancer cells.

INTRODUCTION

The genus Euphorbia is the largest in the spurge family, comprising more than 2000 species (Jassbi, 1977). Some species of the genus Euphorbia have been used as medicinal plants for the treatment of skin diseases, gonorrhea, migraine, and intestinal parasites and as wart cures (Singla and Kamala, 1990). Diterpenoids are the majority of the genus with many different core frameworks such as jatrophanes, lathyranes, tiglianes, ingenanes, myrsinols, etc. In addition, sesquiterpenoids, phloracetophenones, cerebrosides, glycerol, flavonoids, and steroids were also obtained. The compounds isolated from genus Euphorbia and extracts perform many different activities, including antiproliferation, modulability of multidrug resistance, cytotoxic activity, antimicrobial and anti-inflammatory activity, etc (Shi et al., 2008).

Though the cancer therapy is in advance, side effects due to the non-specific cytotoxicity of drugs and resistance to treatment represent a great problem in the cancer treatment. Therefore, development and search of novel and effective anticancer agents have become very important issues (Cameron and Bell, 2004). As we know, natural compounds have provided many effective anticancer agents in current use. Currently, over 50% of drug used in clinical trials for anticancer activity were isolated from natural sources or are related to them (Newman and Gragg, 2007).

In the course of our screening strategy for the anticancer compounds from plants, we undertook the present study to evaluate the *in vitro* cytotoxic activity of four plants used in the traditional Chinese medicine for various diseases such as cancer, inflammation or infectious diseases. We made several extracts using organic solvents from the four selected plants of genus Euphorbia, the cytotoxic activity of all these extracts was assayed on two human cancer cell lines, human hepatocyte carcinoma cell line BEL-7402 and human lung cancer cell line A-549, respectively.

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Table 1. Ethnobotanical data and some reported pharmacological activities of plants species used in this study.

Botanical name	Local name	Part plant collected	Traditional use	Pharmacological activies
Euphorbia fischeriana	Maoyan herb Langdu	Roots	Edema, ascites, and cancer (Jiangsu New Medical College, 1986)	Antitumor effects (Yang et al., 2002; Shen et al., 1984)
Euphorbia tirucalli	Lvyushu, Guanggunshu	Aerial part	Having irriant to skin	Antibacterial (Lirio et al., 1998) Antiherpetic (Betancur-Galvis et al., 2002)
Euphorbia humifusa	Xuejianchou	Aerial part	Dysentery, enteritis, and hematochezia (State Administration of Traditional Chinese Medicine, 1999)	anti-HBV (Tian et al., 2009)
Euphorbia antiquorum	Jinggangzuan, Huoleyang	Aerial part	Cutaneous infections, bronchitis, and latex for dropsy (Jain et al., 1991)	Antivirus (Akihisa et al., 2002)

MATERIALS AND METHODS

Plant material

The selected plants were collected in Yunnan Province of China in October, 2009 and were identified by Dr. ZB Guan from the Yunnan Branch Institute of Medicinal Plants, Chinese Academy of Medical Sciences. Voucher specimens are kept in the "Herbarium" of the Institute. A list of related work for the species of plants studied was shown in Table 1.

Preparation of extract

Selected plants were dried at room temperature, powdered and extracted with 95% EtOH for three times, and the extracts were evaporated under reduced pressure at 40℃ to give residues and then suspended in distilled H_2O and partitioned successively with $CHCl_3$, EtOAc, and *n*-BuOH. Every organic solvents extracts were evaporated under reduced pressure at 40℃ to give $CHCl_3$, EtOAc, and *n*-BuOH extracts.

A total of 20 mg of obtained extracts were dissolved in dimethyl sulfoxide (DMSO) to give a solution stock to 20 mg/ml. Extracts were sterilized by filtration using sterile 0.22 µm pore size filters.

In vitro cytotoxic activity assay

Cell lines and culture medium

Two human cancer cell lines were used in this study, human hepatocyte carcinoma cell line BEL-7402 and human lung cancer cell line A-549. Cells were cultured in minimum essential medium (MEM), supplemented with 10% fetal bovine serum. Cultures were maintained at 37℃ in 5% CO_2 and 100% relative humidity atmosphere.

Cytotoxicity assay

Cytotoxicity of 12 extracts against BEL-7402 and A-549 cells were measured using the MTT method (Tao et al., 2007). Briefly, cells in 100 µl of culture medium were plated in each well of 96-well plates (Falcon, CA). Cells were treated in triplicate with graded concentretions (final concentrations ranging from 1 to 100 µg/ml) of extracts at 37℃ for 72 h. A 20 µl aliquot of MTT solution (5 mg/ml) was added directly to the appropriate wells. The cultures were incubated for 4 h and then 100 µl of "triplex solution" (10% SDS/ 5% isobutanol/ 12 mM HCl) was added. The plates were incubated at 37℃ overnight and then measured using a plate reader at 570 nm (VERSA Max, Molecular Devices). The results were all expressed as IC_{50} values as calculated by the Logit method.

RESULTS AND DISCUSSION

The search for new anti-cancer drugs is one of the most prominent research areas of natural products, especially to medicinal plants having clinical application experience. Some diterpenoid compounds from the genus Euphorbia showed significant cytotoxic activities (Wang et al., 2006, 2010). In this study, we collected four plants of the genus Euphorbia, which were often used as remedy to treat cancer. The extracts for selected plants were prepared according to this remedy. We investigated the cytotoxic potential of 12 extracts for four selected plants to support these plants used in traditional medicine for the treatment of cancer.

The cytotoxic activity was evaluated on two human cancer cell lines, BEL-7402 and A-549. The cytotoxic effect of 12 extracts on BEL-7402 and A-549 cell lines was determined using the

Table 2. IC_{50} values of extracts of selected Euphorbia species on human cancer cell lines.

Plant names	Extracts	IC_{50} values[a] (µg/ml) in two cell lines[b]	
		BEL-7402	A-549
E. fischeriana	$CHCl_3$	51.5	8.4
	EtOAc	>100	1.4
	n-BuOH	>100	>100
E. tirucalli	$CHCl_3$	10.1	4.6
	EtOAc	16.9	46.9
	n-BuOH	>100	69.6
E. humifusa	$CHCl_3$	>100	>100
	EtOAc	19.2	>100
	n-BuOH	>100	>100
E. antiquorum	$CHCl_3$	5.4	1.9
	EtOAc	>100	>100
	n-BuOH	>100	>100

[a]extract with IC_{50} value $\leq$ 30 µg/ml considered active (Suffness and Pezzuto, 1999). [b]BEL-7402 (human hepatocyte carcinoma cell); A-549 (human lung cancer cell).

MTT assay. The assay results represented by IC_{50} are summarized in Table 2.

Eight of twelve extracts from four selected plants showed cytotoxic effects in BEL-7402 and A-549 cells (Table 2), according to The American National Cancer Institute assigns a significant cytotoxic effect of promising anticancer product for future bioguided studies if It exerts an IC_{50} value < 30 µg/ml (Suffness and Pezzuto, 1999). Generally, the cytotoxic activity of extracts could be due to the presence in the extracts of active compounds that could probably have highly cytotoxic activities. In this preliminary study, we have focused our interest on crude plant extracts to provide an important basis for further investigation into the isolation, characterization of cytotoxic compounds from the screened medicinal plants.

The $CHCl_3$ and EtOAc extracts from *E. fischeriana* showed strongly cytotoxic activity on A-549 cells with IC_{50} values of 8.4 and 1.4 µg/ml, respectively. Several studies have been reported on the phytochemical and biological properties of *E. fischeriana*. Two *ent*-abietane diterpenoids, jolkinolides A and B, the major components of the roots, showed significant antitumor activities against sarcoma 180 and Ehrlich ascites carcinoma in mice (Liu et al., 1988; Uemura et al., 1977). Wang et al. (2006) reported that two diterpenoid compounds from *E. fischeriana* exhibited significant cytotoxic activity to Ramos B cells with IC_{50} values of 0.023 and 0.0051 µg/ml, respectively. Recently, another diterpenoid isolated from this plant showed potent cytotoxic effect to MDA-MB-231 cells (Wang et al., 2010). It suggested that this plant is a source to find lead compounds with anti-cancer effect. The $CHCl_3$ extract of *E. tirucalli* exhibited significant cytotoxicity on BEL-7402 cells with IC_{50} values of 10.1 µg/ml, and more strong cytotoxicity on A-549 cells with IC_{50} values of 4.6 µg/ml. The EtOAc extracs of *E. tirucalli* show potent cytotoxic activity on BEL-7402 cells with IC_{50} values of 16.9 µg/ml, but no cytotoxic activity on A-549 cells. Previous phytochemical studies of *E. tirucalli* revealed the presence of triterpene (Rasool et al., 1989; Khan et al., 1988); macrocyclic diterpene compounds (Khan and Malik, 1990). The macrocyclic diterpene and triterpene compounds have been shown to possess anticancer activity (Shi et al., 2008; Manez et al., 1997), but the cytotoxic activity of isolated compounds from *E. tirucalli* has not been done well. It is very promising to find potential anticancer lead compounds from *E. tirucalli.*

Only EtOAc extract of *E. humifusa* showed mild cytotoxicity on BEL-7402 cells with IC_{50} values of 19.2 µg/ml, this plant has not been previously investigated for cytotoxicity but only mild activity was observed in this study.

The $CHCl_3$ extract from *E. antiquorum* exhibited strongly cytotoxic activity on BEL-7402 and A-549 cells with IC_{50} values of 5.4 and 1.9 µg/ml, respectively. The EtOAc and n-BuOH extracts showed no cytotoxic activity on two cell lines. Some diterpene and triterpene compounds have been isolated from *E. antiquorum* (Min et al., 1989; Anjaneyulu and Ravi, 1989; Grewali et al., 1990; Akihisa et al., 2002), but the cytotoxicity for extract and isolated compounds of *E. antiquorum* has not been reported. In our continuing research progress, we will focus on the $CHCl_3$ extract of *E. antiguorum* to find lead

compounds with anticancer activity.

Conclusion

According to the research results, It provide important preliminary data to help find lead compounds with cytotoxic activity from active extracts of Euphorbia species. It is necessary to isolate, identify and verify the cytotoxic activities of the active extracts by bioassay-guided protocol to isolate bioactive secondary metabolites with cytotoxic properties.

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