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Short Communication

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IN VITRO CYTOTOXIC ACTIVITY OF EXTRACTS OF *MARCHANTIA CONVOLUTA* ON HUMAN LIVER AND LUNG CANCER CELL LINES

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Abstract

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to test cytotoxicity of the extracts from *Marchantia convoluta* against two human cancer cell lines (H1299 and HepG2). Low concentrations of the ethyl acetate extract had toxic effects on HepG2 cells and inhibited H1299 cells at higher concentration. Inhibition of proliferation and induction of cell death were in a concentration-dependent. However, the petroleum ether and n-butanol extracts of *Marchantia convoluta* showed no cytotoxic activity.

Keywords: Cytotoxicity test; MTT assay; *Marchantia convoluta*

Introduction

Marchantia plants (Chinese name, Di Qian) are well-known traditional Chinese medicinal herbs used extensively to treat tumefaction of skins (JIB, 1990), protect liver and treat hepatitis (Zhu et al., 2004) and as an antipyretic in countryside (Xiao et al., 2004). *Marchantia convoluta* is ample in Guangxi Zhuang Atuonomous District. According to our investigation, flavonoids and sesquiterpenes are the major constituents in *Marchantia convoluta* (Cao et al, 2005;Xiao et al, 2004;Xiao et al, 2005;Zhu et al, 2003;Zhu et al, 2005). There are few reports on the bioactivity of *Marchantia convoluta.* In this study, the effects of extracts of *Marchantia convoluta* as anti-cancer agents were investigated.

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. The high incidence of the liver cancer has been attributed to factors such as persistent infection with hepatitis virus and contact with hepatocarcinogens like nitrosamines and aflatoxins (Henry et al., 2002). Because of the mutlifocal nature of liver carcinoma, most cancer patients are considered nonresectable at the presentation

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of case. In these patients, chemotherapy is the only choice of treatment. Unfortunately, development of drug resistance in tumor after treatment is always a major obstacle to the successful management of liver cancer (Gottesman, 2002). Thus, developing new therapeutic agents that can overcome drug resistance becomes an urgent need for cancer patient.

Materials and Methods

Plant material

The whole plants of *Marchantia convoluta* were collected in Shangling City of Guangxi Zhuang Autonomous District in August 2003. The specimen (No 20041364) was identified by Zhou Zi-jing at Biology Department of Guangxi Chinese Medical University. The leaves, after being washed with water and dried in the shade for several days, were powdered.

Preparation of plant extracts

The powdered plant material (500 g) was extracted three times with 50% ethanol (v/v) (3000 ml). The accumulated alcoholic extract was concentrated to dryness under reduced pressure and extracted with petroleum ether (3×100 ml). The residue was extracted three times with ethyl acetate (3 ×100 ml). Then the residue was extracted three times with n-butanol (100 ml). The filtrate was concentrated to dryness under reduced pressure. The residues were dissolved in ethanol to form stock solutions sterile filtered with filters (0.2 μm) before testing.

In vitro cytotoxic assay
Human cell lines

Human non-small cell lung carcinoma cell lines H1299 and human liver carcinoma HepG2 were used. Cells kindly provided by Dr. Xu of MSKCC, US were cultured in RPMI 1640 medium supplement with 10% heated foetal bovine serum, 1% of 2mM L-glutamine, 50 IU/ml penicillin and 50 μg/ml streptomycin and maintained at 37 in a 5% CO_2 atmosphere with 95% humidity.

MTT assay

Cells were washed with phosphate buffered saline (PBS) free of Magnesium and Calcium. The PBS was decanted and cells detached with 0.025% trypsin-EDTA (Sigma) and PBS was added to a volume of 50 ml. The cell pellet, obtained by centrifugation was suspended in 10 ml of medium to make a single cell suspension, viable cells density being counted by trypan blue exclusion in a haemocytometer and then diluted with medium to give the previously-determined optimal plating densities

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for H1299 and HepG2 respectively. 100 µl/well of these cell suspensions were seeded in 96-well microtiter plates and incubated at 37 ^{o}C to allow for cell attachment. After 24 h the cells were treated with the extracts. Each extract (500 µg/ml) was tested initially against both cancer cell lines. The active extracts were considered to be those hich gave less than 50% survival at 72 h. The active extracts were further diluted in medium to produce 8 concentrations of 15, 30, 40, 50, 100, 200 µg/ml of each extract. 100 µl/well of each concentration was added to the plates in six replicates. At the end of exposure time, the medium was removed and then 3-(4,5-dimethylthiazol-2-yl)-2,5 -diphenyltetrazolium bromide (MTT) assays were performed by the cell titer kit^{TM} (Promega) following the standard procedure. 20ml of MTT (5 mg/ml) in PBS was incubated with cells in a 96-well plate for 2 h at 37 ^{o}C. Subsequently, the medium containing MTT was removed, and 100 ml of acidified isopropanol (0.04 N HCl) added. Spectrophotometric absorbance of each sample was measured at 470 nm using a microplate reader (Bio-Rad, model 3550). The datas were normalized (A570 nm). The mean absorbance was plotted against drug concentration. Three replicate plates were used to determine the cytotoxicity of each extract. The concentration of each extracts reduced cell survival by 50% (IC_{50}) was determined from cell survival curves.

Statistics

Datas were expressed as the mean±SD. from at least three determinations. Statistical analysis was performed using Student's t-test, with $P < 0.001$ as a criterion of significance.

Results and Discussion

The results are presented in Table 1 and Figure 1.

Table 1: Cellular sensitivity of H1299 and HepG2 cells to all extracts

Extracts	IC_{50} (µg/ml)	
	H1299	HepG2
Petroleum ether	>500	>500
Ethyl acetate	100	30
n-butanol	>200	>200

IC_{50}, drug concentration causing a 50% decrease in a survival curve.

The effect of all extracts on different cell lines was studied by measuring cell numbers by MTT assay after treatment of the cultures with each extract for 72 h. The treatment of all cell lines with ethyl acetate extract decreased cell numbers (Figure 1). However, both petroleum ether and n-butanol extracts did not inhibit cell proliferation. As indicated in Table 1, treatment of H1299 and HepG2 cells with ethyl acetate extract resulted in loss of cell viability. However, H1299 and HepG2 cells more resistant to

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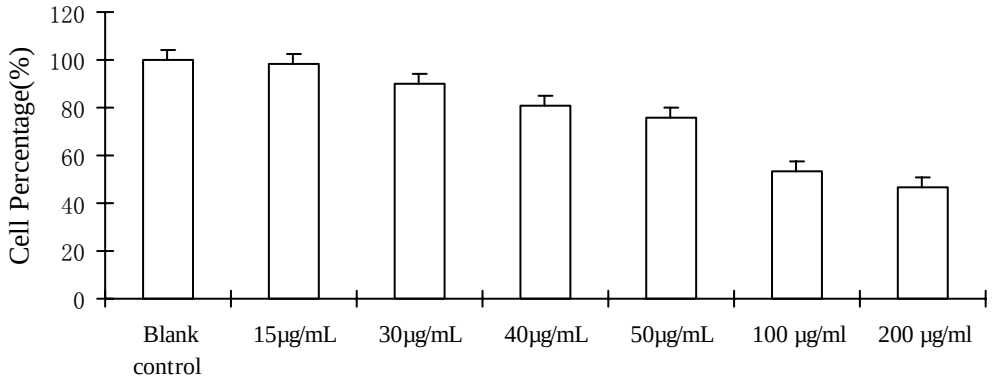
petroleum ether and n-butanol extracts, when compared to their corresponding IC_{50} of H1299 and HepG2 cells.

Figure 1a showed that a high concentration of ethyl acetate extract (100 µg/ml) decreased the number of H1299 cells rapidly. At lower concentrations of ethyl acetate extract (15, 30 and 40 µg/ml), the HepG2 cells numbers started to decrease markedly (Figure 1b). For H1299 cells no inhibition was observed at 15 µg/ml of ethyl acetate extract. These results suggest that at low concentrations the ethylacetate extract has toxic effect on HepG2 cells while the extract inhibited H1299 cells at higher concentrations. Inhibition of proliferation and induction of cell death were concentration - dependent.

The petroleum ether and n-butanol extracts of *M. convoluta* had no effect on the human non-small cell lung carcinoma cell lines H1299 (IC_{50} > 500 and 200 µg/ml, respectively) and human liver carcinoma HepG2 (IC_{50} > 500 and 200 µg/ml, respectively) in all cases.

a



b

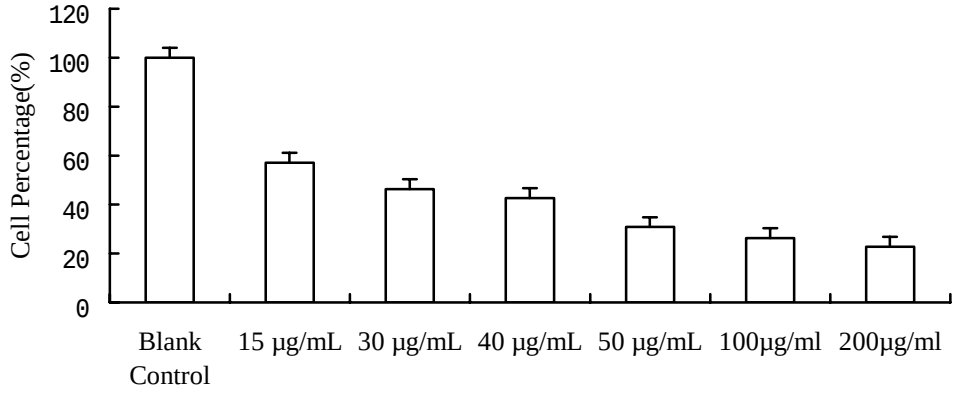


Figure 1: 1a: inhibition of the effect of ethyl acetate extract on H1299
1b: Inhibition effect of ethyl acetate extract on HepG2

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Conclusion

The results obtained in this study indicated that one of the three extracts of *M. convoluta* was preferentially active against cancer cells. The ethylacetate extract of *M. convoluta* were the most cytotoxic against human non-small cell lung carcinoma cell lines H1299and human liver carcinoma HepG2 while petroleum ether and n-butanol extracts of *M. convoluta* showed no cytotoxic effect against cancer lines.

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