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Cytotoxic Activity of Crude Extract from *Costus malortieanus* (Costaceae)

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Abstract: The cytotoxic effects of the crude extract from the root of *Costus malortieanus* were investigated. Polar fraction of the plant was extracted by methanol. The extract was tested for its cytotoxicity effects towards two types of cells, normal embryonic liver cell line (WRL 68) and liver cancer cell line (Hep G2). The cells were treated with different concentrations of the extract. The result showed that the methanolic extract from the root of *C. malortieanus* has low toxicity towards normal cells and high toxicity towards cancer cells.

Key words: *Costus malortieanus* · C totoxicity · Hep G2 · WRL 64

INTRODUCTION

Naturally occuring agents with high effectiveness and less side effects, are desirable as substitutes for chemical therapeutics which have various and severe adverse effects. Natural products that are part of today's shield against diseases include antibiotics (erythromycin and its derivative, clarithromycin), immunosuppressive agents (cyclosporin A and tacrolimus), antitumor agents (paclitaxel and camptothecin derivatives such as topotecan and irinotecan) and antihypercholesterolemic agents (lovastatin and its derivatives) [1]. Renewed interest in investigating natural products has also led to the introduction of several important anticancer compounds such as vinblastine, vincristine, paclitaxel and the semi-synthetic compounds etoposide and teniposide, which are the chemical derivatives of podophyllotoxin, a natural product belonging to the lignan group of compounds [2].

C. malortieanus from the family of Costaceae is commonly known as the step-ladder ginger or step-ladder costus. In Malaysia, it is known as 'setawar jawa'. Within the Costaceae family, there are more than 150 species of plants. The Costaceae family includes four genera, namely *Costus* L., *Tapeinochilos* Miq., *Dimerocostus* O. Kuntze and the monotypic *Monocostus* K. Schum. Costaceae is a tropical monocotyledonous family sister to the gingers, which are from the Zingiberaceae family. *Costus*, which is the largest genus from the family, consists of over 100 species [3].

The present work was carried out to investigate the cytotoxicity of *C. Malortieanus* against normal embryonic liver cells (WRL 68) and liver cancer cells (Hep G2).

MATERIALS AND METHODS

Materials: Solvents used for extractions were hexane and methanol. Purified water was obtained from ultra-pure water purification system. Other chemicals were phosphate buffered saline (PBS), minimal essential media (MEM), trypsin, trypan blue and (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) solution.

Sample Collection: The plant sample of *Costus malortieanus* was collected from Perak in the month of July 2010. A voucher specimen has been deposited in herbarium, Research Institute of Natural Products for Drug Discovery, Faculty of Pharmacy, Universiti Teknologi MARA, Malaysia. The roots were oven-dried for 6 days with the temperature not exceeding 40°C. The dried samples were ground into coarse powder.

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Extraction: 9.4 g of the ground roots were macerated in a column flask with n-hexane, at room temperature for 24 hours and were lixiviated on the next day. The extraction was repeated using the same organic solvent, until the colour of solvent became clear. The solvent was then removed using rotary evaporator. The dried extract was then weighed and kept inside a fume hood prior to use. The ground roots were taken out from the column flask and were air-dried inside the fume hood. Once dried, the roots were extracted again following the same procedure, using methanol.

Cell Culture: Two types of cells were used, normal embryonic liver cells (WRL 68) and liver cancer cells (Hep G2). Handling and maintaining of cells were performed in a biological safety cabinet or tissue culture hood. Most WRL 68 cells were confluent after 24 hours, whereas Hep G2 cells took more than 24 hours to be confluent. The cells were then washed with PBS. In order to detach the cells from the wall of the flask, trypsin was pipetted into the flask. The process of detachment requires incubation for about 5 to 10 minutes inside the cell culture incubator. White clouds indicate that the cells were detached. The cells were later resuspended with MEM for 4 to 5 times. 3000 µl of the cells plus media were collected and were filled into the centrifuge tube. It was then centrifuged for 5 to 10 minutes, with the speed of 4000 rpm. The supernatant formed was discarded into waste and the cells were resuspended with MEM. The cells and media were divided into two and each was pipetted into two small flasks that have been previously filled with MEM. Finally, the cells were incubated for 24 hours.

Cell Counting: Cell counting was performed using a standard trypan blue cell counting technique. Once the centrifuged cells were resuspended with MEM, 10 µl of the cells plus media were pipetted into the eppendorf tube. Next, trypan blue was added into the tube and the mixture was resuspended for a few times. The stained cells were dropped onto a hematocytometer, which is a microscope counting chamber or device. The stained cells were then observed under the microscope. Living cells do not absorb the tryphan blue and appear as shining white dots with blue background. Died or burst cells absorb the die and appear as blue dots. Cell counting was performed by counting the cells in two or three different set of boxes that appear on the hematocytometer.

Cell Plating: The average number of cells was used to calculate the percentage viability of cells to be plated into 60 wells. The percentage viability of cells was calculated using the formula:

$$\% \text{ viability of cells} = \frac{2\times10^{4}\times \text{total volume of media}}{}$$

From the calculation, the amount of media to be mixed with the cells was also calculated. 50 µl of MEM was pipetted into each well that was marked as 'media', 'media + cell' as the control, 5 different concentrations from highest to lowest and 'media + solvent'. Then, 50 µl of the cells plus media that were mixed earlier were plated into each well. The plate was incubated for 24 hours to grow the cells.

Treatment of Cells with *Costus malortieanus* Methanol Root Extract: The extract was diluted with methanol to the concentration of 20 mg/ml. After filtration, 5 different concentrations of the extract were prepared in 5 amber eppendorf tubes. They were prepared by diluting with MEM. The highest concentration was 2 mg/ml, followed by 1 mg/ml, 0.5 mg/ml, 0.1 mg/ml and the lowest concentration being 0.01 mg/ml. After 24 hours of incubation, the media from each well of the plate, except for the wells marked 'media' was pipetted out using the micropipette. 50 µl MEM was pipetted into each of the 'media' wells and 100 µl MEM was pipetted into each of the 'media+cell' wells. 100 µl of the 5 different concentrations were filled into their respective wells. The 'media+solvent' wells were filled with 100 µl of 10% methanol which was diluted with the media. The plate was again incubated for 24 hours to let the extract to take effect on the cells.

MTS Assay: Cytotoxicity was determined by using (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) assay. The media was pipetted out from each well. The wells were then washed with 30 µl of PBS, then 100 µl MEM was filled into each well. 20 µl of MTS solution was pipetted into each well, followed by incubation for 2 hours. After 2 hours, the cell viability was determined by measuring the absorbance at 490 nm using the computer-connected microplate reader. The results can also be observed; yellow colour indicates the cells died whereas brown colour indicates that the cells are alive.

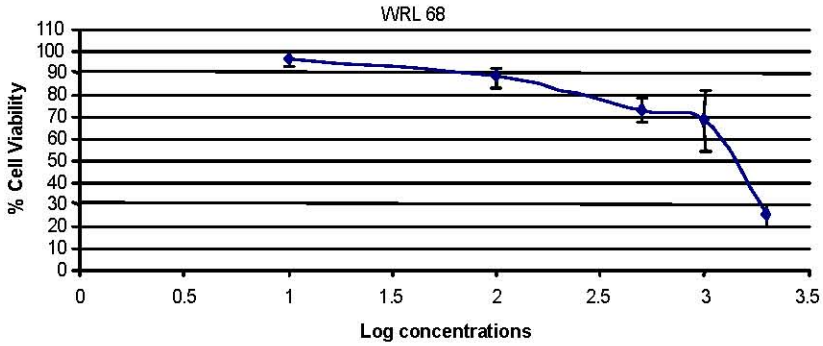

Fig. 1: Effect of Costus malortieanus methanolic root extract on WRL 68 cell viability. Data is presented as mean standard deviation ± 27 (n=3)

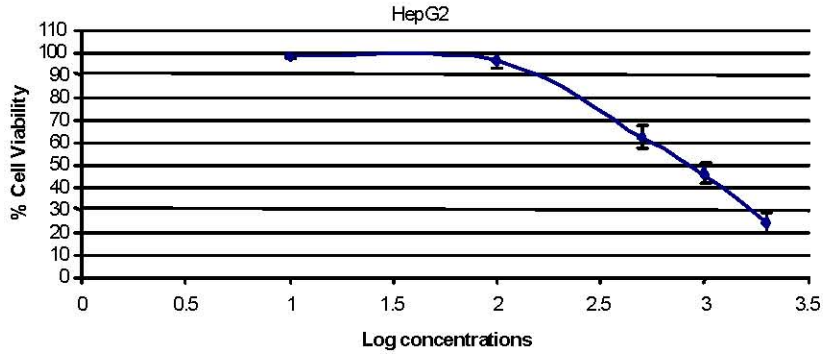

Fig. 2: Effect of Costus malortieanus methanolic root extract on Hep G2 cell viability. Data is presented as mean standard deviation ± 19 (n=3)

RESULTS AND DISCUSSIONS

To our knowledge, this is the first toxicological study of *C. malortieanus* extract. The earlier phytochemical work on Costaceae family reported the isolation of furostanol glycoside from the rhizomes of *Costus spicatus* [4]. A more recent investigation reported the identification of flavonoids, phenols, anthraquinones, cardiac glycosides, terpenoids, alkaloids and tannins [5]. Beside the phytochemical studies, crude extract of *Costus lucanusianus* showed oxytocic effect on non-pregnant rat [6]. Despite the phytochemical studies and biological activity, active constituents isolated from this plant have not been examined to a large extent.

The evidences presented here have shown for the first time that *C. Malortieanus* crude extract could inhibit the growth of cancer cells on Hep G2 cell lines. This warrants the need for bioactivity-guided isolation of the bioactive compound(s) in *C. malortieanus*.

Cytoprotective Effects of *C. malortieanus* Root Extract on WRL 68 Cell Lines: Figure 1 shows the cytoprotective effect of *C. malortieanus* methanolic root extract on the normal embryonic liver cell lines (WRL 68). The graph of percentage of cell viability versus log concentration was plotted to determine at what concentration the extract killed the cells and represent significant toxic effect towards the cells. Five different concentrations were used for this purpose: 2, 1, 0.5, 0.1 and 0.01 mg/ml. IC_{50} of *C. malortieanus* methanolic root extract against normal embryonic liver cells (WRL 68) was 1260 µg/ml. This was based on the average of 3 sets of experiments.

Cytotoxic Effects of *C. malortieanus* Root Extract on Hep G2 Cell Lines: Figure 2 shows the cytotoxic effect of *C. malortieanus* methanolic root extract on the liver cancer cell lines (Hep G2). The graph of percentage of cell viability versus log concentration was plotted to determine at what concentration the extract affected the cells or cause significant toxic effect towards the cells. Similarly, five different concentrations were used for this purpose: 2, 1, 0.5, 0.1 and 0.01 mg/ml. The IC_{50} of *C. malortieanus* methanolic root extract against liver cancer cells (Hep G2) was 800 µg/ml. This was based on the average of 3 sets of experiments.

The IC_{50} value of of *C. malortieanus* methanolic root extract against normal embryonic liver cells (WRL 68) was 1260 µg/ml, whereas the IC_{50} value of *C. malortieanus*

methanolic root extract against liver cancer cells (Hep G2) was 800 µg/ml. This suggests lower toxicity of the extract towards WRL 68 cells as compared to Hep G2 cells because higher concentration of the extract is required to inhibit or kill 50% of the WRL cells. Low toxicity towards normal cells and high toxicity towards cancer cells propose a good anticancer property of the plant extract. It also shows that the plant extract possesses a cytotoxic effect on cancer cells without causing toxicity to normal cells.

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