Cytotoxic activity of *Alpinia murdochii* Ridl.: A mountain ginger species from Peninsular Malaysia

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ABSTRACT

Background: *Alpinia murdochii* (Zingiberaceae) is a wild ginger species restricted to mountain areas of Peninsular Malaysia. Due to rapid development and deforestation activities, this species is becoming rare. This is the first report of the cytotoxic activity of *A. murdochii*. **Objective:** The present study aimed to investigate the cytotoxic effect of leaves and rhizomes of *A. murdochii* against selected human cancer cell lines by using *in vitro* cytotoxicity assay. **Materials and Methods:** The leaves and rhizomes of *A. murdochii* were extracted in hexane, dichloromethane (CH_2Cl_2), and methanol (MeOH) prior to cytotoxic activity assessment against selected human cancer cell lines, namely MCF7 (hormone dependent breast carcinoma cell line), HT29 (colon carcinoma cell line), and SKOV-3 (ovarian cancer cell line) by using *in vitro* neutral red cytotoxicity assay. **Results:** The hexane and CH_2Cl_2 extracts of both leaves and rhizomes exhibited remarkable cytotoxic effect against SKOV-3 cells with the IC_{50} values in the range of 5.2-16.7 µg/ml. **Conclusion:** Based on the preliminary data obtained in the present study, the leaves and rhizomes of *A. murdochii* may be viable therapeutic or preventive candidates for the treatment of ovarian cancer.

Key words: Cancer cell line, neutral red cytotoxicity assay, SKOV-3 cells, Zingiberaceae

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INTRODUCTION

Up to the present time, mortality that results from the common forms of cancer is still excessively high in Malaysia. According to the Malaysian Cancer Statistics, a total of 21,773 cancer cases were diagnosed among Malaysians in Peninsular Malaysia in the year 2006.[1] Researchers are increasingly turning their attention to natural products, looking for new clues to develop better anti-cancer drugs. The use of natural products as anti-cancer drugs are now well-known and have been repeatedly presented and discussed.

The family Zingiberaceae is among the plant families, which are widely distributed throughout the tropics, mostly in Southeast Asia. Many *Alpinia* species are considered medicinal herbs and have been reported to possess antioxidant, anti-inflammatory, anti-cancer, immunostimulating, hepatoprotective, and antinociceptive activities.[2] The present authors have also previously reported the excellent cytotoxic activity of several *Alpinia* species, such as *Alpinia scabra*[3] and *Alpinia mutica*.[4]

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Alpinia murdochii is a wild Zingiberaceae species restricted to mountain areas of Peninsular Malaysia. It grows up to about 1.5 m tall and is aromatic in almost all of its parts. The flowers are spotted crimson, orchid-like and borne in an inflorescence. Due to rapid development and deforestation activities, this species is becoming rare in Malaysia. The present study aimed to investigate the cytotoxic effect of leaves and rhizomes of *A. murdochii* against selected human cancer cell lines using *in vitro* cytotoxicity assay. To our knowledge, there has been no previous investigation on the cytotoxic activity of *A. murdochii*. Such study may also provide information for the evaluation, sustainability and conservation of the rich biodiversity in Malaysia.

MATERIALS AND METHODS

Plant material

The fresh leaves and rhizomes of *A. murdochii* were collected from Genting Highland, Pahang, Malaysia. The plants were identified by Professor Dr. Halijah Ibrahim of Institute of Biological Sciences, Faculty of Science, University of Malaya, Malaysia and a voucher specimen (Herbarium No.: HI 1420) was deposited at the herbarium of the Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia.

Extraction for preliminary cytotoxic investigation

The hexane, dichloromethane (CH_2Cl_2), and methanol (MeOH) extracts of the leaves and rhizomes were prepared according to the method we previously reported.[3] The leaves and rhizomes of *A. murdochii* were firstly extracted with hexane at room temperature for 3 days after dried and ground to fine powder to give hexane extract and hexane-insoluble residue. The hexane-insoluble residue of leaves and rhizomes were then extracted with CH_2Cl_2 to give CH_2Cl_2-soluble extracts and CH_2Cl_2-insoluble residue. The CH_2Cl_2-insoluble residues were further extracted with MeOH to give MeOH extracts. All the extracts were dissolved in dimethylsulfoxide (DMSO) prior to cytotoxicity assay.

Cell lines and culture medium

Hormone-dependant breast carcinoma cell line (MCF7), colon carcinoma cell line (HT29), and ovarian cancer cell line (SKOV-3) were purchased from the American Tissue Culture Collection (ATCC, USA). The MCF7 and HT29 cells were maintained in RPMI 1640 medium (Sigma) and SKOV-3 cells in Dulbecco's Modified Eagle's Medium (DMEM; Sigma), supplemented with 10% fetal bovine serum (FBS, PAA Lab., Austria), 100 µg/ml penicillin or streptomycin (PAA Lab., Austria), and 50 µg/ml of fungizone (PAA Lab., Austria). The cells were cultured in a 5% CO_2 incubator (Shel Lab water-jacketed) kept at 37°C in a humidified atmosphere.

In vitro neutral red cytotoxicity assay

The *in vitro* cytotoxic activity was studied using neutral red cytotoxicity assay, which was previously described.[5] Firstly, cells were plated in 96-well microplates (Nunc) and treated with different concentrations (concentrations ranging from 0.1 to 100 µg/ml) of extracts. The plates were then incubated for 72 h in CO_2 incubator at 37°C. After 72 h, the culture medium were replaced with medium containing 50 µg/ml of neutral red and the plates were further incubated for another 3 h. The media were removed and cells were washed with washing solution after incubation. The neutral red dye was eluted from the cells by adding 200 µl of resorb solution and incubated for 30 min with rapid agitation on a microplate shaker. Absorbance was taken using microplate reader at 540 nm. Cytotoxicity of each extract is expressed as IC_{50} value, which is the concentration of extracts that cause 50% inhibition or cell death, averaged from the three experiments, and was obtained by plotting the percentage inhibition versus concentration of extracts. *Cis*-platin was used as a positive control in the present study.

Statistical analysis

All samples were prepared in triplicate for comparison of values. All data were recorded as means ± standard deviation. Statistical analysis was carried out with Microsoft Excel 2010.

RESULTS AND DISCUSSION

Extraction yield

The extraction yields of leaves and rhizomes using different solvents are summarized in Table 1. The leaves and rhizomes were dried before extraction to avoid the presence of water in the extracts. The moisture content in the rhizomes was higher than the leaves as the yield of the dried and ground rhizomes (18.33%) was much lower than leaves (29.44%). CH_2Cl_2 extract gave the highest yield for the leaves (6.04%) while the MeOH extract gave the highest yield for the rhizomes (3.85%). MeOH was used as the extraction solvent due to its polarity and its known ability to extract compounds such as phenolic compounds, flavonoids, and other polar materials.[6]

Cytotoxicity of the extracts

Cytotoxicity assays are widely used in *in vitro* toxicology studies. In the present study, the cytotoxic activity of leaf and rhizome extracts of *A. murdochii* were evaluated using the neutral red assay, with *cis*-platin as positive control [Table 2]. The neutral red cytotoxicity assay is based on the initial protocol described by Borenfreund

Table 1: Yield of leaf and rhizome extracts of *Alpinia murdochii*

Parts	Samples/extracts	Weight (g) (%)
Leaves	Fresh samples	900
	Dried and ground plant material	265 (29.44)
	Hexane extract	2.38 (0.90)
	CH_2Cl_2 extract	16.0 (6.04)
	MeOH extract	8.15 (3.08)
Rhizomes	Fresh samples	1800
	Dried and ground plant material	330 (18.33)
	Hexane extract	3.75 (1.14)
	CH_2Cl_2 extract	9.03 (2.74)
	MeOH extract	12.71 (3.85)

MeOH: Methanol; CH_2Cl_2: Dichloromethane

Table 2: *In vitro* cytotoxic activity (IC_{50} µg/ml) of leaf and rhizome extracts of *Alpinia murdochii* against various cancer cell lines

Parts	Extracts	IC_{50} values (µg/ml)		
		MCF7	HT29	SKOV-3
Leaves	Hexane	>100.0	>100.0	10.8±1.4
	CH_2Cl_2	36.5±0.5	31.2±0.3	16.7±0.3
	MeOH	>100.0	>100.0	83.2±0.3
Rhizomes	Hexane	>100.0	34.5±1.3	15.0±0.0
	CH_2Cl_2	28.5±0.5	28.0±0.0	5.2±0.1
	MeOH	>100.0	>100.0	26.3±2.5
Cis-platin[a]		2.4±0.6	5.0±0.0	1.4±0.0

[a]*Cis*-platin was used as positive reference compound; the values expressed are mean±standard deviation of triplicate measurements. MeOH: Methanol; CH_2Cl_2: Dichloromethane; MCF7: Hormone dependent breast carcinoma cell line; HT29: Colon carcinoma cell line; SKOV-3: Ovarian cancer cell line

and Puerner[7] with some modifications and it determines the accumulation of the neutral red dye in the lysosomes of viable and uninjured cells. According to United States National Cancer Institute plant screening program, a plant extract is generally considered to have active cytotoxic effect if the IC_{50} value, following incubation between 48 h and 72 h, is 20 μg/ml or less.[8]

As shown in Table 2, all extracts of *A. murdochii* were selectively toxic against the SKOV-3 cells, which reached IC_{50} values at relatively low concentration compared to other cancer cells. The hexane and CH_2Cl_2 extracts of leaf and rhizome extracts displayed excellent inhibition against SKOV-3 cells (IC_{50} values of 10.8, 16.7, 15.0, and 5.2 μg/ml, respectively). Whilst, the MeOH extracts on the other hand were found to demonstrate weak cytotoxicity profile (IC_{50} >20.0 μg/ml in all cancer cells tested). This may implicate that the cytotoxic active compounds in *A. murdochii* may be present in less polar solvents (i.e., hexane and CH_2Cl_2). This finding is similar with the cytotoxicity profile of *A. scabra* and *A. mutica* which reported previously[3,4] that the less polar extracts (i.e., hexane, CH_2Cl_2, and ethyl acetate) showed better cytotoxic activity against the tested cell lines than the polar extracts.

This preliminary result shows that *A. murdochii* may be a valuable candidate as chemotherapeutic agent against ovarian cancer since the current anti-cancer drugs in the market have serious cardiotoxic effect.[9] The active ingredients in hexane and CH_2Cl_2 extracts may lead to valuable compounds that may have the ability to kill ovarian cancer cells. Attempts to carry out chemical investigations of the hexane and CH_2Cl_2 extracts are now underway.

In the present study, the stock materials of the test extracts and compounds were dissolved in 100% DMSO. The small amount of DMSO present in the wells (maximum 0.5%) was proven not to affect the experiments (data not shown). Houghton and Raman[10] also reported that at concentrations below 3% v/v, DMSO is usually not toxic to the cells.

CONCLUSION

Based on the preliminary data obtained in the present study, the leaves and rhizomes of *A. murdochii* may be viable therapeutic or preventive candidates for the treatment of ovarian cancer. The findings from the cytotoxic activity of *A. murdochii* extracts also provide some scientific support toward the utilization of selected *Alpinia* species in the treatment of inflammatory conditions and as anti-cancer agents in East Asian medicine.[11]

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