Anticancer activity of phenolic compound of Pandanus odorattissimus against MDB-MB-468 cell lines

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ABSTRACT

The present study was conducted to investigate the anti cancer activity of phenolic compound 4-(4-(3,4- dimethoxyphenyl) hexahydrofuro [3,4-c]furan-1-y1)-2-methoxyphenyl acetate isolated from Pandanus odorattissimus against MDB-MB-468 cell lines. The IC₅₀ concentration of phenolic compound was determined by MTT assay. Three concentrations were used: 5, 10 and 20 µg/ml. The results showed 5.79±0.09 inhibition of cytotoxicity at a dose of 20 µg/ml of phenolic compound and values were taken for further study. Thereafter, the apoptotic effect was evaluated by measuring the DNA fragmentation in MDB-MB-468 breast cell line and L929 normal fibroblast. Based on the results obtained in this study, it can be concluded that phenolic compound has significant anticancer activity against MDB-MB-468 cell lines and it might be good therapeutic value for further investigation to develop natural compounds as antitumor agents.

Key words: Cytotoxic activity, apoptotic activity, Pandanus odorattissimus L., MDB-MB-468 cell lines.

INTRODUCTION

Cancer is a major public health problem in the world. Breast cancer is the second leading cause of cancer deaths among women (Siegel et al., 2014). Chemotherapy is one of the commonly-used strategies in breast cancer treatment. This therapy is usually associated with adverse side effects, ranging from nausea to bone marrow failure (Cancer Research UK, 2013). Therefore, finding natural compounds from plants may provide an alternative cancer treatment.

During 1960s, the National Cancer Institute (USA) began to screen plant extracts with antitumor activity (Monks et al., 2002). As rich sources of novel anticancer drugs, natural compounds isolated from medicinal plants have received increasing interest ever since (Cai et al., 2004).

Breast cancer is the second largest cancer after lung cancer in the world and the most common malignancy among women (Kummalue et al., 2007). Breast cancers can be classified by stage, pathology, grade, and expression of estrogen receptor (ER), progesterone receptor (PR), or human epidermal growth factor receptor (Her2/neu). MDA-MB-468 cells are characterised as ER-, PR-, and Her2/neu-negative/basal-A mammary carcinoma (Engel et al., 2005). MDA-MB-468 cells were derived from the pleural effusions of 51-year-old female patients. MDA-MB-231 cells were derived from a Caucasian female, while MDA-MB-468 cells were derived from an African American female (Cailleau et al., 1978; Umadevi et al., 2013).

The search for cancer drugs from natural sources started with discovery of Podophyllotoxin in late 1960s, which further lead to the discoveries of vincristine, vinblastine, camptothecin and taxol (Gordon and David, 2005). Nature counts more than 1000 species of plant varieties which possess significant anti-cancer properties. Taxol, one of the most outstanding agents has been found beneficial in the treatment of refractory ovarian, breast and other cancers. Podophyllotoxin, synthetic modification of this molecule led to the development of Etoposide known to be effective for small cell cancers of the lungs and testes (Lee, 1993). The discovery of medicinally important herbs and their...
Mechanism of action would provide an alternative and effective treatment towards the cancer prevention. Since decades, plethoras of drugs have been developed from potent compounds which are isolated from medicinal plants.

Traditional medicine and knowledge of Ayurveda help in the discovery of new drug having high activity and low toxicity for cancer therapy. Initial research focuses on the isolation of bioactive lead compounds, chemical modification and improving other pharmacological profiles.

*Pandanus odorattissimus* L is said to be a restorative, deodorant, indolent and phylactic, promoting a feeling of well being and acting as counter to tropical lassitude. It may be chewed as a breath sweetener or used as a preservative in foods. It is also said to possess healthful properties, including antiviral, anti-allergy, antiplatelet, anti-inflammatory, antioxidant and antitumor activity (Kirtikar and Basu, 2000).

The aim of this study was to determine the anti cancer activity of phenolic compound 4-(4-(3,4-dimethoxyphenyl) hexahydrofuro [3,4-c]furan-1-yl)-2-methoxyphenyl acetate isolated from *P. odorattissimus* against MDB-MB-468 cell lines to its temperature and solid matter was removed by filtration. After this preliminary step, the same plant material was extracted in boiling distilled water under the same condition and the maceration was obtained following the aforementioned process at room temperature 28°C overnight. The solvent was removed by rotary evaporation. The yield (w/w) of the infusion and maceration of methanol was 3.78 and 1.78, respectively in terms of newly collected plant materials.

**Preparation of stock solution**

The dried seed extract was dissolved in 1 ml of DMSO (0.1% v/v) and made up to 10 ml with complete media (MEM) to give stock solution of compound 10 mg/ml.

**Extract dilution**

Stock solution was diluted with complete media to obtain the concentrations of 5, 10 and 20 µg/ml. All were stored in air tight container until tested.

**Cell culture**

MDA-MB-468 (basal-A mammary carcinoma) breast cancer cell lines and L929 normal fibroblast cell lines were obtained from NCCS, Pune. MDA-MB-468 breast cancer cell lines and L929 normal cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM), while L929 normal cells were cultured in Roswell Park Memorial Institute 1640 (RPMI-1640) medium supplemented with 10% v/v foetal bovine serum (FBS), 100 U/mL of penicillin, and 100 µg/mL of streptomycin as a complete growth medium. Cells were maintained in 25 cm² flasks and incubated in a humidified incubator (CO₂ Water-Jacketed IncubatorNuAire, Fernbrook Lane, Plymouth, USA) at 37°C with 5% CO₂.

**Cytotoxicity analysis by MTT assay**

The preliminary cytotoxicity analysis was done using methyl tetrazolium (MTT). A mitochondrial enzyme in the living cells, namely succinate hydrogenase, cleaves the tetrazolium ring, converting the methyl tetrazolium (MTT) to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells. The assay detects living, but not dead cells and signal generated is dependent on the degree of activation of the cells. This method was therefore used to measure cytotoxicity, proliferation or activation (Paul, 1975; Mosmann, 1983).

Cytotoxicity testing was performed using 3- (4,5-
dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma). In this assay, cells were harvested after reaching 80% confluence. Before starting the MTT assay, cells were optimised at different seeding densities ranging from 2.0 × 10^3 to 1.0 × 10^6 cell/mL in light to determine appropriate seeding number for the experiment. Each well of the microtiter plate (96-well) was filled with 100 µL of cell suspension MDA-MB-468 in complete growth medium. After 24 h of incubation, cells were treated with compound of different concentrations ranging from 5 to 20 µg/mL, with a total well volume of 200 µL with technical replicates. Microtiter plates were further incubated for 72 h with plant extracts. After 72 h of incubation, 20 µL of MTT (a stock solution of 5mg/mL in PBS) was added to each well and the plates incubated for 4 h at 37°C. Medium from each well was carefully removed without disturbing the MTT crystals in wells. The MTT formazan crystals were dissolved by the addition of 1M HCl and 100mM isopropanol to each well. After solubilizing the purple formazan, absorbance was measured at a wavelength of 575 nm:

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\% \text{ survival} = \frac{\text{Mean experimental absorbance}}{\text{Mean control absorbance}} \times 100
\]

DNA fragmentation

The cells were incubated with an IC50 concentration of 20 µg/ml for 5 days. The treated cells were collected every day by trypsination. DNA was extracted once, with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) and once with chloroform:isoamyl alcohol (24:1). The DNA was precipitated with a two-third volume of cold isopropanol followed by centrifugation. The DNA pellet was washed once in 70% ethanol and resuspended in deionized water containing 0.1 mg/mL. DNA was analyzed by 1.5% agarose gel electrophoresis (Oberhammer et al., 1993).

Statistical analysis

Student's t-test was used to analyze intergroup differences. Experiments were repeated at least three times, and data was represented as the mean ± SD. A p-value of less than 0.05 was considered to be statistically significant.

RESULTS

The phenolic compound 4-(4-(3,4-dimethoxyphenyl) hexahydrofuro[3,4-c]furan-1-yl)-2-methoxyphenyl acetate isolated from *P. odorattissimus* is shown in Figure 1.

**Determination of IC50 concentration using MTT assay**

The IC50 concentration of phenolic compound of *P. odorattissimus* in MDB-MB-468 cells was determined by MTT assay. The inhibition activity of MDB-MB-468 cells was plated and treated with different concentration such as 5, 10 and 20 µg/mL. The IC50 value was determined based on cell viability rates. The 5.79±0.09 inhibition was observed in concentration of 20 µg/mL which has been taken as IC50 value and fixed for our further study (Figures 2 and 3).

The DNA fragmentation of 20 µg/ml on breast cancer cell lines

A DNA fragmentation assay was used to determine whether the action of 20 µg/ml concentration of compound was associated with apoptosis or not. In this experiment, all tested cell lines were incubated with 20 µg/ml at the IC50 concentration. DNA fragmentation was found MDA-MB-468 and L929 cells at 1, 2, 3, 4 and 5 days after exposure. Therefore, the cytotoxic effect of 20 µg/ml was selective for breast cancer cell lines and mediated through the induction of apoptosis (Figure 4).

**DISCUSSION**

Medicinal plants constituents a common alternative for prevention of cancer and treatment in many countries around the world (Guilford and Pezzuto, 2000). In the present study, it was shown that phenolic compounds inhibit cancer cells by metabolizing enzymes that alter metabolic activation of potential carcinogens. The mechanism of action of anti-cancer activity of phenols is by disturbing the cellular division during mitosis at the telophase stage. It has also been reported that phenols reduce the amount of cellular protein and mitotic index and colony formation during cell proliferation of cancer cells (Soobratte et al., 2006). So they are regarded as promising anticancer agents against most human cancers (Tan et al., 2011; Kumar et al., 2011).

MTT assay is a universally accepted *in vitro* method for screening the drugs having cytotoxic activity. *In vitro* cytotoxic activity against MDB-MB-468 cell line at different concentration of phenolic compound of *P. odorattissimus* was evaluated in the present study. It was shown that phenolic compound inhibited the growth of MDB-MB-468 breast cancer cell lines and had strong cytotoxicity in a concentration-dependent manner. Cytotoxic effect against the MDB-MB-468 cell line is considered as a prognostic anticancer activity. IC50 value of phenolic compound is 20 µg/mL.

The normal fibroblast was used, cell is very difficult to culture. Therefore, the present study was designed to test only extracts that are known to show high toxicity with breast cancer cell lines and L929 used as a representative of a normal cell. Mouse fibroblast L929 is a normal cell line,
which is recommended by international standards for testing medical devices (Klaus et al., 2009) and responds more sensitively than primary cells (Tests for Cytotoxicity: In vitro Methods; 1992). A plant extract that will act successfully as an anti-cancer drug should kill cancer cells without causing excessive damage to normal cells, such as L929, while MDA-MB-468 cells is p53 mutations (Schedle et al., 1995). Furthermore, MDA-MB-468 cells is ER negative and classified as high-grade and basal type (Lacroix et al., 2006). Our results showed that 20 µg/ml had more cytotoxic effects on MDA-MB-468, a high-grade cancer. Surprisingly, it appeared less toxic in normal fibroblast cells than breast cell lines. These results suggest that all fractions were more selective for breast cells than normal cells.
This phenomenon can be generally detected by agarose gel electrophoresis, as shown in Figure 3. The results showed that the DNA MDA-MB-468 and L929 cells treated with 20 µg/ml were observed within five days. Therefore,
DNA ladder formation indicated that the cytotoxic effect of 20µg/ml caused inhibition in the growth of breast cancer and normal fibroblast cells through apoptosis. 20 µg/ml also inhibited growth in normal breast cells, but not to the point of death through apoptosis (Potchanapond et al., 2015). In the L929 cells, 20 µg/ml inhibited cell growth within four days and induced cell death on Day 5 after incubation. Although treated L929 cells showed DNA ladders at Day 5, the incubation time was longer and the dose was higher than in the breast cancer cell lines. Thus 20µg/ml seemed to be safe for normal cells.

**CONCLUSION**

The medicinal plant constitutes a common alternative for cancer prevention and treatment in many countries around the world. The compound has been isolated from *P. odorattissimus* endangered plant species for the treatment of various human ailments for many years. In the present study, the results of anticancer activity of compound showed a potent cytotoxic activity against MDB-MB-468 breast cancer cell lines. Thus the inhibition of cancer after the treatment of pure extract of phenolic compounds 4-(4-(3,4-dimethoxyphenyl)hexahydrofuro[3,4-c]furan-1-yl)-2-methoxyphenyl acetate on the MDB-MB-468 cell line indicates that the compound is a potent drug for the cancer treatment.

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**REFERENCES**


