Cytotoxic and apoptosis effects of *Pandanus odorattissimus* extracts on human breast cancer cell line (MCF-7).

**ABSTRACT**

The present study was to investigate the cytotoxic activities of *Pandanus odorattissimus* extract against MCF-7 cell lines. MCF-7 cell were cultured in MEM medium and incubated with different concentrations 12.5, 25, 50 and 100 µg/ml of *P. odorattissimus* extract for 72 h, respectively. The MCF-7 cells were treated with petroleum ether and the growth was inhibited at 12.5 µg/ml (33, 34 and 34%), 25 µg/ml (36, 35 and 37%), 50 µg/ml (44, 45 and 45%) and 100 µg/ml (52, 53 and 54%) after 24, 48 and 72 h treatments, respectively. The cell growth inhibition increased when MCF-7 cells were treated with chloroform extracts at 12.5 µg/ml (31, 32 and 34%), 25 µg/ml (37, 38 and 37%), 50 µg/ml (47, 48 and 49%) and 100 µg/ml (52, 53 and 54%) after 24, 48 and 72 h treatments, respectively. Similar pattern of cell growth was observed when the methanol extracts was 12.5 µg/ml (32, 36 and 39%), 25 µg/ml (42, 45 and 46%), 50 µg/ml (45, 50 and 55%) and 100 µg/ml (57, 61 and 62%) after 24, 48 and 72 h treatments, respectively. The methanol extracts IC<sub>50</sub> values of 100, 50 and 25 µg/ml determined for 24, 48 and 72 h treatment, respectively, indicated that after 72 h treatment, the extract exhibited potent cytotoxicity against MCF-7 cells.

**Key words:** *Pandanus odorattissimus* extract, MCF-7 cells, cytotoxicity, apoptosis.

**INTRODUCTION**

Breast cancer is one of the most common causes of cancer in females globally (WCR, 2008). Breast cancer is the second leading cause of cancer death and the most common form of cancer affecting women worldwide. It was observed that breast cancer accounts for 23% of all newly occurring cancers in women worldwide and represents 13.7% of all cancer deaths due to breast cancer in males and females. It is the most frequent cancer in both developed and developing regions, but the rate of human breast cancer is higher in developing countries in comparison to developed nations (Ferlay et al., 2000). Over the past several decades, there has been a particular interest in the role of medicinal plant extracts in cancer prevention. The search for natural products for cancer therapy is an area of great interest. Plants are rich sources of chemically diverse compounds with several beneficial properties to human health. Consequently, about 50% of the anticancer therapeutic agents known are derived from plants (Balunas and Kinghorn, 2005). Plants have a great importance in many cultures. Humans use them for their basic needs: feeding, clothing, sheltering, hunting and nursing. As source of medicines, plants have formed the basis for sophisticated traditional systems and continue providing mankind with new remedies. In recent years, the interest in folk medicine has highly increased. It is a fact that 25% of all medical prescriptions are based on substances derived from plants or plant derived synthetic analogues (Sara et al., 2009). Traditional medicine and knowledge of Ayurveda help in the discovery of new drug leads with high activity and low toxicity for cancer therapy, while initial research focuses on the isolation of bioactive lead compounds, chemical modification and improving of other pharmacological profiles (Lee, 1993).

Natural products and related drugs are used to treat 87%
of all categorized human diseases including bacterial infection, cancer and immunological disorders (Newman and Cragg, 2007). About 25% of prescribed drugs in the world originate from plants (Rates, 2001) and over 3000 species of plants have been reported to have anticancer properties (Graham et al., 2000). About 80% of the population in developing countries relies on traditional plant based medicines for their primary health care needs (FAO, 2004).

Based on studies on the chemical structures, most of the isolated compounds belong to steroids, triterpenes, saponins, flavonoids, alkaloids, tannins and phenolics, all of which have a wide range of therapeutic possibilities (Bandaranayake, 1998).

_Pandanus odorattissimus_ L. is said to be a restorative, deodorant, indolent and phylactic promoting a feeling of well-being and acting as a 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 anti-viral, anti-allergy, anti-platelet, anti-inflammatory, antioxidant and antitumor activity (Kirtikar and Basu, 2000).

_P. odorattissimus_ L. is a native to South Asia and India has the tradition of alternative therapies; there are no procedures to test the safety and efficacy of traditional remedies and standardize their effective cure. For these reasons, it is essential to increase our efforts in the area of medicinal plant research and exploit it efficiently for the benefit of humanity.

In this study, the cytotoxic effects and cell death mechanisms of the plant _P. odorattissimus_ extracts, on human breast carcinoma cell line (MCF-7) were investigated.

**MATERIALS AND METHODS**

**Collection of plant material**

Aerial leaf parts of _P. odorattissimus_ L. were collected from Gurmitkal, near Gulbarga, north Karnataka, India. The botanical identification was made by Dr. Shiddamallya N., Scientist, National Ayurveda Dietetics Research Institute (NADRI), Bangalore and A voucher specimen deposited in the department (RRCBI- 12749).

**Preparation of plant extracts**

The extract was obtained by infusion and maceration from 200 g of plant material. The material was weighed, chopped and extracted with solvent. The infusion was prepared with 50 g of dried leaves in 2 × 200 ml of Petroleum ether, Chloroform and Methanol with respective to its temperature and the solid matter removed by filtration. After this preliminary step, the same plant material was extracted in boiling distilled water at the same condition and the maceration obtained following the aforementioned process at room temperature 28°C overnight. The solvent was removed by rotary evaporation. Microculture tetrazolium (MTT) assay of this colorimetric assay is based on the capacity of mitochondria succinate dehydrogenase enzymes in living cells to reduce the water soluble substrate 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) into an insoluble, blue colored formazen product which is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells, the level of activity is a measure of the viability of the cells (Wilson, 2000).

**Preparation of extraction for MTT assay**

Using a sensitive balance 5 mg of each extract was weighed and put in Eppendorf tubes. 50 μl of DMSO were added to the extract and the volume completed to 1 ml with distilled water obtaining a concentration of 5 mg/ml. The mixture was vortexed and stirred by magnetic stirrer to obtain a homogenous solution.

**Cell line and culturing medium**

MCF-7 (Human Breast Cancer) cells were cultured in a culturing flask containing a complete medium consisting of 10% fetal bovine serum and 90% minimal essential medium (MEM) and then incubated at 37°C. The cells were sub-cultured twice a week.

**Cell counting**

Cell counts were done using the improved Neubaure chamber. The cover slip and chamber were cleaned with detergent, rinsed thoroughly with distilled water and swapped with 70% ethanol and then dried. An aliquot of cell suspension was mixed with equal volume of 0.4% trypan blue in a small tube. The chamber was charged with cell suspension. After cells had settled, the chamber was placed under light microscope. Using 40X objective, cells in the 41 argecornor square (each containing 16 small square) were counted. The formula used for calculating cells is given as:

\[
(\text{Cells/ml})_N = \frac{\text{Number of cells counted} \times \text{Dilution factor} \times 10^4}{4}
\]

**Detection of DNA fragmentation**

MCF-7 cells (6 × 10^4 cells /ml) were cultured in 8 well-slide chamber and incubated in CO₂ incubator at 37°C. After
overnight incubation, the cells were treated with the extracts at IC$_{50}$ concentrations for 72 h. Untreated control cells were treated with DMSO. Cells were subsequently incubated for 24, 48 and 72 h. After treatment, cells were washed with Phosphate Buffered Saline (PBS) twice and subsequently processed according to the DeadEnd™ Colorimetric Apoptosis Detection System (Promega, USA) protocol as described by the manufacturer’s instruction.

**Statistical analysis**

The significance of differences were determined using one way analysis of variance (ANOVA) and Dunnett’s Multiple Comparison Test using SPSS 20.0 software.

**RESULTS**

**Cytotoxic effects**

Cytotoxicity study was carried out using petroleum ether, chloroform and methanol extracts of *P. odorattissimus* on human breast carcinoma cell line MCF-7. In all cases, there was a dose-dependent inhibition of MCF-7 cell growth especially when the cells were treated with higher concentrations. The cells were treated with petroleum ether extract where the levels of inhibition increased at higher concentrations. The MCF-7 cell growth was inhibited at 12.5 µg/ml (33, 34 and 34%), 25 µg/ml (36, 35 and 37%), 50 µg/ml (44, 45 and 45%) and 100 µg/ml (52, 53 and 54%) after 24, 48 and 72 h treatments, respectively. The extracts produced potent cytotoxic activity at 72 h treatment with IC$_{50}$ value of 25 µg/ml (Figure 1).

The cell growth inhibition increased when MCF-7 cells were treated with chloroform extracts at 12.5 µg/ml (31, 32 and 34%), 25 µg/ml (37, 38 and 37%), 50 µg/ml (47, 48 and 49%) and 100 µg/ml (52, 53 and 54%) after 24, 48 and 72 h treatments, respectively. However, the IC$_{50}$ values of the extract was 25 µg/ml at 48 h respectively, suggesting that the chloroform extracts also produced cytotoxic activity on MCF-7 cells after 72 h treatment (Figure 2).

Similar pattern of cell growth was observed when the methanol extracts (Figure 3) was 12.5 µg/ml (32, 36 and 39%), 25 µg/ml (42, 45 and 46%), 50 µg/ml (45, 50 and 55%) and 100 µg/ml (57, 61 and 62%) after 24, 48 and 72 h treatments, respectively, at extract concentrations. The IC$_{50}$ values of 100, 50 and 25 µg/ml determined for 24, 48 and 72 h treatment, respectively, indicated that after 72 h treatment, the extract exhibit potent cytotoxicity against MCF-7 cells.

**Apoptosis assay**

All the three crude extracts of *P. odorattissimus* produced potent cytotoxicity on MCF-7 cell line after 72 h treatment. All extracts were therefore subjected to apoptosis assay and the concentrations of IC$_{50}$ at 72 h used to treat the cells for 24, 48 and 72 h. Figure 4 shows that petroleum ether and chloroform extracts triggered DNA fragmentation in MCF-7 cells judging from the presence of stained nuclei. Similarly, the methanol extract produced dark stained nuclei of MCF-7 cells at all the three time points clearly indicating the presence of DNA fragmentation in methanol. It is widely demonstrated that DNA fragmentation is the
Figure 2: Cell growth inhibition for *Pandanus odorattissimus* chloroform extracts against MCF-7 cell at 24, 48 and 72 h, respectively.

Figure 3: Cell growth inhibition for *Pandanus odorattissimus* methanol extracts against MCF-7 cell at 24, 48 and 72 h, respectively.

hallmark of apoptosis (Bowen et al., 1999). These results strongly suggest that all the three extracts of *P. odorattissimus* killed MCF-7 cells through apoptosis.

DISCUSSION

Cancer is a worldwide major problem which claimed more than 6 million lives on a yearly basis. In the past few decades the incidence of breast cancer has increased considerably in the developed countries (Parkin, 1998). It was observed that breast cancer accounts for 23% of all newly occurring cancers in women worldwide and represent 13.7% of all cancer deaths due to the breast cancer in males and females. Natural products play an important role in the discovery of new drugs. More than half of the newly approved drugs are of natural product origin or designed based on the structure of natural product, whereas the synthetic and synthetic with natural product mimic compounds constitute 40% of the new drugs (Newman and Cragg, 2007).

Over the past decades, there has been a particular interest in the role of medicinal plant extracts in cancer prevention. Plants are rich sources of chemically diverse compounds with several beneficial properties to human health. Consequently, about 50% of the anticancer therapeutic agents are derived from plants (Balunas and Kinghorn, 2005).

Identification of medicinal plants with significant cytotoxic potential useful for the development of cancer
therapeutics has gained increasing importance in the last decade, and research in this field is expanding (Al-Kalaldeh et al., 2010).

It was widely reported that the induction of apoptosis is one of the active strategies to arrest the proliferation of cancer cells. Apoptosis is the major focus and target for cancer research since the cells killed through this mode of cell death do not induce an inflammatory reaction which may lead to various adverse side effects (Steele, 2003). One of the hallmarks of apoptosis is the fragmentation of genomic DNA (Elmore, 2007). The presence of dark brown stained nuclei of MCF-7 cells treated with the extracts strongly suggests that the cells are killed through apoptotic pathway.

Pandanus is a very important medicinal plant with certain varieties sometimes preferred for particular treatments. Leaves, especially the basal white section of young leaves and roots are used. In Kiribati, Pandanus leaves are used in treatments for cold/flu, hepatitis, dysuria, asthma, boils and cancer, while the roots are used in a decoction to treat hemorrhoids. In Hawaii, the main parts used in making traditional medicines are the fruits, male flowers and aerial roots (Meilleur et al., 1997). These are used individually or in combination with other ingredients to treat a wide range of illnesses, including digestive and respiratory disorders. The root is used in Palauto to make a drink that alleviates stomach cramps, while the leaves are used to alleviate vomiting (Rosario and Esguerra, 2003).

Antitumour activity of ethanol extract of Pandanus was evaluated against Dalton's Ascitic Lymphoma (DAL) tumour model on dose-dependent manner. The activity was assessed using survival time, average increase in body weight, haematological parameters and solid tumour

Figure 4: The presence of apoptotic cells in the MCF-7 cell line after the treatment of Pandanus odorattissimus: control, petroleum ether extract, chloroform extract and methanol extract. The image was observed under light microscope using 40X magnification. Apoptotic cells with stained nuclei are marked by arrows.
volume. Oral administration of alcoholic Pandanus extract increased the survival time and decreased the average body weight of the tumour bearing mice. After 14 days of inoculation, EPF was able to reverse the changes in the haematological parameters, protein and PCV consequent to tumour inoculation. Oral administration of EPF was effective in reducing solid tumour mass development induced by DAL cells. The results showed that EPF possess significant activity in dose-dependent manner (Mani et al., 2008).

Preliminary qualitative chemical studies indicated the presence of lignan, isoflavones, phenolic contents, steroids, saponin, terpenoids, glycosides, tannins, flavonoids and phenolic in the extract, while isoflavones, polyphenol, namely, lignan, are responsible for regulating the rat fertility and may perhaps be helpful for regulating and enhancing the human fertility.

On the basis of biological activities of P. odoratissimus crude extract and derived phytochemicals and their uses as pharmacological agents in traditional and modern research are possible but will first require more clinical trials and product development. The current evidence is largely limited to correlation between identified phytochemicals and mode of action for any pharmacological activity.

Mechanisms of action studies are expected to lead the way into the discovery of new agents with improved and intriguing pharmacological properties. This could be achieved by molecular modeling studies involving interaction of bioactive phytochemicals from P. odoratissimus with their respective molecular and therapeutic targets. The extract of P. odoratissimus could be further explored in the future as a source of useful phytochemicals for the pharmaceutical industry (Adkar et al., 2014).

Conclusion

In this study, the extracts of P. odoratissimus were investigated for their cytotoxic effects on the human breast cancer cell line, MCF-7. The petroleum ether and chloroform extracts of P. odoratissimus showed a moderate cytotoxic activity, whereas the methanolic extract produced the potent cytotoxicity with IC₅₀ values of less than 30 µg/ml at 72 h. All the crude extracts exhibited potent cytotoxicity on MCF-7 cells through apoptosis. The dark stained nuclei of MCF-7 cells at the three time points clearly indicate the presence of DNA fragmentation in methanol extracts of P. odoratissimus.

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REFERENCES


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