ANTIOXIDANT ANALYSIS AND SCREENING OF ANTIPIROLFERATIVE EFFECT OF LEAF AND LATEX EXTRACTS OF CARICA PAPAYA ON BREAST CANCER CELL LINE (MCF-7)

Abstract

Papaya (Carica papaya.L) is known for its nutritional and medicinal properties throughout the world. It acts as a multi faceted plant. FRAP assay proved chloroform and acetone leaf extract have maximum reducing power. Latex has high reducing power than leaf extracts, due to presence of higher content of flavanoids. The studies on Anticancer effect on Breast cancer cell line (MCF-7), for acetone leaf extract 125 μg/ml concentration and for latex extract 62.5 μg/ml concentration is the minimum lethal dose that kills approximately 50% of cells. The morphological observation confirmed the apoptosis nature of papaya extracts on cells as their membrane kept intact and no membrane permeabilization was observed. The extracts of papaya significantly decreased the growth rate and cell survival of breast cancer cell lines. On the whole the results suggest among all four leaf extracts, acetone leaf extract showed better activity on all evaluation studies. But compared to acetone leaf extract, latex extract proved to have maximum activity on all analysis due to the presence of highest content of phytocompounds.

Keywords: Carica papaya, Antioxidant activity, anticancerous activity, MCF-7 cancer cell line.

Introduction

Nature has been a source of medicinal agents since times immemorial. The importance of herbs in the management of human ailments cannot be over emphasized. It is clear that the plant kingdom harbors an exhaustible source of active ingredients invaluable in the management of many intractable diseases. Furthermore, the active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive. However, these complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components (Shariff, 2001). The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003). The complementary components give the plant as a whole a safety and efficiency much superior to that of its isolated and pure active components (Ahmad, 2001).

Carica papaya belongs to the family Caricaceae. The plant is also described in a documented property forms and it act as analgesic, amebicide, antibacterial, cardiotonic, cholagogue, digestive, emenagogue, febrifuge, hypotensive, laxative, pectoral, stomachic and vermifuge. It is distributed throughout Asia, Nigeria etc (Afolayan, 2003). Recent research on papaya leaf tea extract has demonstrated cancer cell growth inhibition. It appears to boost the production of key signaling molecules called Th1-type cytokines, which help regulate the immune system. Cancer is a leading cause of death worldwide, it is a dreadful disease, and combating this disease is of great importance to public health. There is a necessity for search of new natural extracts of plant source and compounds with cytotoxic activity as the treatment of cancer with the available anticancer drugs is often unsatisfactory due to the problem cytotoxicity to the normal cells. Recent Phytochemical examination of plants which have a suitable history of use in folklore for the treatment of cancer has often resulted in the isolation of principles with anti cancer activity studied by Afolabi et al. (2007).
This study was carried out to evaluate anti-cancer effect of *C. papaya* extracts on MCF7 (breast) cancer cells and also to investigate the antioxidant property of the leaf and latex extracts.

**Materials and method**

**Determination of antioxidant activity**

**Ferric reducing antioxidant power (FRAP Assay)**

The FRAP assay was done according to Benzie and Strain (1996). In ferric reducing antioxidant power assay, 1 ml of test sample of extract in different concentration were mixed with 1 ml of 0.2 M sodium phosphate buffer (pH 6.6) and 1 ml of 1% potassium ferricyanide in separate test tubes.

The reaction mixtures were incubated in a temperature-controlled water bath at 500°C for 20 min, followed by addition of 1 ml of 10% trichloroacetic acid. The mixtures were then centrifuged for 10 min at room temperature. The supernatant obtained (1 ml) was added with 1 ml of deionised water and 200 μl of 0.1% FeCl₃. The blank was prepared in the same manner as the samples except that 1% potassium ferricyanide was replaced by distilled water. The absorbance of the reaction mixture was measured at 700 nm.

**In vitro assay for anti-cancer activity (MTT assay)**

Cell line and culture: *MCF*-7 cell lines were obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in Minimal Essential Medium supplemented with 10% FBS, penicillin (100 U/ml), and streptomycin (100 μg/ml) in a humidified atmosphere of 50 μg/ml CO₂ at 37 °C. The study was done in Life Tech Research Centre, Chennai.

Cells (1 × 10⁵/well) were plated in 24-well plates and incubated in 37°C with 5% CO₂. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24 hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or MEM without serum. 100 μl/well (5 mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide (MTT) was added and incubated for 4 hours. After incubation, 1 ml of DMSO was added in all the wells.

The absorbance at 570nm was measured with UV- Spectrophotometer using DMSO as the blank. Measurements were performed and the concentration required for a 50% inhibition (IC50) was determined graphically (Mosmann, 1983).

The % cell viability was calculated using the following formula:

\[
% \text{ cell viability} = \frac{A_{570} \text{ of treated cells}}{A_{570} \text{ of control cells}} \times 100
\]

Graphs are plotted using the % of Cell Viability at Y-axis and concentration of the sample in X-axis. Cell control and sample control is included in each assay to compare the full cell viability assessments.

**Result and discussion**

**Ferric ion reducing antioxidant power assay of leaf and latex solvent extracts of *Carica papaya*.L.**

Antioxidant functions are associated with decreased DNA damage, diminished lipid peroxidation, maintain immune function and inhibited malignant transformation of cells (Gropper *et al.*, 2009). Reducing power is an indicative of reducing agent having the availability of atoms which can donate electron and react with free radicals and then convert them into more stable metabolites and terminate the radical chain reaction (Jadhav and Deshpande, 2010).

The antioxidant activity of leaf and latex extract were shown in Fig 1. The four different solvents of leaf extracts were screened to identify the maximum reducing power between them.
The presence of flavonoids reacts with the free radicals to stabilize and terminate from free radical chain reaction.

A wide variety of phenolic substances derived from edible plants have been reported to retain marked antioxidant and anti-inflammatory activities, which contribute to their chemopreventive potential (Surh, 1999).

**Anticancer effect of papaya leaf (acetone extract) and latex (water extract) on breast cancer (MCF-7) cell line**

We have adapted a method originally described by Mosmann (1983) and further developed to measure the cell survival/proliferation, to produce a sensitive and reliable colorimetric method for the quantitative evaluation of macrophage cytotoxicity tumor targets *in vitro*.

In studied cell lines, the *Carica papaya* extract decreased cell viability in longer time exposure in a dose dependent manner. The more concentrated extract led to higher motility of cell line. Although, Mcf-7 cell line required longer exposure time to reach the motility. For acetone leaf extract it was observed that 125 μg/ml concentrations is the minimum lethal dose that kills approximately 50% of cells (Table 1 and Figure 2). Aqueous extract of papaya flesh (0.01-4% v/v) shows significant inhibitory effect on proliferation of MCF-7 cells p<0.05 (Garica-Solics, 2009). For latex extract it was observed that 62.5 μg/ml concentrations is the minimum lethal dose that kills approximately 50% of cells (Table 2 and Figure 3). The morphological observation confirmed the apoptosis nature of papaya extracts on cells as their membrane kept intact and no membrane permeabilization was observed.

The results revealed that both the leaf (acetone extract) and latex (water extract) extracts of papaya significantly decreased the growth rate and cell survival of breast cancer cell lines. The extract induced cell death regarding natural cell growth rate. MCF-7 cell line naturally has a higher growth rate, thus higher growth inhibition of MCF-7 cell line by the leaf and latex extracts of papaya was confirmed from the Figure 4 & 5.

![Figure 1: Ferric ion reducing antioxidant power assay of leaf and latex solvent extracts of *Carica papaya*.L.](image-url)
Table 1: Anticancer effect of Papaya Leaf (acetone extract) on MCF-7 cell line

<table>
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<tr>
<th>S.No</th>
<th>Concentration (µg/ml)</th>
<th>Dilutions</th>
<th>Absorbance (O.D)</th>
<th>Cell viability (%)</th>
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Figure 2: Graphical representation of anticancer effect of Papaya Leaf (acetone extract) on MCF-7 cell line
Table 2: Anticancer effect of Papaya Latex (water extract) on MCF-7 cell line

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<th>S.No</th>
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**Figure 3:** Graphical representation of anticancer effect of Papaya Latex (water extract) on MCF-7 cell line
Normal MCF-7 Cell line

Toxicity- 1000 µg/ml

Toxicity- 125 µg/ml

Toxicity- 62.5 µg/ml

Toxicity- 31.2 µg/ml

Figure 4: Anticancer effect of Papaya Leaf (acetone extract) on MCF-7 cell line
Normal MCF-7 Cell line

Figure 5: Anticancer effect of Papaya Latex (water extract) on MCF-7 cell line.
Conclusion
FRAP assay indicate that plant extracts of papaya are a significant source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses. The anticancer cell line study reports, that both the leaf (acetone extract) and latex (water extract) extracts of papaya significantly decreased the growth rate and cell survival of breast cancer cell lines. Papaya promotes immune system. Papaya is potent cancer fighter that is highly effective against hormone related to cancer as well as other cancer. Papaya can stop the growth of cancer cell by halt metastasis and normalized cell cycle. On the basis of generated information from the present study, it may lead to development of potential bio-product in the treatment of different human ailments and could be exploited in the management of various disease ailments.

References