

Evaluation of Antiproliferative activity of Hydro-ethanolic extract of unripe fruit of *carica papaya* l. using various cell lines

Praveena.P¹, Jethinlalkhosh. J. P², Victor Arokia Doss.D³

¹Research and Development Centre, Bharathiar University, Coimbatore, India

²Research and Development Centre, Bharathiar University, Coimbatore, India

³Department of Biochemistry, P S G College of Arts and Science, Coimbatore, India
praveenapadmam@gmail.com¹

Abstract :

Plant based concoctions has become a significant element of various medical systems around the world. Majority of the plants studied have shown effective medicinal values. This study was undertaken to investigate the antiproliferative activity of Carica papaya L. The hydroethanolic extract of unripe fruit of C.papaya L (HEECP) was prepared. The qualitative and quantitative phytochemical analyses were performed. HT29 (colon cells) and HeLa (cervical cancer) were initially used for screening the antiproliferative activity of the crude extract by MTT assay. The extract showed pronounced activity against HeLa cell line suggesting interaction and inhibition of various components of cell cycle regulators. From the results obtained, it was concluded that Carica papaya L can be considered as a potent anticancer agent.

Keywords: *Carica papaya L, antiproliferative, cell cycle regulators*

1. Introduction

Cancer of the cervix is the third most common cancer with estimated one lakh new cases in 2016 and about 1.04 lakh during 2020. Cervical cancer, mainly caused by Human Papillomavirus infection, is a leading cancer in Indian women and the second most common cancer in women worldwide. While statistics for cancer are getting deadlier by the day, it is estimated that one out of every three people are at risk of contracting the disease. Plant based medications have now become a significant element of indigenous medical systems around the world. Majority of the plants studied have shown effective medicinal values. In the recent years efforts are being made to identify herbal contraceptives which are biologically safe and also cost effective. Several studies have proven that *Carica papaya* Linn (*C.papaya*) has commendable medicinal properties. *Carica papaya* Linn belongs to family Caricaceae and is commonly known as papaya in English and Papita in Hindi. The plant is recognized by its weak and usually unbranched soft stem

yielding copious white latex and crowded by a terminal cluster of large and long stalked leaves, is rapidly growing and can grow up to 20m tall. It is cultivated for its fruits. Papain is a proteolytic enzyme which finds numerable industrial uses. It is used in meat tenderizes and chewing gums. The leaves are used for treatment of malaria, dengue, and jaundice. Both leaves and fruits of *C.papaya* possess medicinal properties like anti-inflammatory hypoglycaemic, anti-fertility, abortifacient, hepatoprotective, wound healing, antihypertensive and antitumor activities¹. Evidences from several studies suggest that the fruits of *C.papaya* have antifertility properties. Consumption of ripe papaya during pregnancy is not dangerous, however unripe and semi ripe papaya contain high amount of latex that produces marked uterine contraction that could be unsafe for consumption during pregnancy^{2,3}. The crude papaya latex contains a uterotonic principle consisting of a combination of enzymes, alkaloids, flavanoids and other substances, which evoke sustained contractions of the uterus by acting mainly on the alpha adrenergic receptor population of the uterus at different stages⁴. Papaya leaves has been seen as a potential source of useful food and drug items. The presence of alkaloids is being effectively used as an anti-malaria agent^{5,6}. According to the book 'Nature cure for cancer' there are many reports that cancer sufferers have been healed by drinking papaya leaf concentrate⁷.

2 .Materials and Methods

2.1. Plant material

C.papaya were collected from in and around Kodakara,Thrissur,Kerala. They were identified and certified by the Taxonomist, Botanical Survey of India (BSI), Coimbatore, Tamil Nadu, India (Plant identification no.-BSI/SRC/5/23/2013-14/Tech/683).

2.2.Extraction Procedure

Crude plant extract was extracted by Soxhlet extraction method. About 100 grams of plant material was uniformly packed into a thimble and extracted with 350 ml of 50% ethanol as solvent. The process of extraction continued till the solvent in siphon tube of an extractor became colourless. The solvent extract was filtered and dried using rotary evaporator. This fraction of *C.papaya* L (HEECP) was taken for further studies. The extract was subjected to phytochemical analysis.

2.3.Invitro cytotoxicity determination by MTT assay

HT29 (colon cancer) and HeLa (cervical cancer) cells were initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecos modified Eagles medium.The cell lines were cultured in 25 cm² tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). Cultured cell lines were kept at 37°C in a humidified 5% CO₂ incubator^{8,9}. The viability of cells were evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method.

2.3.1. Cells seeding:

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension (5×10^4 cells/well) was seeded in 96 well tissue culture plate and incubated at 37°C in a humidified 5% CO₂ incubator.

2.3.2. Preparation of plant extracts and compound stock:

1 mg of the extract was added to 1ml of DMEM and dissolved completely by cyclomixer. The extract solution was filtered through 0.22 µm Millipore syringe filter. After 24 hours the growth medium was removed, freshly prepared each plant extracts in 5% DMEM were five times serially diluted by two fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µg in 100µl of 5% MEM) and each concentration of 100µl were added in triplicates to the respective wells and incubated at 37°C in a humidified 5% CO₂ incubator.

2.3.3. Cytotoxicity Assay by Direct Microscopic observation:

Entire plate was observed at an interval of each 24 hours; up to 72 hours in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

2.3.4. Cytotoxicity Assay by MTT Method:

Fifteen mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells were removed and 30µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then incubated at 37°C in a humidified 5% CO₂ incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT solubilisation Solution (DMSO was added and the wells were mixed gently by pipetting up and down in order to solubilise the formazan crystals. The absorbance values were measured by using microplate reader at a wavelength of 570 nm.

The percentage of growth inhibition was calculated using the formula:

$$\% \text{ of viability} = \frac{\text{Mean OD Samples} \times 100}{\text{Mean OD of control group}}$$

3. Results and Discussion

The solvent extraction of the unripe fruit of *C.papaya* was performed the crude fraction was obtained. Phytochemical analysis was performed and the results are shown in table no.1.the

results showed the presence of carbohydrates, protein, flavanoids, alkaloids, saponins, tannins, glycosides etc. The viability of cells treated with HEECP was found to reduce in concentration-dependent manner. The percentage viability has been shown in Table-1 and Table-2. Higher extract concentrations exhibited stronger antiproliferative activity.

Table-1: Percentage viability of HT29 cell line treated with HEECP

Sample Concentration ($\mu\text{g/ml}$)	Average OD at 540nm	Percentage Viability
Control	1.306	100
6.25	0.9916	75.92
12.5	0.9337	71.49
25	0.8406	64.36
50	0.4737	36.27
100	0.2661	20.37

Table-2 Percentage viability of HeLa cell line treated with HEECP

Sample Concentration ($\mu\text{g/ml}$)	Average OD at 540nm	Percentage Viability
Control	0.8514	100
6.25	0.5967	70.08
12.5	0.5311	62.37
25	0.4678	54.94
50	0.4178	49.07
100	0.3915	45.98

On direct microscopic observations detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity. The IC_{50} value was found as $83.42\mu\text{l/ml}$ for HT-29 cell line and $75.88\mu\text{l/ml}$ for HeLa cell line. The results indicated that HEECP had significant antiproliferative activity.

4. Conclusion

In the present study, crude fraction of unripe fruit of *C.papaya* was prepared. The qualitative analysis was performed. The antiproliferative activity was screened using HT-29 and HeLa cell line. The extract showed dose dependent activity in both the cell lines. The results suggest that *C.papaya* has significant antiproliferative activity. While evaluation of potentially toxic agents often depends on animal bioassay data to predict risk in humans, other experimental approaches will be necessary to ascertain the effective dosage of unripe papaya for exhibition of the antiproliferative activity.

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ABOUT AUTHOR(S)



Mrs. P Praveena: Is presently working as Assistant Professor, Department of Biotechnology, Sahridaya College of Engineering and Technology, Thrissur, Kerala. Currently she is pursuing her Ph. D from Department of Biochemistry, Research and Development Centre, Bharathiar University, Coimbatore, Tamil Nadu, India, under the guidance of Dr. D Victor Arokia Doss. Her major research area and publications are in the field of Biochemistry, Clinical toxicology and Phytochemistry.



Mr. Jethinlalkhosh J P : Is working as Quality Control Assistant in Food Corporation of India, Palakkad, Kerala, India. Prior to that, he worked as an Assistant Professor, Department of Biotechnology and Applied Microbiology, St. Thomas College, Pala, Kottayam, Kerala, India. Currently he is pursuing his Ph.D from Department of Biochemistry, Research and Development Centre, Bharathiar University, Coimbatore,

Tamil Nadu, India, under the guidance of Dr. D Victor Arokia Doss. He has research experience in the field of Phytochemistry, Pharmacology, Clinical Biochemistry and Toxicology, Molecular Biology and Cancer Biology and is having publications in many international peer reviewed journals.



Dr. Victor Arokia Doss: Is working as an Associate Professor in the Department of Biochemistry, P S G Arts and Science College, Coimbatore, Tamil Nadu, India since 1994. He has research experience in the field of Clinical Biochemistry and Toxicology, Molecular Biology, Cancer Biology, Immunology, Protein Chemistry and Bioinformatics. Over the years he has authored and co-authored more than 80 research articles in international peer reviewed journals.