



Research Article

THE BIOACTIVE COMPOUNDS OBTAINED FROM THE PAPAYA (CARICA PAPAYA) ACT AS POTENTIAL ANTICANCER AGENTS AGAINST THE HUMAN PROSTATE CANCER CELL LINE DU-145

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ABSTRACT:

The papaya is the fruit of the plant *Carica papaya*, the sole species in the genus *Carica* of the plant family Caricaceae. The papaya is a large, tree-like plant, with a single stem growing from 5 to 10 m (16 to 33 ft) tall, with spirally arranged leaves confined to the top of the trunk. The lower trunk is conspicuously scarred where leaves and fruit were borne. It consists of high content of zeaxthin, proteolytic enzymes like papain and chymo papain, vitamin K, β -carotene, other phytoconstituents like polyphenols, sugars, aromatic amino acids and sulphur containing amino acids, phytosterols, starch and nutrients e. g P, S, K, Ca, Fe, Mg etc and had good health protective effects. The main objective of the present work is to evaluate the anticancer activity of various extracts of fruit of *Carica papaya* against Human Prostate cancer cell line DU-145. DU-145 and PC3 human prostate cancer cell lines are the "classical" cell lines of prostatic cancer DU145 cells have moderate metastatic potential compared to PC3 cells which have high metastatic potential. The DU145 cell line was derived from brain metastasis. SRB assay was used to analyze the cell growth inhibition. From the present study it had been displayed that CFE, ELE and MLE, all were exhibiting the potential capability to kill the cancer cell when compared with standard drug 5-FU. The cell growth inhibition by various extracts of fruit of *Carica papaya* was varied due to the presence of varying concentration of bioactive compounds. CFE had displayed the highest cell growth inhibition (94.52 %) at 6.25 μ g (IC_{50} = 2.2 μ g/ml) due to the presence of polyphenols (flavanoids), ELE with the 93.83% growth inhibition at 6.25 μ g (IC_{50} = 2.4 μ g/ml) and MLE with the 92.80% % growth inhibition at 6.25 μ g (IC_{50} = 2.6 μ g/ml).

KEYWORDS: Zeaxthin, Phytoconstituents, DU-145, Metastatic potential, SRB, IC_{50}

INTRODUCTION

The papaya is the fruit of the plant *Carica papaya*, the sole species in the genus *Carica* of the plant family Caricaceae. The papaya is a large, tree-like plant, with a single stem growing from 5 to 10 m (16 to 33 ft) tall, with spirally arranged leaves confined to the top of the trunk.

The lower trunk is conspicuously scarred where leaves and fruit were borne. The leaves are large, 50–70 cm (20–28 in) in diameter, deeply palmately lobed, with seven lobes. Unusually for such large plants, the trees are dioecious. The tree is usually unbranched, unless lopped. The flowers are similar in shape to the flowers of the Plumeria, but are much smaller and wax-like. They appear on the axils of the leaves, maturing into large fruit - 15–45 cm (5.9–17.7 in) long and 10–30 cm (3.9–11.8 in) in diameter. The fruit is

ripe when it feels soft (as soft as a ripe avocado or a bit softer) and its skin has attained an amber to orange hue. Carica papaya was the first transgenic fruit tree to have its genome deciphered[1][2]

Papaya plants come in three sexes: "male," "female," and "hermaphrodite." The male produces only pollen, never fruit. The female will produce small, inedible fruits unless pollinated. The hermaphrodite can self-pollinate since its flowers contain both male stamens and female ovaries. Almost all commercial papaya orchards contain only hermaphrodites [3]. Gaining in popularity among tropical fruits worldwide, papaya is now ranked third with 11.22 Mt, or 15.36 percent of the total tropical fruit production, behind mango with 38.6 Mt (52.86%) and pineapple with 19.41 Mt (26.58%). Global papaya production has grown significantly over the last few years, mainly as a result of increased production in India [4]

Global papaya production is highly concentrated, with the top ten countries averaging 86.32 percent of the total production for the period 2008–2010. India is the leading papaya producer, with a 38.61 percent share of the world production during 2008–2010, followed by Brazil (17.5%) and Indonesia (6.89%). Other important papaya producing countries and their share of global production include Nigeria (6.79%), Mexico (6.18%), Ethiopia (2.34%), Democratic Republic of the Congo (2.12%), Colombia (2.08%), Thailand (1.95%), and Guatemala (1.85%). Originally from southern Mexico (particularly Chiapas and Veracruz), Central America, and northern South America, the papaya is now cultivated in most tropical countries. In cultivation, it grows rapidly, fruiting within three years. It is, however, highly frost-sensitive, limiting its production to tropical climates. Temperatures below -2°C (29°F) are greatly harmful if not fatal. In Florida and California, growth is generally limited to southern parts of the states. In California, it's generally limited to private gardens in Los Angeles, Orange, and San Diego counties. It also prefers sandy, well-drained soil as standing water will kill the plant within 24 hours[5]

The literature survey revealed that the papaya extracts were possessed a wide range of pharmacological activities viz Age-related macular degeneration, Asthma prevention, Cancer, Bone health, Diabetes, Digestion, Heart disease, Inflammation, Skin and healing and antimicrobials etc. The objective of the present work is to evaluate the anticancer activity of fruit extracts of Carica papaya.

MATERIALS AND METHODS

Drugs and Chemicals

The standard drug 5-fluorouracil purchased from Local Retail Pharmacy Shop and solvents and other chemicals were used from Institutional Store and were of AR grade. The cell culture Human Prostate cancer cell line DU-145 was provided by National Centre for Cell Science (NCCS), Pune and were grown in Eagles Minimum Essential Medium (EMEM) which contained 10% fetal bovine serum (FBS). All cells were maintained at 37°C , 100% relative humidity, 5% CO_2 , 95% air and the culture medium was changed twice a week.

Extraction[6]

Weigh 20 g of papaya paste (ripen can be mashed to prepare a paste) into a 250 ml round-bottomed flask. Add 50 ml of methanol and 60 ml of dichloromethane. Heat the mixture under reflux for 5 min on stem-bath with frequent shaking. Filter the mixture under suction and transfer the filtrate to a separatory funnel. Wash this mixture containing bioactive compounds with three portions of 150 ml each with sodium chloride solution. Dry the organic layer over anhydrous magnesium sulfate. Filter and evaporate most of the solvent in vacuum without heating. Same procedure is followed for the extraction of MLE and CFE extracts.

Preliminary Phytochemical Screening [7][8][9]

Preliminary Phytochemical Screening has to be carried out for the identification of reducing sugars, pentoses, disaccharides, polysaccharides, proteins and amino acids phytosterols, polyphenols and carotenoids etc.

EVALUATION OF IN VITRO ANTICANCER ACTIVITY BY SRB ASSAY

Principle [10][11]

Sulphorodamine B (SRB) is a bright pink aminoxanthine dye with two sulfonic acid group. Under mild acidic conditions SRB dye binds to basic amino acid residues in trichloro acetic acid (TCA) fixed cells to provide a sensitive index of cellular protein content that is linear over a cell density range of visible at least two order of magnitude.

Procedure [12]

The monolayer cell culture was trypsinized and the cell count was adjusted to $0.5-1.0 \times 10^5$ cells/ml using medium containing 10% new born sheep serum. To each well of the 96 well microtitre plate, 0.1 ml of the diluted cell suspension (approximately) 10,000 cells was added. After 24 hrs, when a partial monolayer was formed, the supernatant was flicked off, washed once and $100\ \mu\text{l}$, $50\ \mu\text{l}$ and $25\ \mu\text{l}$ of different concentration of extracts of fruits of *Meyna spinosa* Roxb were added to the cell in microtitre plate. The plates were incubated at 37°C for 72 hrs in 5% CO_2 incubator, microscopic examination was carried out and observations were recorded every 24 hrs. After 72 hrs, $25\ \mu\text{l}$ of 50% TCA was added to wells gently such that it forms a thin layer over the test extracts to form overall concentrations 10%. The plates were incubated at 40°C for 1 hr. The plates were flicked and washed five times with tap water to remove traces of medium sample and serum and were then air dried. The air dried plates were stained with $100\ \mu\text{l}$ SRB and kept for 30 mnts at room temperature. The unbound dye was removed by rapidly washing four times with 1% acetic acid. The plates were then air dried. $100\ \mu\text{l}$ of 10 mM Tris base was then added to the wells to solubilise the dye. The plates were shaken vigorously for 5 mnts. The absorbance was measured using microplate reader at a 540 nm. The % growth inhibition was calculated by the following formula:

$$\% \text{ cell growth inhibition} = 100 - \left\{ \frac{At - Ab}{Ac - Ab} \right\} \times 100$$

At = Absorbance value of test compound

Ab = Absorbance value of blank

Ac = Absorbance value of control

RESULTS AND DISCUSSION

Phytochemical screening

Preliminary Phytochemical screening of various extracts of papaya had shown the presence of various bioactive compounds such as carbohydrates (MLE, ELE and CFE), aminoacids and peptides (MLE, ELE and CFE), phytosterols (CFE), carotenoids (MLE, ELE and CFE) and polyphenols (CFE).

Biological screening

The results for cell growth inhibition by the extracts such as MLE, ELE and CFE against DU-145 cell lines for various concentrations is shown in (Table 1, Table 2 Table 3) As the concentration increases there is an increase in the cell growth inhibition and it was found that CFE with the highest 94.52 % growth inhibition at 6.25 µg (IC₅₀ = 2.2 µg/ml), ELE with the 93.83% growth inhibition at 6.25 µg (IC₅₀ = 2.4 µg/ml) and MLE with the 92.80% % growth inhibition at 6.25 µg (IC₅₀ = 2.6 µg/ml). In the USNCI screening program a compound is generally considered to

have in vitro anticancer activity, if the IC₅₀ value following incubation between 48 hrs and 72 hrs is less than 4 µg/ml or 10 µM³⁵. In the present study IC₅₀ values below 4 µg/ml were displayed by MLE, ELE and CFE of papaya. The IC₅₀ value of standard drug 5-FU was found to be 1.91 µg/ml with 96.54 % growth inhibition. Flavanoid and terpinoid compounds possessed the highest anticancer activity obtained from the natural sources.

IC₅₀ Determination [13]

IC₅₀ is the acronym for “half maximal inhibitory concentration”. IC₅₀ value indicates the concentration needed to inhibit a biological or biochemical function by half (e.g. inhibition of enzymes, affinity to cell receptors). In pharmaceutical research, it is a frequently used unit to specify the in vitro potency of a drug or a NCE. Amongst others, determination of IC₅₀ is commonly calculated via linear interpolation: The activity of an enzyme is determined after exposure to a series of inhibitor concentrations. IC₅₀ is calculated by the following formula:

$$IC_{50} = \frac{(50\% - \text{Low Inh}\%) \times (\text{High Conc} - \text{Low Conc})}{(\text{High Inh}\% - \text{Low Inh}\%)}$$

Low Inh% / High Inh%: % inhibition directly below / above 50% inhibition

Low Conc / High Conc : Corresponding concentrations of test compound

Table 1: For percentage (%) of cell Growth Inhibition of Methanolic Extract (MLE) of Fruits of *Carica papaya* on DU-145 Cell lines by SRB Assay:

Serial no.	Concentration of the Extracts	Absorbance of extracts	Inhibition of cell growth (%)
1	6.25 µg/ml	0.021	92.80
2	12.5 µg/ml	0.029	90.06
3	25 µg/ml	0.032	89.05
4	50 µg/ml	0.036	87.67
5	2.5 µg/ml (5-FU)	0.0101	96.54
6	Control	0.292	0

Table 2: For percentage (%) of cell Growth Inhibition of Ethanolic Extract (ELE) of Fruits of *Carica papaya* on DU-145 Cell lines by SRB Assay:

Serial no.	Concentration of the Extracts	Absorbance of extracts	Inhibition of cell growth (%)
1	6.25 µg/ml	0.018	93.83
2	12.5 µg/ml	0.027	90.75
3	25µg/ml	0.030	89.72
4	50 µg/ml	0.035	88.01
5	2.5 µg/ml (5-FU)	0.0101	96.54
6	Control	0.292	0

Table 3: For percentage (%) of cell Growth Inhibition of Chloroform Extract (CFE) of Fruits of *Carica papaya* on DU-145 Cell lines by SRB Assay

Serial no.	Concentration of the Extracts	Absorbance of extracts	Inhibition of cell growth (%)
1	6.25 µg/ml	0.016	94.52
2	12.5 µg/ml	0.025	91.43
3	25 µg/ml	0.028	90.41
4	50 µg/ml	0.032	89.04
5	2.5 µg/ml (5-FU)	0.0101	96.54
6	Control	0.292	0

Figure 1:Percentage (%) of cell Growth Inhibition by MLE, ELE and CFE of Fruits of *Carica papaya* on DU-145 Cell line.

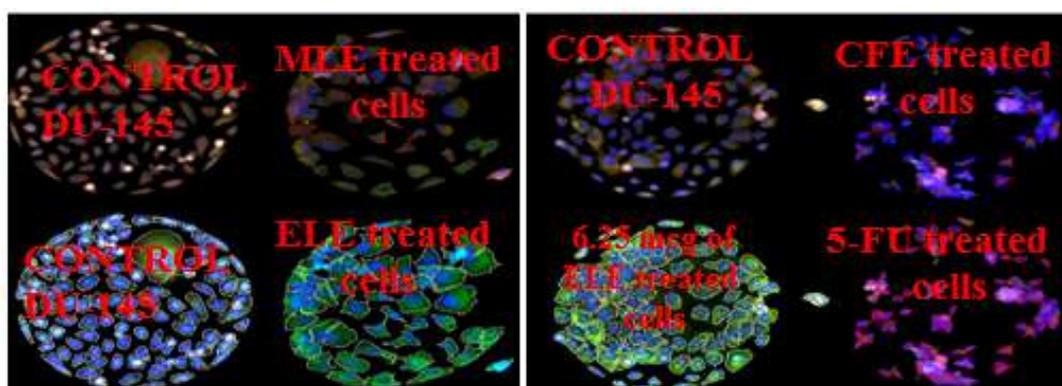


Figure 2: :Percentage (%) of cell Growth Inhibition by MLE, ELE and CFE of Fruits of *Carica papaya* on DU-145 Cell line at 12.5 µg.

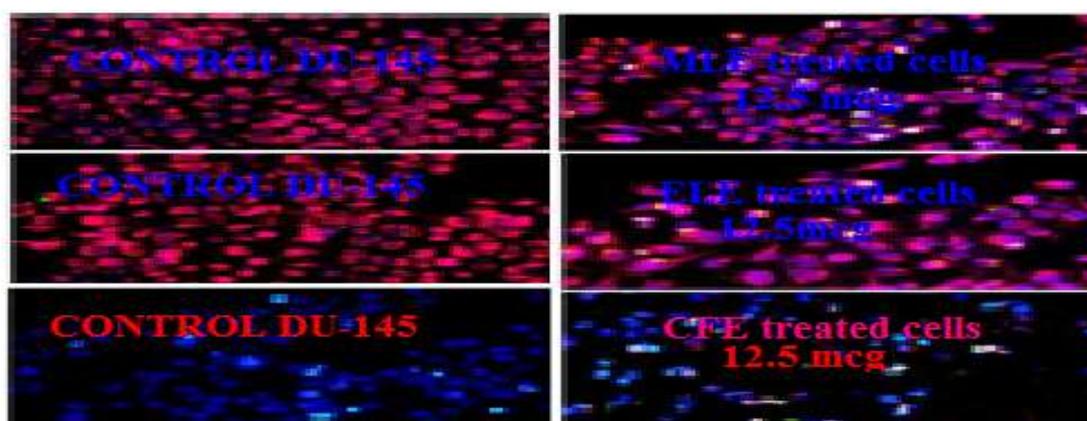
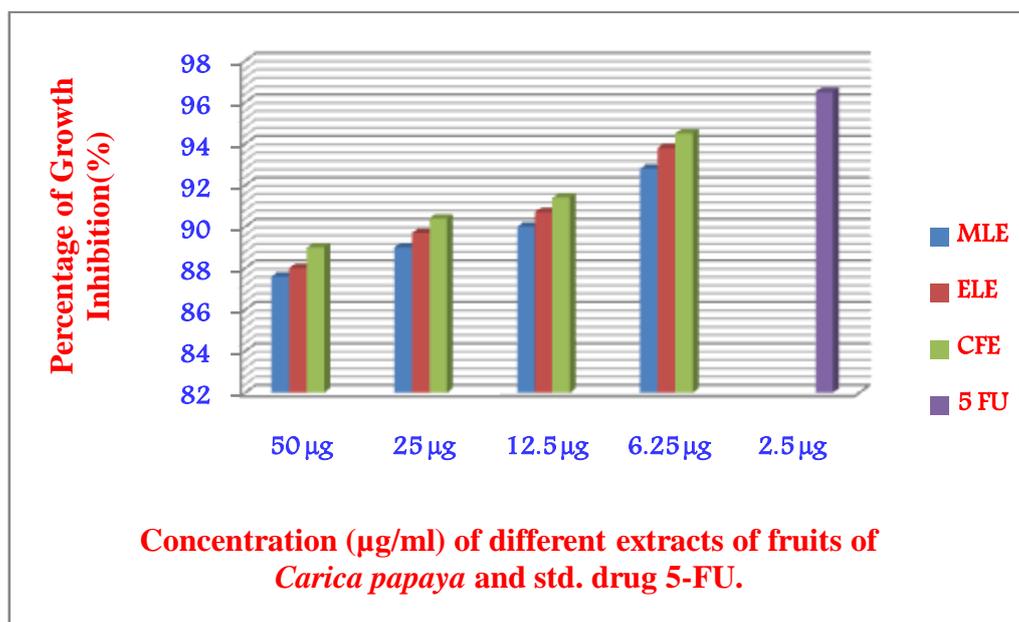


Figure 3: Graphical representation of Percentage (%) of cell Growth Inhibition by MLE, ELE and CFE of Fruits of *Carica papaya* on DU-145 Cell line at different concentration.



CONCLUSION

The results obtained from the *in-vitro* studies performed by SRB assay using the DU-145 cell lines displayed that the various extracts of fruit of *Carica papaya* (MLE, ELE and CFE) possessed a very good anticancer activity. From the present studied it had been concluded that CFE, ELE and MLE, all were exhibiting the potential capability to kill the cancer cell when compared with standard drug 5-FU. The cell growth inhibition by various extracts of fruit of *Carica papaya* was varied due to the presence of varying concentration of bioactive compounds. CFE had displayed the highest cell growth inhibition (94.52 %) at 6.25 µg ($IC_{50} = 2.2 \mu\text{g/ml}$) due to the presence of polyphenols (flavonoids), ELE with the 93.83% growth inhibition at 6.25 µg ($IC_{50} = 2.4 \mu\text{g/ml}$) and MLE with the 92.80% % growth inhibition at 6.25 µg ($IC_{50} = 2.6 \mu\text{g/ml}$).

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