

IN VITRO ANTICANCER ACTIVITY OF *PUNICA GRANATUM* FRUIT JUICE EXTRACT AGAINST OVARIAN CANCER AND OSTEOSARCOMA CELL LINES

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ABSTRACT

The plant has a long history of numerous traditional and ethno botanical application in diverse cultures. *Punica granatum* (Lythraceae) is traditionally used for anti-fungal, antioxidant, antimicrobial, anti-cancer and anti-bacterial activity. In the present study, *In vitro* anticancer activity of acetone extract of pomegranate juice was carried out in PA-1 cell line and MG 63 cell line through MTT assay method. The IC50 value of *Punica granatum* fruit extract was found to be 50.91 for ovarian cancer cell line (PA-1) and 52.76 for osteosarcoma cell line (MG 63). This has concluded that the pomegranate juice extract possess anticancer effect on ovarian cancer and osteosarcoma cell line due to the presence of flavonoids.

KEYWORDS: *Punica granatum*, osteosarcoma cell line, ovarian cancer, pomegranate juice, flavonoids, anthocyanins, anticancer.

INTRODUCTION

Cancer is a class of disease in which a group of cells give rise to uncontrolled group invasions (destruction of adjacent tissue) and metastasis (spread to other parts). Cancer can affect people of any age and the probability increases with age. It is characterised by progressive, persistent, purposeless, and uncontrolled proliferation of tissues. Cancer is the major cause of death worldwide.^[1,2] In these years, plant based products, nutraceuticals and food supplements comprising the complementary and alternative therapies have gained a big share in the drug market in the developed countries. In recent years, extensive research has focused on the anti-carcinogenic potential of chemical constituents like triterpenes, flavonoids and polyphenols.^[3,4,5]

MATERIALS AND METHODS

Plant Collection: The fruit was collected from Koyambedu market, Chennai district, Tamil nadu (India). It was authenticated by professor Dr. M. Kannan Siddha Central Institute, Chennai.

Processing of Plant Material: The collected fruit was washed with tap water. The fruit was cut, squeezed, collected and filtered.

Materials Required

Pomegranate fruit, knife, squeezer, beaker, Whatmann

filter paper (grade 1), Measuring cylinder, Pipette.

Preparation of Extract

Pomegranate fruit was peeled and taken out. The juice was squeezed, filtered (using Whatmann filter paper – grade 1) and collected in a small beaker.

Acetone of 70% was prepared with distilled water. The juice and acetone was mixed in the ratio of 1:20 respectively. i.e., 2ml of juice is mixed with 40ml of acetone. The mixture was let to stand undisturbed for about 4 days until the acetone gets evaporated. The crude extract thus formed is weighed and tested for the presence of different chemical constituents.^[6,7]

Phytochemical Investigation of Extracts: The juice of *P. granatum* was extracted using acetone. Qualitatively tested for different phytochemical constituents namely alkaloids, glycosides, carbohydrates, flavonoids, tannins, saponins, phenolic acids and proteins by following the standard procedure of Veena sharma et al.^[8,9]

In-vitro Cytotoxicity – MTT Assay: PA-1 and MG 63 cell lines were obtained from National Centre for Cell Sciences, Pune (NCCS). The cells were maintained in DMEM 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.

Cells (1×10^5 /well) were plated in 24-well plates and incubated in 37°C with 5% CO₂ condition. After the cell reaches the confluence, the various concentrations of the samples were added and incubated for 24hrs. After incubation, the sample was removed from the well and washed with phosphate-buffered saline (pH 7.4) or DMEM without serum. 100µl/well (5mg/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, 1ml 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. The % cell viability was calculated using the following formula^[10]:

$$\% \text{ Cell viability} = \left(\frac{\text{A570 of treated cell}}{\text{A570 of control cell}} \right) \times 100$$

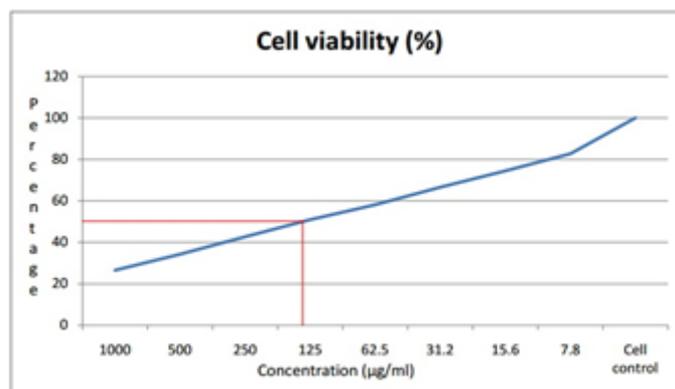
RESULTS AND DISCUSSION

Table 1: Phytochemical Analysis.

S. No	Phytochemical Parameter	Acetone Extract
1.	Alkaloids	-
2.	Carbohydrates	+
3.	Glycosides	-
4.	Proteins	-
5.	Phenolic acids	+
6.	Flavonoids	+
7.	Saponins	-
8.	Tannins	+

Table 2: Anticancer effect of *Punica granatum* on PA-1 cell line.

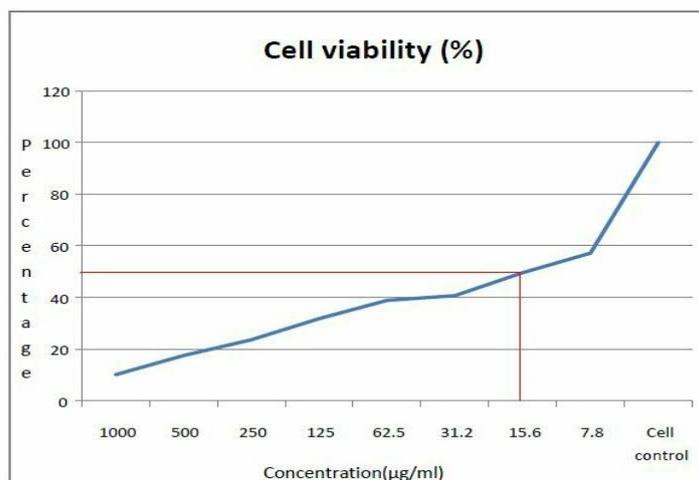
S. No.	Concentration (µg/ml)	Dilution	Absorbance (O.D)	Cell Viability (%)	IC ₅₀ Value
1	1000	Neat	0.144	26.47	50.91
2	500	1:1	0.186	34.19	
3	250	1:2	0.232	42.64	
4	125	1:4	0.277	50.91	
5	62.5	1:8	0.316	58.08	
6	31.2	1:16	0.362	66.54	
7	15.6	1:32	0.405	74.44	
8	7.8	1:64	0.450	82.72	
	Cell control	-	0.544	100	



Graph 1: Graphical representation of concentration dependent cell viability of *Punica granatum* on PA-1 cell line.

Table 3: Anticancer effect of 5-FU on PA-1 cell line.

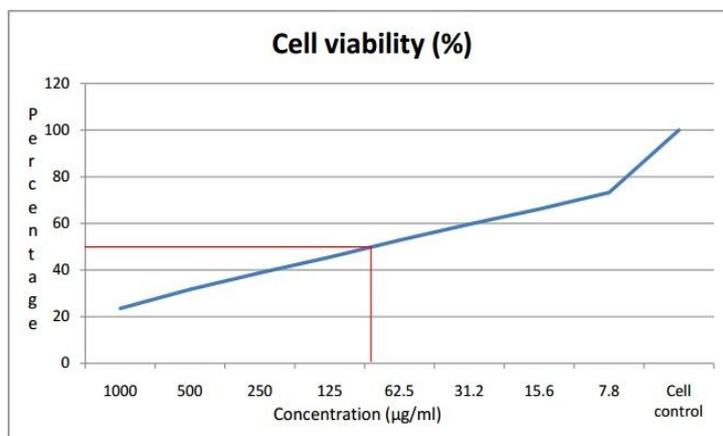
S. No.	Concentration (µg/ml)	Dilution	Absorbance (O.D)	Cell Viability (%)	IC ₅₀ Value
1	1000	Neat	0.096	10.20±0.476	15.8
2	500	1:1	0.167	17.32±0.265	
3	250	1:2	0.225	23.28±0.175	
4	125	1:4	0.300	31.50±0.312	
5	62.5	1:8	0.370	38.49±0.353	
6	31.2	1:16	0.388	40.37±0.337	
7	15.6	1:32	0.473	49.26±0.380	
8	7.8	1:64	0.545	57.10±0.289	
	Cell control	-	0.954	99.99±0.090	



Graph 2: Graphical representation of concentration dependent cell viability of 5-FU on PA-1 cell line.

Table 6: Anticancer effect of *Punica granatum* on MG 63 cell line.

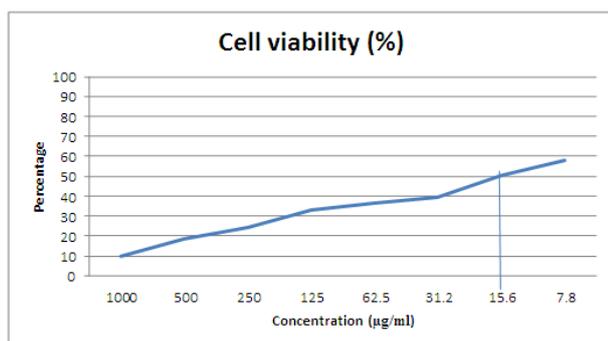
S. No.	Concentration (µg/ml)	Dilutions	Absorbance (O.D)	Cell Viability (%)	IC50 Value
1	1000	Neat	0.288	23.45	52.76
2	500	1:1	0.401	31.65	
3	250	1:2	0.476	38.76	
4	125	1:4	0.558	45.43	
5	62.5	1:8	0.648	52.76	
6	31.2	1:16	0.732	59.60	
7	15.6	1:32	0.812	66.12	
8	7.8	1:64	0.899	73.20	
	Cell control	-	1.228	100	



Graph 3: Graphical representation of concentration dependent cell viability of MG 63 cell line.

Table 7: Anticancer activity of 5-FU on MG 63 cell line.

S. No.	Concentration (µg/ml)	Dilution	Absorbance (O.D)	Cell Viability (%)	IC50 Value
1	1000	Neat	0.086	9.98±0.476	15.6
2	500	1:1	0.153	18.36±0.265	
3	250	1:2	0.221	24.28±0.175	
4	125	1:4	0.298	33.12±0.312	
5	62.5	1:8	0.360	36.48±0.353	
6	31.2	1:16	0.710	39.34±0.337	
7	15.6	1:32	0.422	50.31±0.380	
8	7.8	1:64	0.534	58.12±0.289	



Graph 4: Graphical representation of concentration dependent cell viability of 5-FU on MG 63 cell line.

CONCLUSION

The fruit *Punica granatum* (Lythraceae) was claimed to be useful for cancer but the claim has not been scientifically validated. The phytochemical investigation of *P.granatum* fruit juice extracted with acetone revealed the presence of flavonoids, phenolic acids, carbohydrates and tannins. *In vitro* anticancer activity has been carried out to establish their potency by MTT assay method. From the obtained IC₅₀ values, it was concluded that *Punica granatum* fruit juice possess anticancer activity. In future, it will be interesting not only to isolate the active chemical constituents but also to determine the mechanism of action of the same by using different models.

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