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Cytotoxic activity of leaves extract of pomegranate (*Punica granatum* L.)

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Abstract

Cancer is a pathological condition where excessive and abnormal cell growth leads to widespread invasion within the body to affect various organ functions. It is known that chemotherapeutic agents are themselves possible candidates of cancer generation as they can kill normal cells. Nowadays, there is an immense interest for the search herbal formulation with cancer-preventive effect because of the problems, generated with existing chemotherapeutic regimens. Various medicinal plants are becoming increasingly popular for the investigation of novel anticancer drugs. Traditional herbal medicinal plants have served as prime resources for the development of complementary medicine in anticancer therapy. Studies have demonstrated that Pomegranate may use as a natural remedy to chemical treatment due to its capability against a wide range of pathogens. There are wide ranges of phytochemical properties that have demonstrated anticancer activities in pomegranate. The present study aimed to evaluate the anticancer activity of leaves extract of Pomegranate on HT29 and MCF7 cancer cells by the MTT assay method. The maximum cell death of HT29 cells by the leaves extract of Pomegranate was $70.56 \pm 0.90\%$ at $250 \mu\text{g/mL}$ concentration. The maximum cell death of MCF7 cells by the leaves extract of Pomegranate was $72.75 \pm 0.57\%$ at $250 \mu\text{g/mL}$ concentration.

Keywords: cytotoxicity, HT29 cells, MCF7 cells, MTT assay

Introduction

Cancer is a multistage process resulting in an uncontrolled and abrupt division of cells and is one of the leading causes of mortality. Natural products are viewed as more biologically friendly, that is less toxic to normal cells [1].

The most recent and successful secondary metabolites, including alkaloid, diterpene, triterpene, and polyphenolic type compounds have potential anticancer activity. Each compound's natural source, their therapeutic targets, as well as the main structural modifications that can improve anticancer properties to show the role of plants as a source of effective and safe anticancer drugs [2].

Cancer is becoming a high-profile disease in developed and developing countries, and its treatment is a struggle with some successful cases. Nevertheless, the drugs developed by synthesis and used in chemotherapy have limitations mainly due to their toxic effects on non-targeted tissues and consequently furthering human health problems [3].

Therefore, there is a demand for alternative treatments, and the naturally-derived anticancer agents are regarded as the best choice. secondary metabolites are themselves suitable anticancer agents leading to the development of new clinical drugs with also new anticancer mechanisms of action. Some have already become cases of success for the pharmaceutical industry. Additionally, they are excellent lead compounds, by which, through structural modifications, alternative formulations, and/or using increasingly effective delivery systems, their pharmacological potential is enhanced [4].

Polyphenols research in fruits is increasing now, because of their anticancer activity and potential health benefits. The study aimed to evaluate the anticancer potential of leaves extract of Pomegranate (*Punica granatum*) and its inhibition of cancer progression. Pomegranate has demonstrated anti-proliferative, anti-metastatic, and anti-invasive effects on various cancer cell lines by *in vitro* as well as *in vivo* animal models or human clinical trials.

Although several clinical trials are in progress for identifying the pomegranate as a candidate for various cancer treatments, the anticancer evaluation of leaves extract was not much reported and is necessary to validate its therapeutic value in anticancer therapy [5].

Pomegranate (*Punica granatum*) belonging to the family Punicaceae, is a small tree or shrub and is believed to be indigenous of India, Afghanistan, and Iran. However, it can be seen growing naturally in mild warm valleys as well as outer hills of the Himalayan range. The Pomegranate fruit possesses therapeutically important constituents, varying slightly in different parts of the fruit itself [6]. In the past decade, numerous studies on pomegranate constituents have been published. The results suggest that pomegranate components have antioxidant, anti-carcinogenic, and anti-inflammatory components, which are effective in the prevention and treatment of cancer and other chronic and infectious diseases [7].



Fig 1: Pomegranate (*Punica granatum*)

Description

Macroscopy

Tree The pomegranate is a neat, rounded shrub or small tree that can grow to 20 or 30 ft., but more typically to 12 to 16 ft. in height. It is usually deciduous, but in certain areas, the leaves will persist on the tree. The trunk is covered by a red-brown bark which later becomes gray. The branches are stiff, angular, and often spiny. There is a strong tendency to sucker from the base. Pomegranates are also long-lived. The vigor of a pomegranate declines after about 15 years [8].

Leaves

The pomegranate has glossy, leathery leaves that are narrow and lance-shaped [9].

Flowers

The attractive scarlet, white or variegated flowers are over an inch across and have 5 to 8 crumpled petals and a red, fleshy, tubular calyx which persists on the fruit. The flowers may be solitary or grouped in twos and threes at the ends of the branches. The pomegranate is self-pollinated as well as cross-pollinated by insects. Cross-pollination increases the fruit set. Wind pollination is insignificant [10].

Fruit

The nearly round, 2-1/2 to 5 in. wide fruit is crowned at the base by the prominent calyx. The tough, leathery skin or rind is typically yellow overlaid with light or deep pink or rich red. The interior is separated by membranous walls and white, spongy, bitter tissue into compartments packed with sacs filled with sweetly acid, juicy, red, pink, or whitish pulp or aril [11]. In each sac, there is one angular, soft or hard seed. High temperatures are essential during the fruiting period to get the best flavor. The 15 Pomegranate may begin to bear in 1 year after planting out, but 2-1/2 to 3 years is more common. Under suitable conditions, the fruit should mature some 5 to 7 months after bloom [12].

Materials and Methods

Preparation of Extract

The Pomegranate leaves were collected at Kundrathur, Chennai, Tamilnadu, India. The leaves were washed, shade dried for 10 days, and then powdered by the mechanical blender. About 20 g of powdered leaves were soaked in 200 mL of methanol and kept at room temperature for 72 h. The supernatant was filtered and condensed through a rotor evaporator at 50 °C, which yields greenish gummy extract.

Cytotoxic activity

Chemicals and reagents

MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) Invitrogen, USA. Acridine orange was obtained from Sigma, USA. All other fine chemicals were obtained from Sigma, Aldrich.

Cell culture

HT29 (colon cancer) and MCF7 (breast cancer) cells were obtained from NCCS (National Centre For Cell Science, Pune) were cultured in Rose-well Park Memorial Institute (RPMI) medium, supplemented with 10% fetal bovine serum, penicillin/streptomycin (250 U/mL), gentamycin (100µg/mL) and amphotericin B (1mg/mL) obtained from Sigma Chemicals, MO, USA. All cell cultures were maintained at 37 °C in a humidified atmosphere of 5% CO₂. Cells were allowed to grow to confluence over 24 h before use.

MTT assay

Cell viability was measured with the conventional MTT reduction assay method, as described previously with slight modification. Briefly, HT29 and MCF7 cells were seeded at a density of 5×10³ cells/well in 96-well plates for 24 h, in 200 µL of RPMI with 10% FBS. Then culture supernatant was removed and RPMI containing various concentrations (0.781-250 µg/mL) of methanol leaves extract of Pomegranate was added and incubated for 48 h. After treatment, the cells were incubated with MTT (10 µL, 5 mg/mL) at 37 °C for 4 h and then with DMSO at room temperature for 1 h. The plates were read at 595 nm on a scanning multi-well spectrophotometer. Data are represented as the mean values for three independent experiments [13].

$$\text{Cell viability (\%)} = \left[\frac{\text{Mean OD}}{\text{Control OD}} \right] \times 100$$

Results and Discussion

Cancer is a frightful disease and represents one of the biggest health-care issues for the human race and demands a proactive strategy for the cure. Plants are reservoirs for novel chemical entities and provide a promising line for research on cancer [14].

Nevertheless, plants and plant-derived products is a revolutionizing field as these are Simple, safer, eco-friendly, low-cost, fast, and less toxic as compared with conventional treatment methods. Phytochemicals are selective in their functions and acts specifically on tumor cells without affecting normal cells.

Carcinogenesis is a complex phenomenon that involves many signaling cascades. Phytochemicals are considered suitable candidates for anticancer drug development due to their pleiotropic actions on target events with multiple manners. The research is in progress for developing potential

candidates (those that can block or slow down the growth of cancer cells without any side effects) from these phytochemicals. Many phytochemicals and their derived analogs have been identified as potential candidates for anticancer therapy. An effort has been made through this

comprehensive review to highlight the recent developments and milestones achieved in cancer therapies using phytochemicals with their mechanism of action on nuclear and cellular factors [15].

Table 1: Cytotoxic activity of methanol leaves extract of Pomegranate on HT29 (colon cancer) cells.

S. No	Concentration $\mu\text{g/mL}$	% of cell death
		HT29
1	0.781	25.64 \pm 0.77
2	1.562	35.13 \pm 1.54
3	3.125	47.90 \pm 0.79
4	6.25	50.62 \pm 0.15
5	12.5	55.03 \pm 0.76
6	25	56.10 \pm 1.11
7	50	64.41 \pm 1.72
8	100	68.87 \pm 0.79
9	250	70.56 \pm 0.90

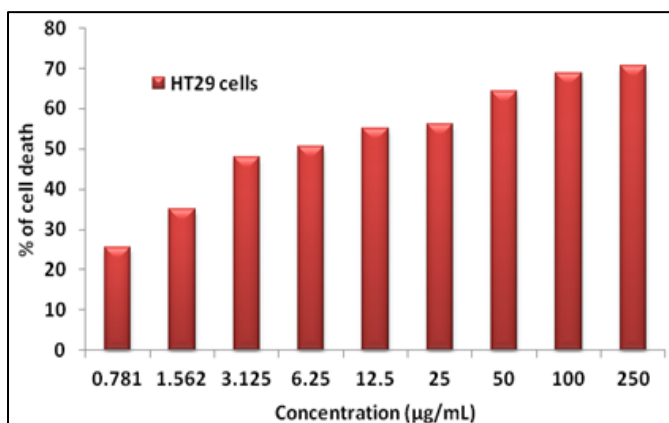


Fig 2: Cytotoxic activity of methanol leaves extract of Pomegranate on HT29 (colon cancer) cells.

Table 2: Cytotoxic activity of methanol leaves extract of Pomegranate on MCF7 (breast cancer) cells.

S. No	Concentration $\mu\text{g/mL}$	% of cell death
		MCF7
1	0.781	45.38 \pm 0.78
2	1.562	46.18 \pm 0.19
3	3.125	48.23 \pm 0.19
4	6.25	55.00 \pm 1.63
5	12.5	59.71 \pm 1.08
6	25	62.82 \pm 1.61
7	50	66.17 \pm 1.18
8	100	71.32 \pm 2.64
9	250	72.75 \pm 0.57

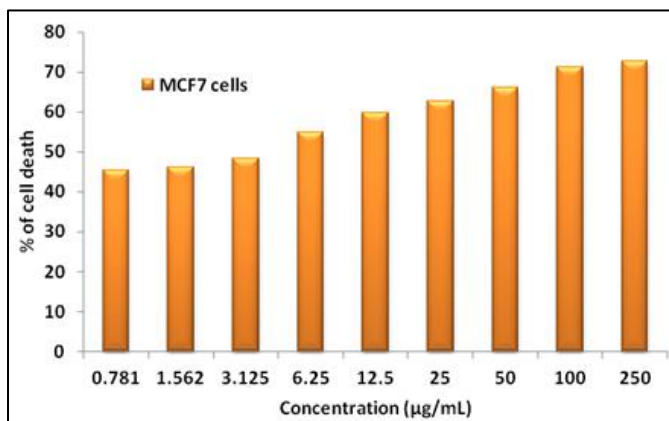


Fig 3: Cytotoxic activity of methanol leaves extract of Pomegranate on MCF7 (breast cancer) cells.

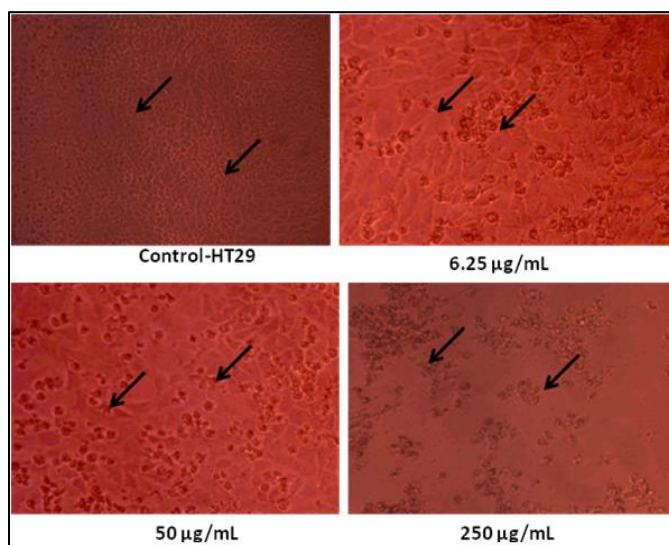


Fig 4: Morphological changes of HT29 cells after treatment by the leaves extract of Pomegranate

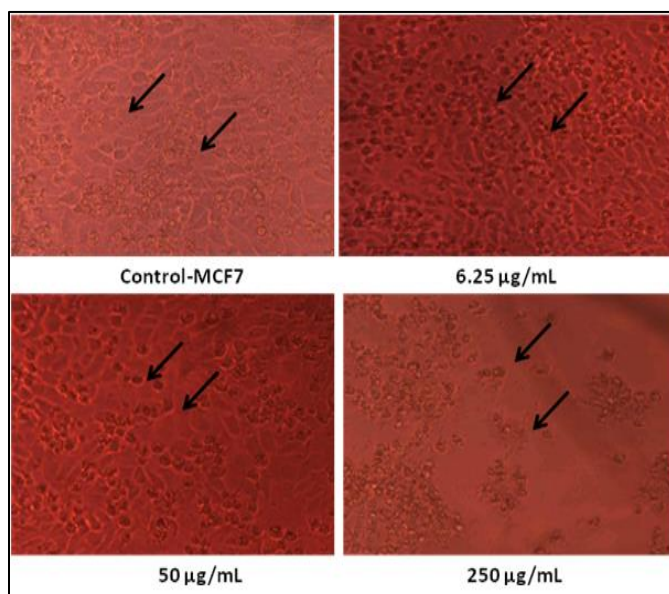


Fig 5: Morphological changes of MCF7 cells after treatment by the leaves extract of Pomegranate

The cytotoxic activity of leaves extract of Pomegranate results showed that variable in cellularity against HT29 and MCF7 cancer cells. The maximum cell death of HT29 (colon cancer)

cells by the leaves extract was $70.56 \pm 0.90\%$ at $250 \mu\text{g/mL}$ concentration and the IC_{50} was $6.17 \mu\text{g/mL}$ concentration (Table 1; Fig. 2). The maximum cell death of MCF7 (breast cancer) cells by the leaves extract was $72.75 \pm 0.57\%$ at $250 \mu\text{g/mL}$ concentration and the IC_{50} was $3.24 \mu\text{g/mL}$ concentration (Table 2; Fig. 3). The majority of cells have round or ovoid shapes, but sometimes they can be polygonal or elongated after treating the leaves extract. The microscopic image revealed that the leaves extract of Pomegranate has a significant effect on treated HT29 and MCF7 cells compared to untreated cells (Fig 4; Fig 5). At $250 \mu\text{g/mL}$ concentration, enlargement of the nucleus of cells was observed with variable sizes and shapes and the dead cells increase with increasing concentration of the leaves extract.

Conclusion

The rising burden of cancer worldwide calls for an alternative treatment solution. Herbal medicine provides a very feasible alternative to western medicine against cancer. The *in vitro* studies showed cancer cell inhibition through DNA damage and activation of apoptosis-inducing enzymes by the secondary metabolites in the plant extracts. Herbal medicine has become a very safe, non-toxic, and easily available source of cancer-treating compounds. Many studies have reported that different plants of medicinal herbs showed promising anticancer potential and inhibition of enzymes that stop tumor growth. The cytotoxic studies are mainly performed in human cell lines and it is highlighted that the herbal plants play an important anticancer role through their different classes of secondary metabolites. However, the study of the Pomegranate plant should not limit and some parts of which are still unexplored. Studies are needed to highlight the mechanism of anticancer action of explored and unexplored parts of Pomegranate.

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