

Cytotoxicity activity of *Punica granatum* L. (Punicaceae) extracts against cancer cell lines

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ABSTRACT

Despite the great advances that have been made in cancer therapy, the treatment of cancer remains poor due to resistance developed by the cancer cells to conventional drugs. So the search for new alternatives is needed. Medicinal plants considered one of the main sources of drugs used in pharmaceutical industry. *Punica granatum* has been used in traditional medicine since ancient times. Therefore, we aimed to investigate anticancer activity of the *P. granatum* leaf and flower extracts against the K562 (chronic myelogenous leukemia), HL-60 (promyelocytic leukemia) and U937 (leukemic monocyte lymphoma) cell lines. The aqueous and ethanolic extracts of *P. granatum* showed various degree of cytotoxicity but generally in a dose dependent manner. IC50 values demonstrate that HL60 cell lines are more sensitive than K 562 and U937 cells lines toward *P. granatum*. These results highlights potent anticancer activity of *P. granatum*.

Key words: *Punica granatum*; Punicaceae; Cytotoxicity; K562; HL-60; U937

INTRODUCTION

P. granatum (Punicaceae) commonly known as pomegranate is a small tree which is grown in Iran, India, USA, and most near and far east countries [26]. Pomegranate has been used widely in traditional medicine of many countries worldwide for curing ailments like helminthiasis, dysentery, diarrhea, stomachic, respiratory disorders and hemorrhage [22,24].

In addition, many scientific reports showed that *P. granatum* have antioxidant [9,19], anti-atherosclerotic [4], antibacterial [6], antiviral [27] and anticancer [3,2] properties. The constituents of *P. granatum* include gallo catechins, delphinidin, cyanidin, gallic acid, ellagic acid, pelargonidin and sitosterol, which are very well known for their therapeutic properties [13].

According to the world health organization (WHO), cancer is a leading cause of death worldwide and there are many difficulties associated with its treatment, the most common of which include drug resistance, toxicity and the low specificity of currently available cytotoxic drugs [7]. Therefore, world is witnessing huge scientific and commercial interest in looking for new anticancer agents from natural sources [11].

Natural products derived from plants still provide a source of new potential cancer chemotherapeutic agents [14]. In the current study, we investigate the effects of the aqueous and ethanolic extracts from *P. granatum* against the K562 (chronic myelogenous leukemia), HL-60 (promyelocytic leukemia) and U937 (leukemic monocyte lymphoma) cell lines.

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MATERIALS AND METHODS

2.1. Plant material and preparation of extracts:

P. granatum leaves and flowers were purchased from a local herbal store. The samples were identified and authenticated by a pharmacognosist. Aqueous and ethanolic extracts of *P. granatum* were prepared using the method described by Abdel-Barry *et al.* [1]. Briefly, dried leaves and flowers were grinded by electric grinder to a fine powder. 50 g of the powder were suspended in 500 ml of distilled water and ethanol, then stirred magnetically for 24 hours at 40 °C. Subsequently, each suspension was filtered (Whatman No.1) and concentrated under reduced pressure at 40°C. The water extract was freeze-dried, yield of aqueous and ethanolic extracts were 18.23 and 13.15% respectively. The preliminary phytochemical tests revealed the presence of Tannins, saponins, flavonoids, alkaloids in *P. granatum* [23,8].

2.2. Cell culture and cytotoxicity:

K562, HL60 and U937 cell lines were cultured in IMDM supplemented with 10% fetal calf serum and gentamycin (625 µl/l). Experiments were conducted on cells seeded into 96-well culture plates at densities 10⁵ cells/ml, cells were grown and maintained in a humidified incubator at 37°C and in 5% CO₂ atmosphere.

Stock solutions of extracts was dissolved in dimethyl sulfoxide (DMSO) and diluted with complete Iscoves' Modified Dulbecco's Medium (IMDM) to give final concentrations ranging from 100 - 1000 µg/ml. Cytotoxic effects of the extracts were evaluated by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Briefly, 10 µl of plant extract concentrations 100, 250, 500, 750, 1000 µg/ml was added to wells each one as triplicate, while the negative control was treated with complete IMDM medium only. Then, cells were plated at 104 cells/well and incubated at 37°C in a 5% humidified CO₂ incubator for 72 h. control wells were prepared with no extracts. At least 3 independent experiments were conducted. After incubation period, acidified medium was aspirated from wells and MTT (5 mg/ml) was added. Plates were incubated at 37°C for 3 h. Then 100 µl of acidic (0.04 M HCl) isopropanol alcohol was added to the wells to solubilize the formazan crystals. Microplates were left in dark overnight and absorbance was measured using an ELISA multiwell spectrophotometer at 540 nm with a reference wavelength of 620 nm. The mean optical density (OD) ±SD for each group of replicates was calculated. The inhibitory rate of cell growth was calculated using the formula: % GrowthInhibition = (1– OD extract treated)/OD negative control × 100 [20]. The concentration required to kill 50% of the cell population or IC₅₀ was calculated from dose-response curves.

3-Results:

The cytotoxicity effect of *P. granatum* leaves and flowers extracts were tested in vitro by MTT assay. Figures 1 and 2 shows the inhibition of K562, HL60 and U937 cell lines when treated with aqueous and ethanolic extracts of *P. granatum* in different concentrations ranged from 100-1000 µg/ml. Extracts showed varied degree of inhibition against used cancer cell lines, the most effected cells were HL 60.

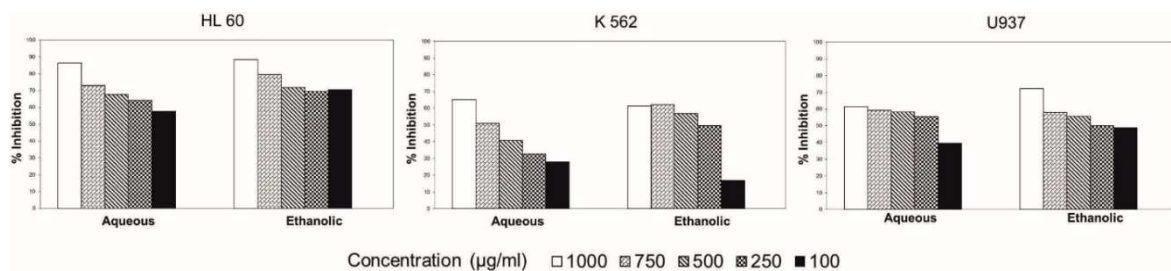


Fig. 1: Cytotoxic Effect of *P. granatum* leaves extracts on HL60, K562 and U937 cell lines.

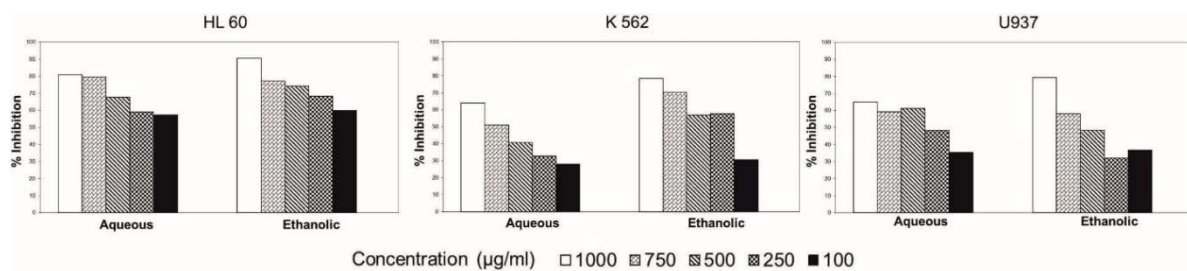


Fig. 2: Cytotoxic Effect of *P. granatum* flowers extracts on HL60, K562 and U937 cell lines.

The IC50 values were calculated for aqueous and ethanolic extracts of *P. granatum* leaves and flowers as shown in Table 1. HL60 cell line were more susceptible than other cell lines which shown varied IC50 values, ethanolic extracts usually were more active than aqueous extracts with some exceptions.

Table 1: IC50 values ($\mu\text{g/ml}$) of anticancer activity for extracts of *P. granatum*

	<i>P. granatum</i> Leaves		<i>P. granatum</i> Flowers	
	Aqueous Extract	Ethanolic Extract	Aqueous Extract	Ethanolic Extract
HL60 cell lines	84.74	72.46	87.71	83.33
K562 cell lines	735.29	267.30	694.44	219.29
U937 cell lines	223.21	255.10	260.41	531.91

Discussion:

Medicinal plants have played an important role in treatment of numerous diseases that affect the health of human beings from ancient times. Cancer is one of the leading causes of death throughout the world. The need to cure cancer and find better ways to combat this disease has raised the need to find anticancer compounds in the plant kingdom. Plant extracts and the bioactive compounds present in them, which are responsible for anticancer activity [21].

The present study has demonstrated that aqueous and ethanolic extracts of *P. granatum* leaves and flowers could show cytotoxicity against HL60, K562 and U937 cells in vitro using the MTT assay. Such activity of *P. granatum* was characterized by the dose-dependent manner.

Numerous in vitro studies have investigated the therapeutic effect of pomegranate extracts against cancer cell lines, like HT-29 colon cancer, KB and CAL-27 oral cancer, MCF-7 and MB-MDA-231 breast cancer and PC-3 prostate cancer cell [16,12,2,25]. *P. granatum* may affect cancer cell lines through additional or synergistic effect of compounds present in the extract by mechanisms like antiproliferative, apoptotic, antioxidant, and possibly anti-inflammatory effects [15,18].

In conclusion, the results of this investigation show that aqueous and ethanolic extracts of *P. granatum* displays cytotoxic activity against different cancer cell lines. This observations show that *P. granatum* can be a potential source for the treatment of cancer at the future.

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