Evaluation The Effect Of Pomegranate (*Punica Granatum*) Seeds And Peels Extracts On Cancer And Normal Cell Lines

Hiba Muhammed Al-Khuzaay [1*]
Department of Science, College of Basic Education, University of Mustansiriyah, Baghdad, Iraq.
hibamuhammed@uomustansiriyah.edu.iq

Abstract:

In this study, crude aqueous and ethanol extracts was prepared from *Punica granatum* seeds and peels, then evaluate the anticancer effects of these extracts on cancer and normal cell lines. Michigan Cancer Foundation-7 (MCF-7) and Ahmed, Murtudha, Jabriyah, 2013 (AMJ13) cancer cell lines and Rat Embryo Fibroblast (REF) normal cell line were used in the *in vitro* study. Cell line exposure times were estimated after 24, 48, and 72 hours, and normal cell line was calculated after only 72 hours of exposure in a micro titration plate under sterile conditions. Anti-cancer property of *Punica granatum* extracts on cancer cell lines has been reported and there was not effect on normal cell line. In this work, the MCF-7, AMJ13 and REF cell line were treated with different concentrations of four type of extracts (15.62, 31.25, 62.5, 125, 250 and 500 µg/mL). and the cytotoxic effect of crude extracts was investigated using the microculture tetrazolium test (MTT). The result obtained showed time and concentration dependent inhibition effect; the higher concentrations gave significantly ($p<0.05$) higher cytotoxic effect at 48 hours of exposure.

Keywords:Antitumor; MTT; *Punica granatum* L.;Cytotoxicity**.**

Introduction:

Cancer is one of the leading causes of death in developed nations and a serious public problem (Al-Khuzaay *et al*., 2019; Ferlay *et al*., 2020). Traditional plants are a valuable source of new cytotoxic agents and are still actively contributing to health concerns (Eghbali *et al*., 2021). The plant kingdom has produced a variety of cancer treatments throughout history and around the globe (Lemonnier *et al*., 2017). Currently, plants are a source of analgesics, anti-inflammatory drugs, anti-spasmodic, anti-asthmatics, anti-arrhythmic drugs, antihypertensive, and antimicrobials that are often used (Al-Khuzaay *et al*., 2019). The pomegranate, or *Punica granatum* L. is a fruit that is high in macro- and micronutrients and has a variety of functional uses, including bioactive xenobiotics. This makes it a "Super Fruit." profile that promotes health (Cortez-Treje *et al*., 2022). In addition to being eaten raw,

the fruit is also frequently consumed in juice, jam, and wine. Pharmacological research has revealed that *Punica granatum* has a variety of active substances with anti-inflammatory, antioxidant, and neuroprotective properties. It has been discovered by researchers that the flavonoids extracted from pomegranate juice have antioxidant activity that is substantially higher than red wine and comparable to green tea (Wong *et al*., 2022). It's interesting to note that pomegranate therapy has captured the attention of numerous researchers across the globe. In addition, studies have demonstrated that pomegranate possesses antimicrobial, anti-proliferative, anti-invasive, anti-metastatic, and apoptotic qualities (Melgarejo-Sanchez *et al*., 2021). Pomegranate peel and seed oil (PSO) are rich in flavonoids and polyphenols with wound-healing and antioxidant qualities (Khwairakpam *et al*., 2018).

This *in vitro* study was used to estimate the effect of the aqueous and ethanolic extracts of seeds and peels of *Punica granatum* on breast cancer cell lines (MCF-7, AMJ13) and normal cell lines (REF).

MATERIALS AND METHODS

Sample collection

Fresh fruits of Iraqi *Punica granatum* were collected from Wasit, Al-Suwaira orchards in October 2023. The seeds and peels parts of the plant were separated, shade dried, and grinded into powder with mortar and pestle. The prepared powder was kept in tight containers protected completely from light.

Extraction

1. Aqueous extraction procedure for *Punica granatum* L. seeds

Pomegranate aqueous extraction was made using 100 g of fresh seeds that were boiled in 250 ml of distilled water for roughly six hours before being homogenized. To get rid of all the leftover materials, the mixture was next filtered using filter paper. It was then heated to 45°C in a hot air oven with a circulation fan and held at 4°C until it was used. The next studies involved dissolving 10 g of plant powder into 100 ml of Phosphate-buffered saline (PBS) (as the solvent), filtering and sterilizing the suspension using a 0.2 µl sterile Millipore filtering system, and storing the stock solutions at 4°C until needed (Abdul gany *et al*., 2010).

2. Aqueous extraction procedure for *Punica granatum* L. peels

Aqueous extract of peels was prepared using the same protocol adopted for aqueous extraction of *P. granatum* seeds with 100 g of dried peels powder.

3. Ethanolic extraction procedure for *Punica granatum* L. seeds

- Ethanolic extract of seeds was carried out by putted 100 g of powdered dry seeds inside the thimble, 500 mL of 70 % ethanol was added and the thimble loaded into the soxhlet´s main chamber and left overnight at room temperature.
- Seeds extraction started in soxhlet, for 4 hours at 40˚ C.
- Seed extract is then dried by evaporator at 40˚ C.
- Weighted the extract then it placed in labeled plastic tube and stored at 20˚ C.
- Briefly, the concentrated plant extracts were dissolved in dimethyl sulphoxide (DMSO) (SIGMA, USA) to get a stock solution of 1000 µg/ml (Abdul gany *et al*., 2010).

4. Ethanolic extraction procedure for *Punica granatum* L. peels

Extract of peels was prepared using the same protocol adopted for ethanolic extraction of *Punica granatum* seeds with 100 g of dried peels powder.

Cell Growth Assay

The impact of pomegranate aqueous and ethanolic extracts on cancer cell lines (MCF-7 and AMJ13) and normal cell line (REF) were determined using an *in vitro* technique. Solutions were created using The Iraqi Center for Cancer and Medical Genetic Research (ICCMGR) procedure. To begin the subculture process, trypsin/versine was applied to these cell lines.

Cytotoxicity Assays

The MTT cell viability assay was performed, the MTT assay is generally performed to assess cell viability. The ability of live cells to convert the yellow tetrazolium salt to purple formazan is measured using the simple, rapid and economical MTT in vitro assay (Basim and Kasim, 2023).

After seeding 1×10^4 cells per well and allowing them to adhere for the whole night in a CO_2 incubator, the cells formed a confluent monolayer. Following treatment with extracts, the cells were cultured at various concentrations (15.62, 31.25, 62.5, 125, 250, and 500 µg/ml) of the pomegranate seeds and peels aqueous and ethanolic extracts . Following the incubation period, each well received 20 µl of a 2 mg/ml solution of MTT,

and the media was withdrawn. After removing the MTT solution, DMSO (130 µl) was added to dissolve the resulting purple formazan crystals. (Abdullah *et al*., 2020).

A microplate reader was used to measure the absorbance at (570 nm), by comparing the absorbance of cells treated with extracts to that of untreated control cells, the percentage of live cells was ascertained. The following formulas were used to determine the percentage of cytotoxicity, or the inhibition rate of cell growth (Al-Shammari *et al*., 2020).

Inhibition % = [(optical density of control wells - optical density of test wells)/ optical density of control wells] × 100

Statically analysis

In the study parameters, the Statistical Analysis System. SAS (2018) program was used to detect the effect of difference factors. The least significant difference –LSD test was used to significant compare between means in this study.

Result

Different types of *P. granatum* extracts were used in current study; including aqueous extract of seeds, ethanolic extract of seeds, aqueous extract of peels and ethanolic extract of peels to estimate their cytotoxic activity against breast cancer cell lines and normal cell line.

- **Crude aqueous extraction of seeds:**

The aqueous extract of the seeds of *P. granatum* gave a light brown sticky product, which became powder upon drying, and the yield was 10 %.

- **Crude ethanolic extraction of seeds:**

The ethanolic extraction of the seeds of *P. granatum* gave a dark brown sticky product, which became powder upon drying with a yield of 12 %.

- **Crude aqueous extraction of peels:**

The aqueous extraction of peels gave a dark brown sticky product dried consistency with a yield of 17 %.

- **Crude ethanolic extraction of peels:**

The ethanolic extraction of peels gave a brown sticky product, which became powder after drying, with a yield of 16 %.

Effect of *P. granatum* extracts on cancer and normal cell lines

The MCF-7, AMJ13 and REF cell lines were exposed to sex concentrations (15.62, 31.25, 62.5, 125, 250, 500) µg/ml of *P. granatum* extracts for 24, 48 and 72 hours durations. The optical density for the cell lines were measured by ELISA reader at 570 nm.

- **Effect of *P. granatum* extracts on AMJ13 cells**

The effect of treating AMJ13 cells with aqueous and ethanolic extracts of *P. granatum* seeds and the effect of aqueous and ethanolic extracts of *P. granatum* peels after 24 hours of exposure are shown in Figure (1), the results revealed that the aqueous extract of seeds had the greatest growth inhibition effect on AMJ13 cell line than the other tested extracts after 24 hours of exposure.

Also the results in Figure 2 revealed that the aqueous extract of seeds had the greatest growth inhibition effect on AMJ13 cell line than the other extracts after 48 hours of exposure. The four types of *P. granatum* extracts showed a time-dependent effect on viability of AMJ13 cells. All concentration have significant cytotoxic effect ($P<0.05$). The highest concentration (500 µg/mL) at the 48 hrs., produced the highest percentage of inhibition thus reaching 77.505 %.

Figure 3 show that the aqueous extract of seeds had the greatest growth inhibition effect on AMJ13 cell line after 72 hours of exposure, rather than the other tested extracts. The growth inhibition percentage for the aqueous extract of seeds at the highest concentration (500 µg/ml) was 59.581 % after 72 hours.

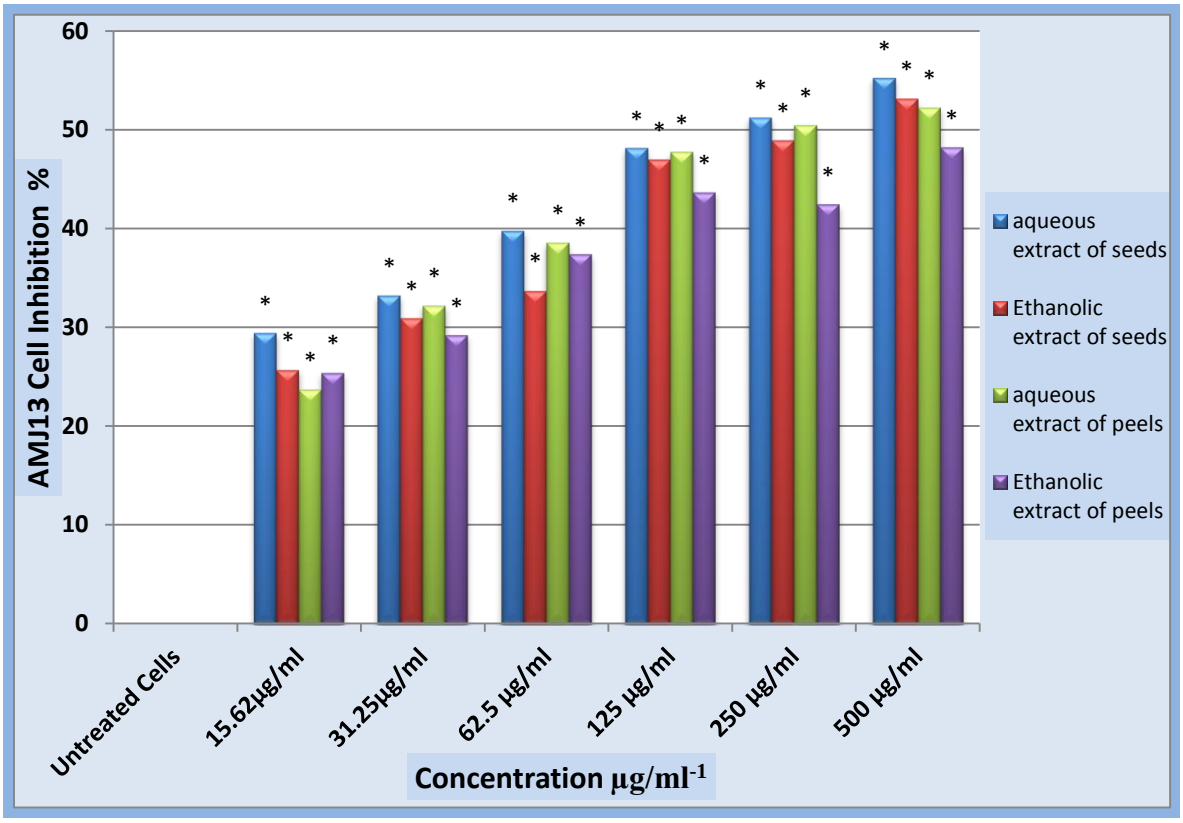

Figure 1. A Comparison Of Growth Inhibition Rates On AMJ13 Cell Line By Aqueous And Ethanolic Extracts Of *P. Granatum* Seeds And Aqueous And Ethanolic Extracts Of *P. Granatum* Peels After 24 Hrs. Of Exposure.

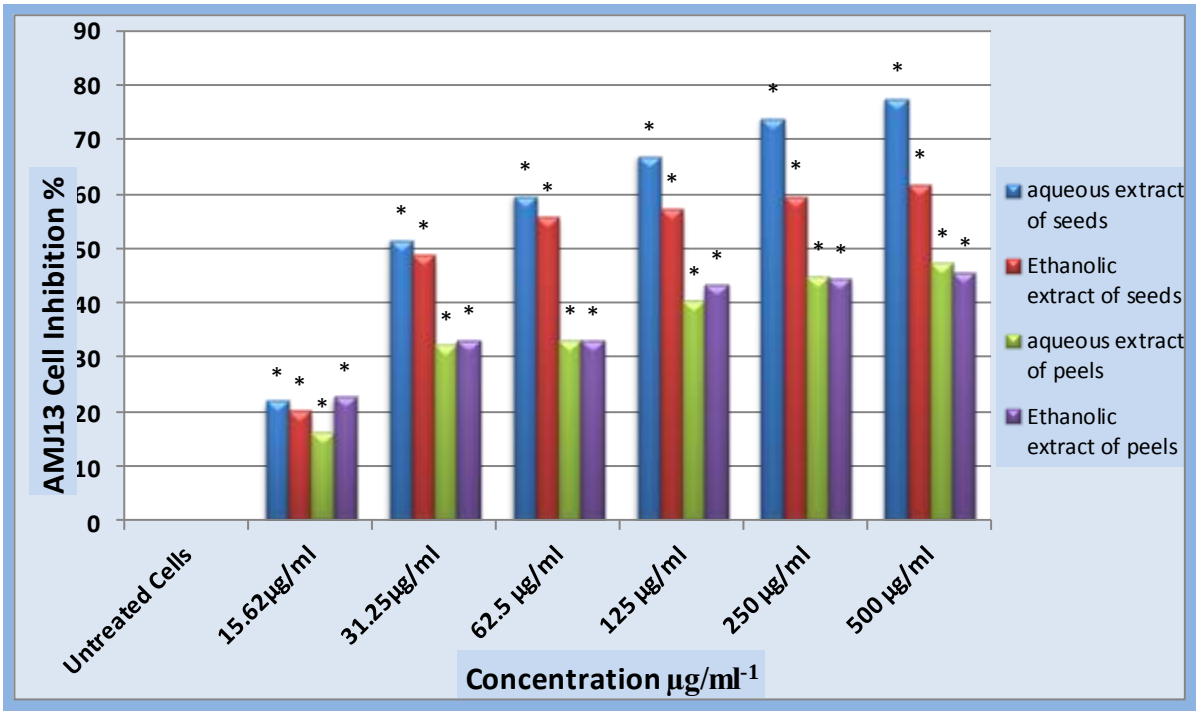

Figure 2. A Comparison Of Growth Inhibition Rates On AMJ13 Cell Line By Aqueous And Ethanolic Extracts Of *P. Granatum* Seeds And Aqueous And Ethanolic Extracts Of *P. Granatum* Peels After 48 Hrs. Of Exposure.

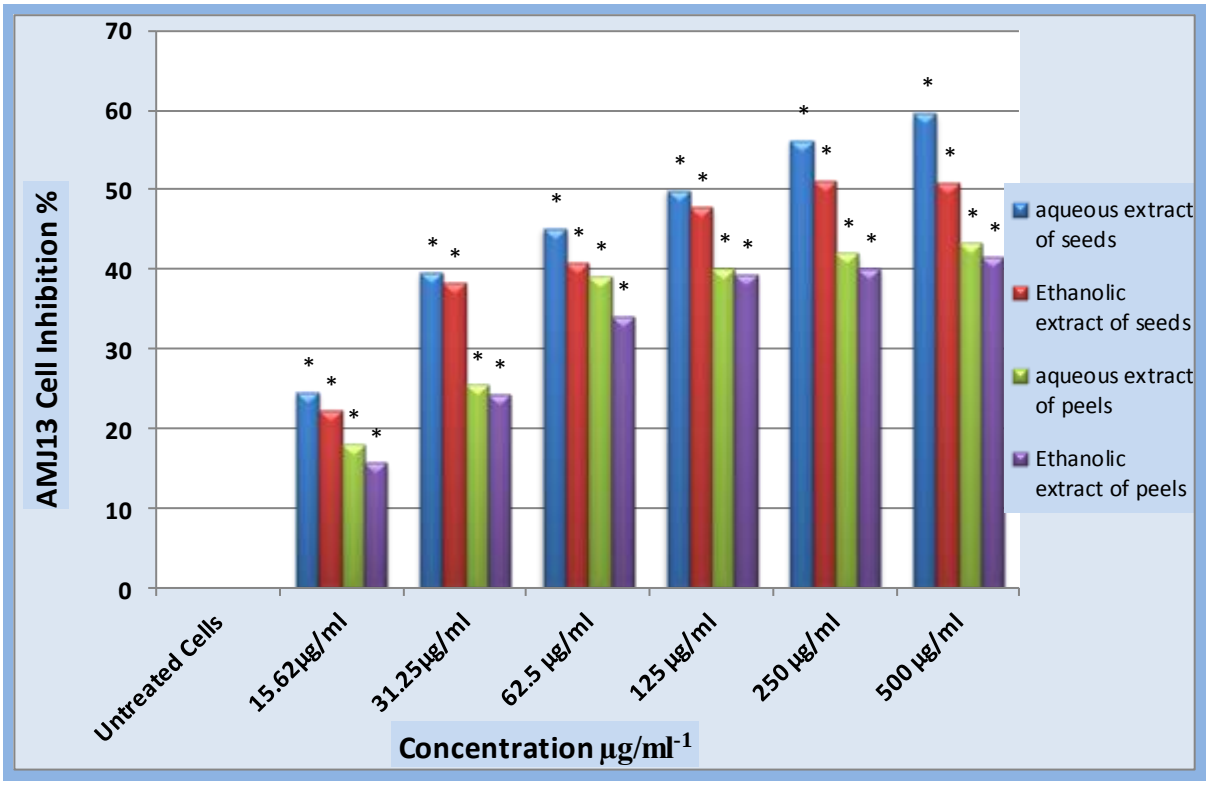

Figure 3. A comparison of growth inhibition rates on AMJ13 cell line by aqueous and ethanolic extracts of *P. granatum* seeds and aqueous and ethanolic extracts of *P. granatum* peels after 72 hrs. of exposure.

- **Effect of *P. granatum* extracts on MCF-7 cells**

The effect of treating MCF-7 cells with aqueous and ethanolic extracts of *P. granatum* seeds, The aqueous and ethanolic extracts of peels after 24 hours of exposure are shown in Figure (4). All concentrations have significant cytotoxic effect ($P<0.05$). Inhibition percentage reached 50.22 % after treatment with 500 µg/ml after 24.

Figure 5 shows that the aqueous extract of seeds had the greatest growth inhibition effect on MCF-7 cell line after 48 hours of exposure, rather than the other tested extracts. The growth inhibition percentage for the aqueous extract of seeds highest concentration was 82.12 % after 48 hours of exposure.

The results that shown in Figure (6) showed All concentrations of all four types of extracts have significant cytotoxic effect ($P<0.05$) after 72 hours of exposure.

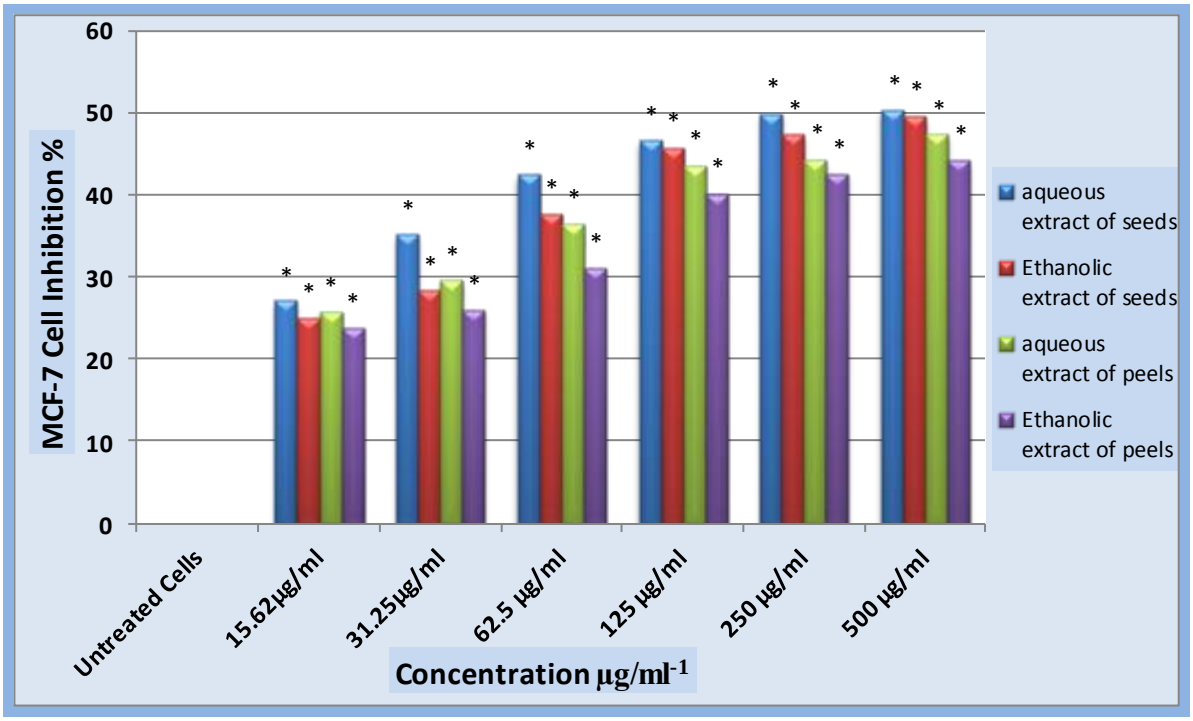

Figure 4. A comparison of growth inhibition rates on MCF-7 cell line by aqueous and ethanolic extracts of *P. granatum* seeds and aqueous and ethanolic extracts of *P. granatum* peels after 24 hrs. of exposure.

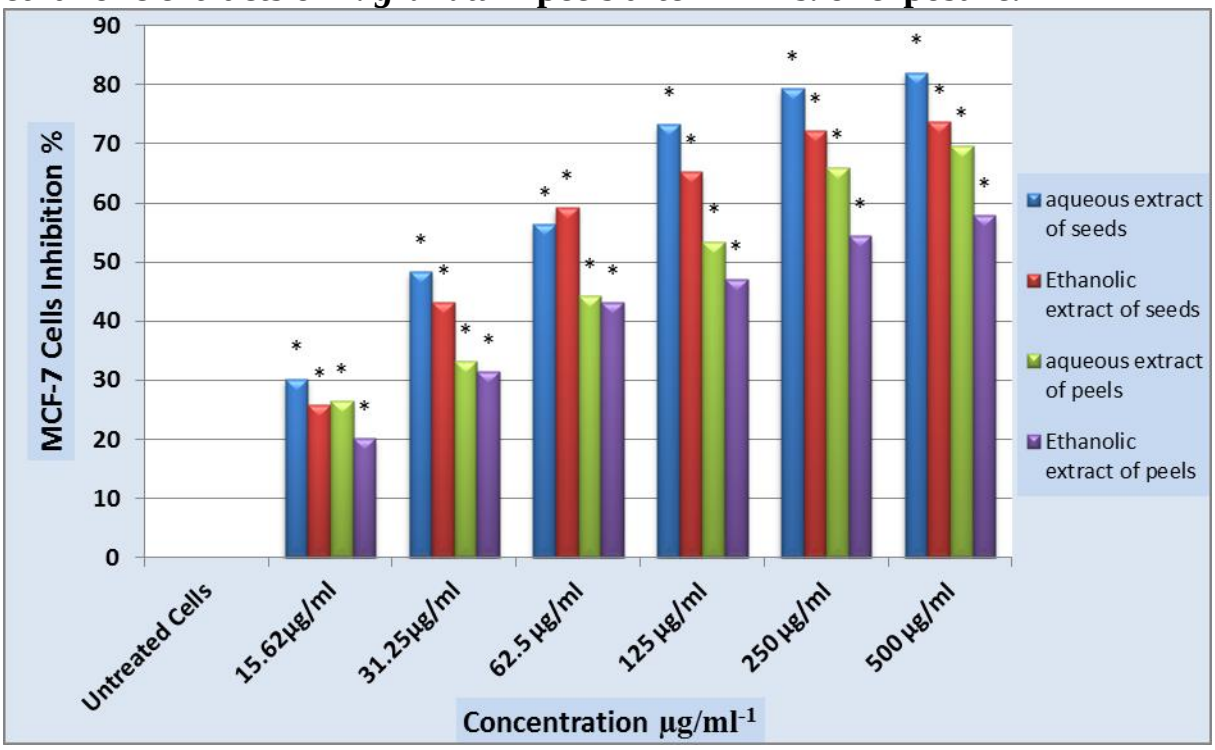

Figure 5. A comparison of growth inhibition rates on MCF-7 cell line by

aqueous and ethanolic extracts of *P. granatum* seeds and aqueous and ethanolic extracts of *P. granatum* peels after 48 hrs. of exposure.

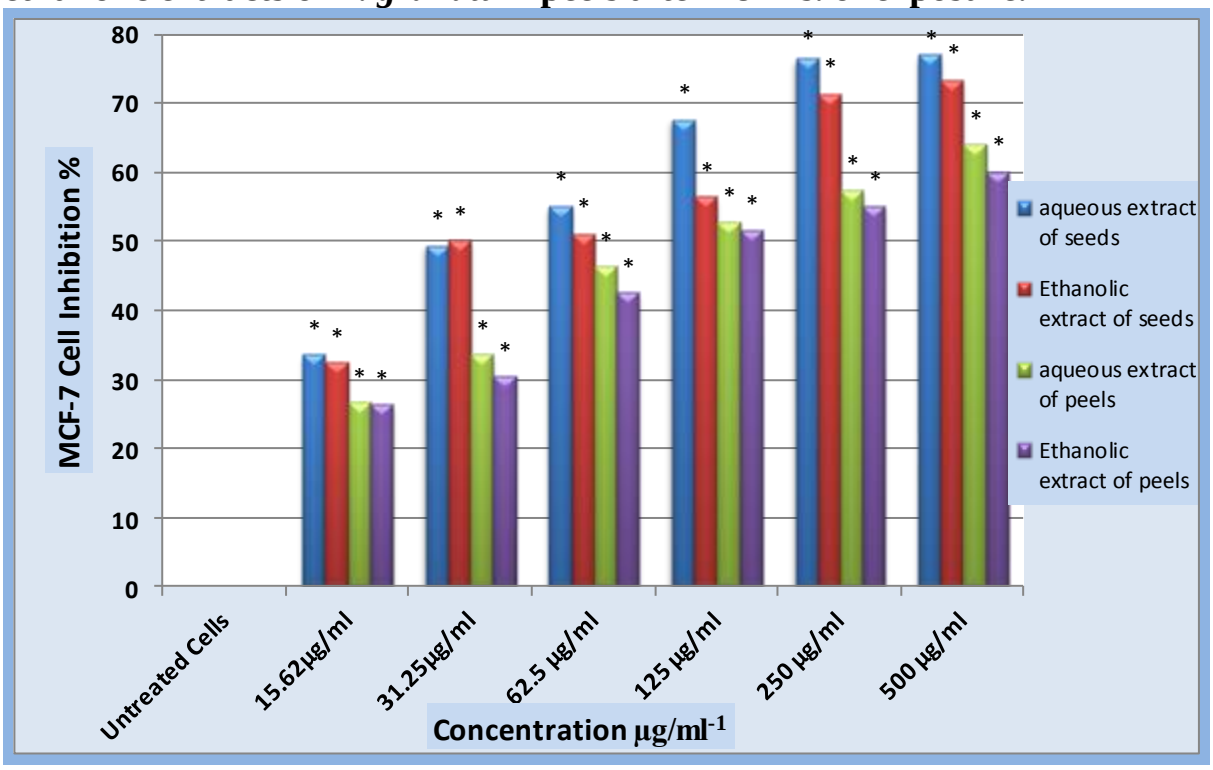

Figure 6. A comparison of growth inhibition rates on MCF-7 cell line by aqueous and ethanolic extracts of *P. granatum* seeds and aqueous and ethanolic extracts of *P. granatum* peels after 72 hrs. of exposure.

- **Effect of *P. granatum* extracts on REF cells**

Normal cell lines (REF) have been treated with sex concentrations from aqueous and ethanolic extracts of seeds and peels in one exposure time (72 hrs.). Statistical results revealed that was not a clear cytotoxic effect for these extracts on REF cells, as shown in Figure (7).

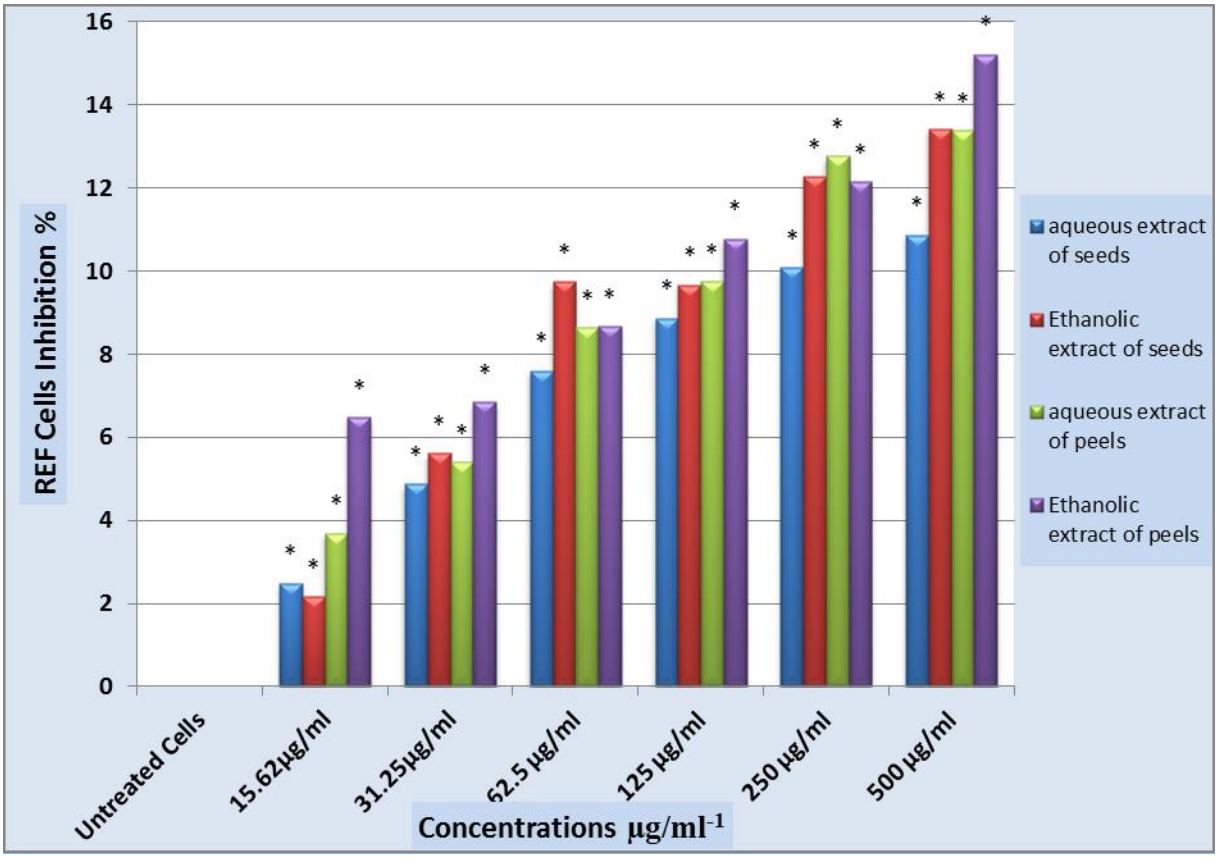

Figure 7. A comparison of growth inhibition rates on REF cell line by aqueous and ethanolic extracts of *P. granatum* seeds and aqueous and ethanolic extracts of *P. granatum* peels after 72 hrs. of exposure.

Discussion

Cancer is the second most common cause of mortality in human history, accounting for one in six deaths worldwide (Sharma *et al*., 2023). The final results from the MTT experiment showed varying anticancer effects on three different cell lines (MCF7, AMJ13, and REF), with considerable variances depending on the dose and duration of exposure. From the figure 1, 2, 3, 4, 5 and 6 it was determined that the aqueous and ethanolic extracts of seeds and peels have a high cytotoxic effect on two cancer cell lines, but the severity of this cytotoxicity differed from the type of extracts and from cell line to cell line. According to figure 7 in normal cells all four types of *P. granatum* extracts does not have a highly cytotoxic effect. According to the all results, the crude aqueous extract of seeds had the most cytotoxic effect in all cancer cell lines, followed by aqueous extract of peels and then ethanolic extract of seeds and peels.

The overall results obtained from that study on the cytotoxic effect of *P. granatum* extracts on cancer cell lines refer to the fact that the highest inhibitory effect of these extracts occurred on MCF-7 cancer cell line, which proves that MCF-7 cell line was very sensitive to the effect of *P. granatum* extracts compared to AMJ13 cell lines at the same conditions.

Our findings revealed that *P. granatum* extracts have an anti-cancer effect and supported the findings of other studies, Shama and his collagenous (2022), confirmed that the extracts from *P. granatum* have cytotoxic effect.

Conclusions

In the present study, aqueous and ethanolic extracts from *P. granatum* seeds and peels were prepared. The extracts from *P. granatum* exhibited a significant antitumor activity against Michigan Cancer Foundation-7 (MCF-7) Ahmed, Murtudha, Jabriyah, 2013 (AMJ13) cancer cell lines in concentration dependent manner. and the obtained results showed that these extracts did not have a cytotoxic effect on Rat Embryo Fibroblast (REF) normal cell line.

References

Abdul gany Z. 2010. The Role of IL-6, IL-10 and IFN-γ mRNA in Women with Recurrent Abortion. Iraqi Journal of Cancer and Medical Genetics 3 (1): 46-53.

Abdullah S, Al-Shammari A, and Lateef S. 2020. Attenuated measles vaccine strain has potent oncolytic activity against Iraqi patient derived breast cancer cell line. Saudi journal of biological sciences, 27 (3): 865-872.

Al-Khuzaay H, Al-Juraisy Y, Alwan A, and Tousson E. 2024. Evaluation of Effect of β-Glucan on Cancer Cell Lines *in vitro*. Al-Mustansiriyah Journal of Science 35 (1): 17-20.

Al-Khuzaay H, Al-Juraisy Y and Al-Halbosiy M. 2019. The activity of aqueous extracts of leaves and roots of dandelion on cancer cell lines. Plant archive. 19 (2): 3933-3936.

Al-Shammari A, Al-Saadi H, Al-Shammari S and Jabir M. (2020). Galangin enhances gold nanoparticles as anti-tumor agents against ovarian cancer cells. AIP Conference Proceedings 2213 (1): 12-18.

Basim S, and Kasim A. 2023. Cytotoxic activity of the ethyl acetate extract of Iraqi Carica papaya leaves in breast and lung cancer cell lines. Asian Pacific Journal of Cancer Prevention. 24 (2): 581.

Cortez-Treje M, Olivas-Aguirre F, Dufoo-Hurtado E, Castaneda-Moreno R, Villegas-Quintero H, Medina-Franco J. and Mendoza S. 2022. Potential

Anticancer Activity of Pomegranate (Punica granatum L.) Fruits of Different Color: In Vitro and In Silico Evidence. Biomolecules.12 (11): 1649.

Eghbali S, Askari S, Avan R, Sahebkar A. 2021. Therapeutic effects of Punica granatum (pomegranate): an updated review of clinical trials. J Nut Metab.

Ferlay J, Ervik M, Lam M and Mery L. 2020. Global Cancer Observatory: Cancer Today. Lyon, France: International Agency for Research on Cancer. 2021. https://gco.iarc.fr/today.

Khwairakpam A, Bordoloi D, Thakur K, Monisha J, Arfuso F, Sethi G, Mishra S. and Kumar A. 2018. Possible use of *Punica granatum* (Pomegranate) in cancer therapy. Pharmacological Research. 133. 53-64.

Lemonnier N, Zhou G, Prasher M, Mukerji M, ChenZ, Brahmachari S, Noble D, Auffray C. and Sagner M. 2017. Traditional Knowledge-based Medicine: A Review of History, Principles, and Relevance in the Present Context of P4 Systems Medicine. Prog. Prev. Med., 2: 11.

Melgarejo-Sanchez P, Nunez-Gomez D, Martínez-Nicolás J, Hernández F, Legua P and Melgarejo P. 2021. Pomegranate variety and pomegranate plant part, relevance from bioactive point of view: A review. Bioresour. Bioprocess.,8:2.

SAS. 2018. Statistical Analysis System, User's Guid. Statistical. Version 9.6th ed. SAS. Inst. Inc. Cary. N.C. USA.

Sharma A, Sharma L, Nandy SK, Payal N, Yadav S, Vargas-De-La-Cruz C. 2023. Molecular aspects and therapeutic implications of herbal compounds targeting different types of cancer. Molecules. 28(2): 750.

Wong T, Strandberg K, Croley C, Fraser S, Venkata K, Fimognari C, Sethi G and Bishayee A. 2021. Seminars in Cancer Biology. Academic Press; Waltham, MA, USA. Pomegranate bioactive constituents target multiple oncogenic and oncosuppressive signaling for cancer prevention and intervention, 73: 265–293.

تقييم تأثير مستخلصات بذور وقشور نبات الرمان (*Punica granatum*) على خطوط الخلايا السرطانية والطبيعية

م.م. هبة محمد عبد الواحد
الجامعة المستنصرية/ كلية التربية الاساسية/ قسم العلوم

مستخلص البحث

في هذه الدراسة تم التعرف على قابلية المستخلص المائي والكحولي لبذور وقشور نبات الرمان*Punica granatum* (*P. granatum*) في التأثير على اثنين من الخطوط الخلوية هما خطي خلايا سرطان الثدي MCF-7 و AMJ13 والخط الخلوي الطبيعي لأجنة الجرذ الليفية REF. اوضحت القيم الناتجة ان التأثير التثبيطي لحيوية الخلايا بشكل عام يعتمد على تركيز المستخلصات الخام والوقت. اظهرت المستخلصات لنبات الرمان تثبيطا عاليا للخلايا السرطانية، حيث التراكيز الأعلى أعطت تأثيراً ساماً للخلايا أعلى معنوياً ($P<0.05$) عند 48 ساعة من التعرض ، بينما لم يكن هناك تأثير لمستخلصات نبات الرمان على خط الخلايا الطبيعي.