

Original Research Article

## Study of the Antioxidant and Anti-Cancer Activity and Biological Activity of (*Punicagranatum L.*) Extract on Pathogenic Microorganisms Using Alkaline Energy Water

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**Abstract:** *Objective:* to characterize and prepare pomegranate extract and study its antioxidant, anti-cancer activity, determine the total phenols and flavonoids, determine the active ingredients using LGC MAS technology. *Materials and Methods:* Preparation of *Punicagranatum* extract in distilled water and alkaline energy water, and characterization of the ingredients in each extract was carried out using GC-MASS spectroscopy; cytotoxicity was measured against MCF-7 cancer cell line. *Results:* it was found that alkaline water extract showed the highest concentration of phenols and flavonoids at a concentration of 34.32 and 59.65 µg/g and the percentage of the two compounds in the GC-MASS assay Hexadecanoic acid, methyl ester, 11- Octadecanoic acid, methyl ester. In alkaline water with the highest concentration (24%), 15.34%) compared to distilled water extract *Punicagranatum*, as it was found to be 25.67 and 39.22, noting that these two compounds are anti-cancer compounds. Furthermore, the study proved that *Punicagranatum* alkaline extract showed a high inhibitory activity for a type of cancer breast cancer cells line MCF-7. *Conclusions:* MCF-7 cell proliferation were significantly decreased with a concentration-dependent response, and it was found that a concentration of 0.5 µg/ml gave an inhibition rate of 93.47%, and *Punicagranatum* extract with alkaline water showed a high effectiveness in inhibiting the following microorganisms: *S. aureus*, *B. cereus*, *E. coli*, *S. typhimureum*, *A. niger*, *C. albicans*, inhibition and antioxidant activity 87.18 at a concentration of 40mg/ml, the concentration of the ingredients are more in alkaline energy water extract than distilled water extract.

**Keywords:** *Punicagranatum* extract, alkaline water, phenols, flavonoids.

## INTRODUCTION

Pomegranate (*Punicagranatum L.*) is one of the oldest recognized edible fruits (Saeed *et al.*, 2018) belonging to the family Punicaceae. It is widely cultivated in many tropical and subtropical countries. Natural products have been shown to be the most effective in terms of their ability to alter the function of cancer-related proteins [1].

Plants have been an important medicine source for thousands of years. Even today, the World Health Organization estimates that up to 80% of people rely primarily on remedies such as herbs for their traditional medicines. Medicinal plants constitute one of the main sources of new medicines and health care products [2]. The use of pomegranate juice, peel and oil has been indicated. Pomegranate has anticancer activities, including interfering with cancer cell proliferation, cell cycle, invasion, and angiogenesis. It may be linked to anti-inflammatory effects. In the past decade, several studies have been published on the components of pomegranate. There are many studies that have been completed regarding the effectiveness of pomegranate in treating colon, breast, and liver cancer [3].

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The current study aimed to prepare a pomegranate extract with alkaline energy water and characterized it using LGC-MAS and studies its antioxidant and anticancer activity; also determine the total phenols, ingredients, and flavonoids.

## MATERIALS AND METHODS

Fresh pomegranate fruits were collected from Diyala farms. The seeds and peels were peeled by hand. The seeds were mixed with distilled water and another mixture was prepared by mixing the seeds with alkaline energy water, by taking 20 g of seeds to 200 ml of solvent (distilled water and alkaline energy water) each separately and mixed with a mixer and centrifuged at 3500 rpm, then keep the extract in fridge until use [4].

### Determination of Total Phenol Content

The determination of the total phenol content was carried out by Folin-Ciocalteu test according to [5]. One mL of the extract sample (5mg 100) in (60% acetone mL solution) was mixed with 200 $\mu$ L of Folin-Ciocalteu reagent and 1mL of aqueous Na<sub>2</sub>CO<sub>3</sub>. The solutions were left at room temperature for 30 minutes and the phenol concentration was determined by colorimetric method at 715 nm. The titration curve was prepared using solutions of gallic acid at concentrations from 1 to 0.01562 mg/mL in 60% acetone. The total phenol content was expressed in terms of gallic acid equivalent (mg/g).

### Determination of the Total Flavonoid Content

The total flavonoid content was estimated using aluminum chloride calorimetry [6]. 0.5 ml of methanol test sample solution (5 mg/100 ml) was mixed with 2 ml of distilled water and 150  $\mu$ l of 5% sodium nitrate. After 6 min, 150  $\mu$ l of 10% aluminum chloride and 2 ml of 1 M NaOH were added and left at room temperature for 15 min. The absorbance of the samples was measured at a wavelength of 510 nm (UV-Visible Ultraspec 2000) and the total flavonoid contents were calculated as rutoside equivalents from the rutoside calibration curve. The titration curve was prepared in the same way using 0.01562-1 mg/ml rutoside solutions in methanol.

### Perform a DPPH Check

The antioxidant activity of the extracts was examined according to (8), different concentrations of the sample were prepared in methanol and 5 ml of each concentration was added to 5 ml of a methanol solution and 0.004% of DPPH was added after the reaction was completed at room temperature for a period of time. Tetrazolium (0.5 g) was dissolved in 100 mL phosphate-buffered saline (PBS) (concentration 5 mg/mL [7].

### Treatment of Cancer Cells

Different concentrations of the assay extract (0.5, 0.25, 0.12, 0.062, 0.031, 0.015) (three replicates) were added to the cells that were grown in a PLATE96-hole transparent flat plate at a density of  $1 \times 10^4$  cells/hole and incubated at 37 °C in an incubator for 24 hours. There were three replicates for each concentration tested, while the last column in the panel is kept as (-ve control) (cells without treatment). Cells were incubated at 37 °C and 5% CO<sub>2</sub>, Cell viability was measured after 48 hours, according to the method prescribed by [8]. The rate of cell growth inhibition (percentage of cytotoxicity was calculated as:

$$(GI) = (A-B)/A \times 100$$

Where, A is the average optical density of untreated pits (control), B is the optical density of treated pits (test cells) [9].

### Statistical Analysis

One-way analysis of variance was used to analyze the data using Anova: one representative by Microsoft Office Excel 2010 Determine the LSD value for a significant value of 5%.

## RESULTS AND DISCUSSION

Table (1) shows the results of the analysis of total flavonoids and total phenols of pomegranate extract with distilled water and energy water. It was found that the concentration of total phenols and the concentration of flavonoids in the pomegranate extract with alkaline energy water are significantly higher than the concentration of the pomegranate extract prepared with distilled water. The high basal potential worked to break down the cell walls and release phenols and flavones into the aqueous medium, which led to an increase in their concentration in the alkaline aqueous medium compared to distilled water, as it was found that the total concentration of phenols and flavones was 22 mg/g and flavones 51.32 mg/g compared to distilled water extract Phenols 15.28 mg/g and flavones 43.52 mg/g. This is consistent with [9, 10], as it was found that when extracting phenols and flavonoids; a different pH of pomegranate fruits gave pH 8 and 9 the highest concentration of the mentioned compounds.

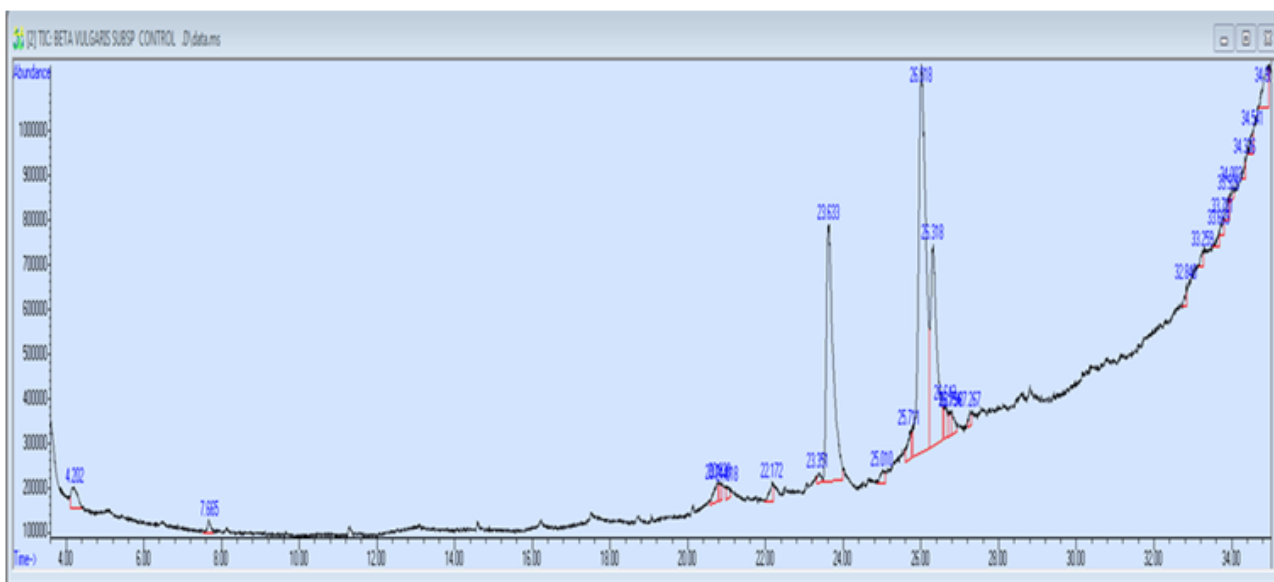
**Table 1: Data analysis of total flavonoids and phenols of *Punicagranatum* extract**

Extract	Total phenol content mg /gm	Flavonoid	P value
Water extract	25.67	39.22	Sig.
Alkaline Energy water	34.32	59.65	Sig.
P value	Sig	Sig.	

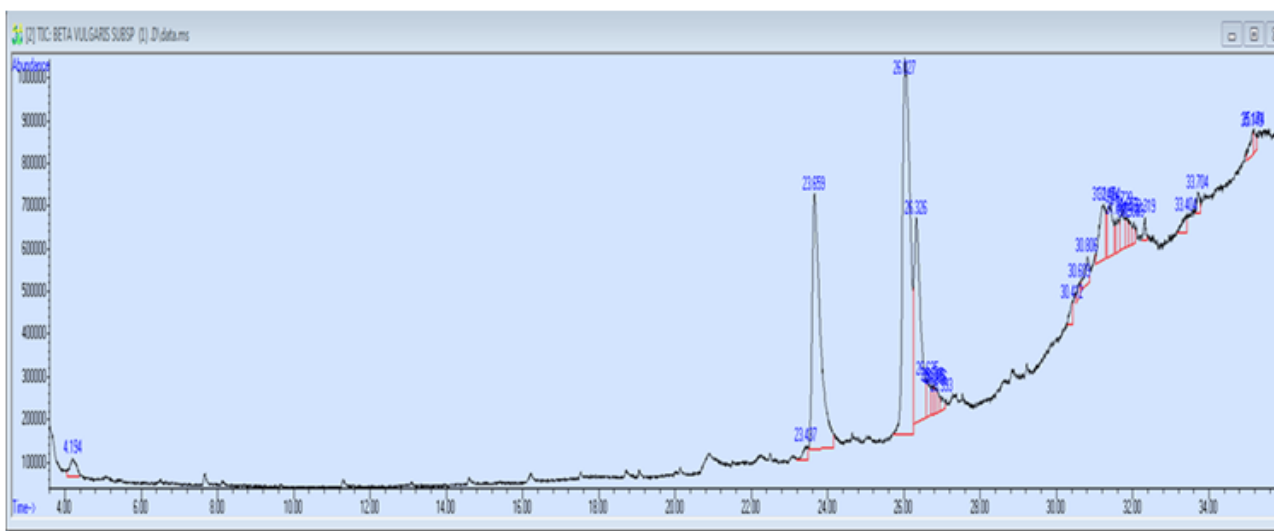
*The mean difference is significant at the 0.05 level*

Figure (1), and Table (2) show the active compounds of *Punicagranatum* extract with distilled water and alkaline water using (GC-MASS) technology. It was found that 1.38%, Pregnan-20-one, 3-hydroxy- (24%) Hexadecanoic acid, methyl ester (17.14%) Heptadecanoic acid, 10-methyl, (15.34%) 6-Octadecenoic acid, methyl ester (1.35%) Oleic acid. The components that appeared in the beetroot extract with alkaline energy water are shown in table 3 and figure 2 are (34.23%) 6-Octadecanoic acid, methyl ester (15.47%), Heptadecanoic acid, 10-methyl, (37.31%) hexadecanoic acid, methyl ester, (2.44%) oleic acid.

It was found that the concentration of 6-Octadecanoic acid, methyl ester, Hexadecanoic acid, methyl ester in the extract of alkaline energy water is higher than in beetroot extract with distilled water in addition to the emergence of new components that differ from it in the extract using alkaline water.



**Figure 1: The active compounds of *Punicagranatum* with distilled water using (GC-MASS)**



**Figure 2: The active compounds of *Punicagranatum* with alkaline water using (GC-MASS)**

**Table 2: The active ingredients of the extract, distilled water of beetroot using the (GC-MASS)**

Pk#	Library/ID	RT	Area%
1	(3-Methyl-oxiran-2-yl)-methanol	4.202	1.73
2	Malic Acid	7.665	0.57
3	Tridecanoic acid, methyl ester	20.744	1.17
4	Tetradecanoic acid, 12-methyl-, methyl ester	20.838	0.53
5	Methyl trans-9-(2-butylcyclopentyl)nonanoate	21.018	0.54
6	cis-13-Octadecenoic acid	22.172	0.92
7	2-Isopropenyl-5-methylhex-4-enal	23.351	0.51
8	Hexadecanoic acid, methyl ester	23.633	24.00
9	cis-Vaccenic acid	25.010	0.84
10	alpha.-Hydroxy-17.alpha.-methyl testosterone	25.711	1.38
11	6-Octadecenoic acid, methyl ester	26.018	17.14
12	Methyl stearate	26.318	17.14
13	cis-13-Octadecenoic acid	26.643	1.35
14	cis-11-Eicosenoic acid	26.754	0.90
15	E,E,Z-1,3,12-Nonadecatriene-5,14-diol	26.796	0.93
16	9-Octadecenoic acid (Z)-, 2-hydroxy-1 (hydroxymethyl)ethyl ester	27.267	0.46
17	Oleic Acid	32.840	0.54
18	13-Octadecenal	33.259	0.55
19	Decyloleate	33.669	0.57
20	13-Octadecenal	33.781	0.61
21	Oleic Acid	33.926	1.05
22	Oleic Acid	34.003	0.44
23	n-Propyl 11-octadecenoate	34.336	0.51
24	Erucic acid	34.541	0.95
25	Fumaric acid, 3-heptyl hexadecyl ester	34.901	2.94

**Table 3: Shows the active ingredients of the *Punicagranatum* alkaline water extract using the GC-MASS technique**

Pk#	Library/ID	RT	Area%
1	2-[2-[2-[2-(tert-Butyldimethylsilyloxy)ethoxy]ethoxy]ethoxy]ethanol	4.194	1.34
2	Oleic Acid	23.437	0.78
3	Hexadecanoic acid, methyl ester	23.659	24.81
4	6-Octadecenoic acid, methyl ester	26.027	34.23
5	Heptadecanoic acid, 10-methyl-, methyl ester	26.326	15.47
6	11-Octadecenoic acid, methyl ester	26.625	1.53
7	3-Hexen-2-one, 3-cyclohexyl-4-ethyl L	26.745	0.74
8	3-Hexen-2-one, 3-cyclohexyl-4-Ll-	26.805	0.68
9	9-Octadecenoic acid (Z)-, methyl ester	26.856	0.65
10	3-Hexen-2-one, 3-cyclohexyl-4-ethyl l-	26.993	0.59
11	Oxiranedodecanoic acid, 3-octyl-, cis-	30.412	0.70
12	Oxiranedodecanoic acid, 3-octyl-, cis-	30.609	0.58
13	3.beta.-Acetoxy-16-isothiocyanatop	30.806	1.34
14	Dodecane, 4,9-dipropyl-3,3-Diethyltridecane	31.242	4.15
15	5,5-Diethyltridecane	31.404	3.12
16	Octadecanoic acid, 13-oxo-, methyl ester	31.601	1.24
17	Tetrahydrofuran-2-carboxylic acid	31.720	2.06
18	3,3-Diethylpentadecane	31.857	0.93
19	Tetrahydrofuran-2-carboxylic acid, dibenzofuran-3-ylamide	31.908	0.72
20	1,3-Dioxolane, 4-ethyl-5-octyl-2,2 - (trifluoromethyl)-, cis	32.019	0.59
21	3,7,11,15-Tetramethyl-hexadecanol,trimethylsilyl ether	32.319	0.56
22	9-Hexadecenoic acid, methyl ester, cis-9-Hexadecenoic acid	33.404	0.86
23	9,12,15-Octadecatrienoic acid, 2 (trimethylsilyloxy)-1-[(trimethylsilyloxy)methyl]ethyl ester	33.704	0.67
24	Oleic Acid	35.140	1.02
25	Tetradecanoic acid, dodecyl ester	35.174	0.62

The results of the tumor cell inhibition assay on breast cancer cells showed that the alkaline water extract showed a significant inhibitory activity for the cancer cells, as it was found that the inhibitory activity of the alkaline pomegranate extract concentrations in this study, we applied the MTT test to evaluate the cytotoxic effect using increasing concentrations of the extracts under study (0.5, 0.25, 0.12, 0.6, 0.031, 0.015 µg/ml). The highest inhibition was shown at 0.5 µg/ml concentration (93.47%) for *Punicagranatum* extract with alkaline energy water compared to distilled water extract, which was 84.96%. The first antitumor drug from plants with the application of cancer chemotherapy was developed five decades ago. Great as: Finn Blasteen and Finn Kristen, table 4 shows the anti-tumor activity of a type on MCF-7 (human breast cancer) cell line.

**Table 4: The results of the inhibitory activity of beetroot extracts (MCF-7) against cancer cells(distilled water, alkaline water)**

Conc. (µg/ml)	A	B	P
0.5	93.47	84.96	Sig
0.25	70.13	77.43	Sig
0.12	62.23	50.30	Sig
0.062	53.78	33.56	Sig
0.031	41.21	18.54	Sig
0.015	30.53	5	Sig
P	Sig	Sig	

A-*Punica water extract (energy alkaline water)* B- *Punica water extract (D.W)*,

Table (5) shows the results of the antimicrobial activity of *Punicagranatum* extracts (distilled water, alkaline water). It was found that *Punicagranatum* extracts with distilled water and alkaline water showed a high activity in inhibiting microorganisms, and it was found that alkaline water extract showed a higher activity in inhibiting microorganisms, as it found the inhibitory activity against the following microorganisms *S. aureus* *B. cereus* *E. coli* *S. typhimureum* *A. niger* *C. albicans* compared to the results of *Punicagranatum* extract with distilled water 15,13.54, 11.42,11.14,11mm diameter damping. This is likely due to the presence of phenols 10, 9.43, 7.12, 5, 10.65, and flavones in higher concentrations in *Punicagranatum* alkaloid extract.

In general, *Punicagranatum* extracts show anti-microbial activity. The extracts showed antibacterial activity against. Table (5) shows the results of the inhibitory activity of microorganisms of beetroot extracts (distilled water, alkaline water).

**Table 5: Results of the inhibitory activity of *Punicagranatum* extracts (distilled water, alkaline water) against microorganisms**

Samples	<i>S. aureus</i> .	<i>B. subtilis</i>	<i>E. coli</i>	<i>S. typhimureum</i>	P
A	15. ±0,24	13.54±0.47	11.42±0.21	11±0.044	14.10
B	10±0.76	9.43±0.17	7.12±0.42	5±0.016	10.65
P	Sig	Sig	Sig	Sig	Sig

A-*Punica water extract (energy alkaline water)* B- *Punica water extract (D.W)*  
The mean difference is significant at the 0.05 level

Table (6) shows the antioxidant activity of *Punicagranatum* extracts with distilled water and alkaline water, it was found that the concentrations of the extracts under study (10,20,30,40 mg/ml) showed significant antioxidant activity, and it was found that the alkaline extract had a higher antioxidant activity than the *Punicagranatum* extract with distilled water. 40 mg/ml showed an 87% antioxidant activity.

**Table 6: Results of the antioxidant activity of *Punicagranatum* extracts (distilled water, alkaline water)**

Concentration mg/ml	A	B	P
10	22.8 ± 0.23	13.87±0.22	Sig
20	34.35±0.27	27.18±0.39	Sig
30	67.43±0.32	42.29±0.34	Sig
40	87.18±0.28	65.16±0.42	Sig
P	Sig	Sig	

The mean difference is significant at the 0.05 level, A-*Punica water extract (energy, alkaline water)* B- *Punica water extract (D.W)*

## CONCLUSIONS

- ✓ MCF-7 cell are significantly decreased with a concentration-dependent response.

- ✓ The concentration of 0.5µg/ml gave an inhibition rate of 93.47%, and *Punicagranatum* extract with alkaline water showed a high effectiveness in inhibiting.
- ✓ The following microorganisms: *S. aureus* *B. cereus* *E. coli* *S. typhimureum* *A. niger* *C. albicans*, inhibition and antioxidant activity 87.18 at a concentration of 40 mg / ml.
- ✓ The concentrations of the ingredients are more in alkaline energy water extract than distilled water extract.

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