Journal of Food and Dairy Sciences

Journal homepage: www.jfds.mans.edu.eg
Available online at: www. jfds.journals.ekb.eg

Phytochemical Constituents, Antimicrobial and Antitumor Effects of Pomegranate Fruit (*Punica granatum* L)

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ABSTRACT

Pomegranate fruit has exposed various health benefits in many recent studies due to its rich content of bioactive chemical compounds. This study was carried out to investigate the content of these bioactive compounds in pomegranate fruit along with its antimicrobial and antitumor effects. Pomegranate peel powder and juice were analyzed for its phytochemical constituent content (total phenols, total flavonoids). Phenols and flavonoids contents were estimated qualitative and quantitative using HPLC technique. Furthermore, antitumor activity was tested in vitro on human cell lines. Likewise, antimicrobial and antioxidant activity were investigated. The results showed that the both ethanolic and aqueous extracts of juice and peels powder have antitumor and antimicrobial activity.Phytochemicals have shown superior content in peels than juice including total polyphenols 61.63 and 15.93 mg/g, and total flavonoids 49.3 and 25.85 mg/g, in peels and juice, respectively. Peels extract has exhibited significant antimicrobial activity higher than aqueous extract of juice at 1000 ppm concentration. Thus, peels and juice extracts demonstrated higher antimicrobial activity against *E.coli*, fungi and yeast. In the test of pomegranate peels and juice extracts for the protective effect against human cell lines, the pomegranate extracts and AgNPs exhibited significant protective effect.In conclusion, our study suggested that pomegranate fruits or its extracts can use as antitumor and antimicrobial agents. Pomegranate fruits are sources of bioactive compounds such as phenolic and flavonoids with potentially high antioxidant activities.

Keywords: Pomegranate , Pomegranate peel , Phytochemicals, Antimicrobial activity ,Cancer therapy

INTRODUCTION

Pomegranate (*Punica granatum L.*) is a small tree of the *Punicaceae* family. During the last years, there have been great increases in the commercial farming of various pomegranate cultivars in most countries of Middle East, Mediterranean Sea, USA, and South Africa (Fawole *et al*., 2011). Additionally, to the value of its fruits, (*Punica granatum*) is further used in many traditional medicine systems against some diseases. Previously mentioned studies revealed several biological activities of pomegranate such as antitumor, antimicrobial, antioxidant (Cassano *et al*., 2011).

Pomegranate fruits indicated that its peels, flowers and juice consisted of different types of antioxidants compounds including anthocyanins, catechins and ellagitannins (Hayrapetyan *et al*., 2012). Antioxidant abilities of pomegranate *in vivo* and *in vitro* have been demonstrated (Singh *et al*., 2002). Additionally to its antioxidant properties, it has antimicrobial, antibacterial, antiviral, antifungal and antimutagenic. Moreover, pomegranate peels have been stated to have marked antioxidant activity ((Negi *et al*., 2003).

Pomegranate peels are an unbeatable part obtained during processing of pomegranate juice. Pomegranate peels is a wealthy source of tannins, flavonoids and other phenolic compounds (Li *et al*., 2006). Antioxidant, anticarcinogenic and antibacterial properties of pomegranate peels have been reported (Al-Zoreky, 2009). However, up to now, there have been no attempts to investigate phytochemical constituents, the antimicrobial and antitumor properties of pomegranate peels extracts and juice.

Current reports show that pomegranate fruits (peel and juice) extracts have strong antitumorigenic effects in prostate cancer; the principle of this study was to estimate phytochemical constituents and antimicrobial activates of pomegranate peels extracts and juice then study the effects of pomegranate peels extracts and juice on breast cancer (MCF-7), liver cancer (HEPG-2) and colon cancer (HCT116) cancer cell lines.

MATERIALS AND METHODS

Material:

Fruit samples.

Fresh pomegranate fruits were obtained from the local market, Mansoura city, Egypt. The fruits were charily washed by tap water. Pomegranate peels was dried away from sunlight to final moisture content of the peels which was set to 9 ± 0.2% and crushed to fine powder using Braun GmbH Grinding (Model, KSM2; Type, 4041). Grinded peels powder was separated to be fine enough to pass through sieve size (75–100 µm).

Methods:

Chemical composition of pomegranate peels

The chemical composition of pomegranate peels was carried out at the accurate analysis Unit, Chemistry department, Faculty of Agriculture, Mansoura University.

The crude protein, moisture, ash, lipids and fibers content were determined according to the method described by AOAC, (2005) using 6.25 as a protein factor. In addition, total carbohydrates were calculated by difference

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DOI: 10.21608/jfds.2019.60208

Preparation of pomegranate peels ethanolic and aqueous extracts

Dried pomegranate peels were used to make ethanolic and aqueous extracts. 100g of peels was added into 500 ml of aqueous 80% ethanol in a soxhlet apparatus for (72h) the solvent was filtered and then evaporate by rotary evaporator apparatus after the extraction. 100g of peels was added into 500ml of water then filtered with the help of Whatman filter paper No.1 and filtrate, and then the aqueous extract was concentrated to nearly dryness under reduced pressure using the rotary evaporator at 45°C to achieve the crude aqueous extract which kept for further investigation (Eidi *et al*., 2007).

Preliminary phytochemical constituents of pomegranate peels and juice extracts.

Qualitative phytochemical tests were carried out on the crude ethanolic and aqueous extracts of pomegranate peels and juice to detect the presence of: terpenes, tannins, flavonoids, saponins, alkaloids, carbohydrate and/or glycosides, phenolic glycosides and resins according to the method reported by Harborne,(1988)

Determination of total flavonoids content.

Total flavonoids content of pomegranate peels was quantitavily estimated according the method stated by Lin and Tang,(2007).

Determination of total polyphenolic Content.

Total phenolic contents of pomegranate peels were determined by using Folin-Ciocalteu reagent method according to Yadav and Agarwala,(2011).

High performance liquid chromatography (HPLC) analysis.

HPLC analysis was conducted in the laboratory of Food Technology Research Institute, Giza, Egypt.

Identification and quantification of flavonoids

Flavonoids were quantified and identified in pomegranate peels and juice extracts by An Agilent 1100 Series HPLC operational with diode array detector. Analysis conditions were described in details in the method described by Mattila *et al*., (2000)

Identification and quantification of polyphenols

Phenolic components were identified in pomegranate peels and juice extracts, and then injected into reversed phase HPLC/diode array detection (Hewlett Packard 1050) with a protector column Alltima C_{18}, 5 mm according to the method described by Goupy *et al*., (1999).

Antioxidant activity (DPPH radical assay)

Free radical scavenging activity of pomegranate peels extracts and juice on the stable radical 2,2-diphenyl-picrylhydrazyl (DPPH) was evaluated according the method described by Li *et al*., (2015).

Antimicrobial activity

The pomegranate peels extracts and juicewere subjected to antimicrobial evaluation using the agar diffusion technique according to the method described by Stuardo and San Martin, (2008). The tested extracts and juice were evaluated against, Gram +ve bacteria (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 35556), Gram -ve bacteria (*Escherichia coli* ATCC 23282 and *Pseudomonas aeruginosa* ATCC 10145), Yeast (*Candida albicans* IMRU 3669) and Filamentous Fungus (*Aspergillus niger* ATCC 16404). The bacteria and yeast were grown on nutrient agar while the fungus was grown on potatoes dextrose agar (PDA) medium (Balouiri *et al*., 2016).

Antitumor activity

In our study, the pomegranate peels extracts and juice were subjected to cytotoxic assessment on human tumour cell line. Doxorubicin was used in this experiment as a positive control. The title extracts were dissolved in 20%Dimethyl sulphoxide (DMSO) in concentration 1mg/ml. Serial dilutions were made attainment final concentration of the compounds to 6.25, 12.5, 25, 50and100 µg/ml. The tumour cell lines were obtained frozen in liquid nitrogen (-180°C) from the American Type Culture Collection (ATCC) and was maintained at the National Cancer Institute, Cairo, Egypt, by serial subculturing. The antitumor activity was measured *in vitro* on human cancer cell line (MCF-7, HCT116 and HEPG2) using sulphorhodamine -B stain (SRB) assay applying the method of Skehan *et al*., (1990)

RESULTS AND DISCUSSION

The present study includes the estimation of chemical properties of bioactive compounds in ethanolic and aqueous extracts obtained from pomegranate peels and juice In addition, study their antimicrobial and antitumor effect in human cancer cell lines of breast, colon, and liver.

Chemical composition of pomegranate peels: -

Data presented in Table (1) showed that the chemical composition of pomegranate peels based on dry weight (g/100g). The pomegranate peels showed high carbohydrates and fibers content (59.98 and 15.5 g/100g, respectively). While moisture, Ash content and crude fat content were 7.2, 2.7 and 3.5 g/100g, respectively. Our findings were in agreement with (Zaki *et al*., 2015).

Phytochemical screening of pomegranate peels and juice:-

The phytomolecules in pomegranate fruits (peel and juice) are a vital starting point for assessing their nutritional, biological and technological aspects. The results of preliminary phytochemical study are mentioned in Table (2). The qualitative examinations of phytochemical molecules of pomegranate peels and juice under study, revealed the existence of tannins, flavonoids, alkaloids, saponins, terpenes and glycosides.

Table 1. Chemical constituents of pomegranate peels (in dry weight basis).

Plant	Chemical composition (g / 100g)					
	Moisture	Ash	Protein	Fat	Fibers	Carbohydrates*
pomegranate peels	7.2	2.7	11.12	3.5	15.5	59.98

*** Carbohydrates was determined by difference**

Table 2. phytochemical profile of pomegranate peels and juice.

Sample	Tannins	Flavonoids	Alkaloids	Saponins	Terpenes	Glycosides
pomegranatePeels	++	++	+++	++	++	+++
pomegranate Juice	+	++	+	+	+	+++

On the whole, there is a huge difference of the phytochemicals between the pomegranate peels and juice. Pomegranate peels contained all compounds of phytochemicals, i.e. tannins, flavonoids, alkaloids, saponins, terpenes and glycosides higher than in pomegranate juice. It is of awareness to note that the pomegranate peels contained higher amount of alkaloids than that in juice. Conversely, pomegranate fruits (peels and juice) enclosed almost equal quantities of glycosides, flavonoids and terpenes. Saponins and tannins quantity of peels was higher than that in juice. In this respect, Elfalleh *et al*., (2012) suggested that the pomegranate bioactive compounds differed according to type of solvent which used to extract these compounds. Additionally, the phytochemicals of pomegranate parts depends on the pomegranate environmental, type, post harvest and handing out factors (Houston, 2005). The detected phytochemical compounds in pomegranate peels and juice in this study are in agreement with the results obtaindby (Farag *et al*., 2014).

Total polyphenols, total flavonoids and Antioxidant activity of pomegranate peels and juice.

Table (3) showed that total polyphenols, flavonoids contents and antioxidant activity of pomegranate peel and juice under investigation. Antioxidants are substrates that take action as inhibitors of the oxidation process and are found to inhibit oxidant chain reactions at small concentrations and there by eradicate the threat of pathological processes (Nasik, 2003).

Table 3. Total polyphenols, total flavonoids and Antioxidant activity of Pomegranatepeels and juice.

Compounds	Peel	juice
Total polyphenols (TPP) (GAE mg/g dry weight)	61.63	15.93
Total flavonoids (TF)((QE mg/g dry weight)	49.32	25.85
Antioxidant activity DPPH method (IC_{50}, µg/ml)	22.30	16.24

Data obtainable in Table (3) showed clearly that, total phenol content in pomegranate peels (61.63 GAE mg/g) is higher than in juice (15.93 GAE mg/g). Also total flavonoids in peels (49.32QE mg/g) are higher than in juice (25. 85QE mg/g). The antioxidant activity of pomegranate parts were also studied we found that IC_{50} DPPH method for pomegranate peels was 22.30µg/ml, While the IC_{50} for juice was 16.24 µg/ml. Comparable results were obtained by Elfalleh *et al*., (2012) and Farag *et al*., (2014). It has been documented that both flavonoids and phenols showed antioxidant activity through scavenging or chelating process. The free radical scavenging properties determined by DPPH was articulated as the IC_{50} value (the efficient concentration which required inhibiting 50% of the initial DPPH free radical). The IC_{50} values of the 2, 2-diphenyl-1-picrylhydrazyl (DPPH) are shown in Table (3). The pomegranate peels produced higher scavenging activity (22.30) µg/g, while juice set up to be scored (16.24) µg/g. On contrast, Elfalleh *et al*., (2012) information reported that the water extract of pomegranate leave display higher antioxidant action than that of peels extract. Alternatively, Singh *et al*., (2001) reported that peels are a good source of antioxidants. Also, Ardekani *et al*., (2011) found that the antioxidant activity of pomegranate fruits (peels and juice) extracts were 10 times higher than the pulp extract. These findings reinforce our results. These results proved that the antioxidant properties of pomegranate peels containing polyphenol and flavonoids compounds are because of their ability to be donors of hydrogen atoms or electrons and to capture the free radicals. The eating of foods containing high amounts of flavonoids and phenols has been showed to lower the danger of various cancers (Knekt *et al*., 2002). Earlier studies have shown that some flavonoids such as quercetin had anticancer properties and were able to inhibit cancer cell growth (Ranelletti *et al*., 2000). It is worth mentioning that the obtained data suggested that pomegranate peels and juice can be practical basically as food addition to hold back oil oxidation and to alleviate from certain diseases through its free-radicals scavenging property.

Phenolic compounds of pomegranate peels extracts and juice (mg/100g):-

Phenolic compounds in pomegranate peels extracts and juice were identified by HPLC technique and the results were cited in Table (4), which illustrates that pomegranate peels extracts (ethanol and aqueous extract) are rich in substantial quantity of phenolic compounds with greater amount of phenolic compounds in pomegranate peels extracts than pomegranate juice which are confirmed to have constructive bioactivity in several biological functions.

From the data that mentioned in the Table (4) we found that the pomegranate peels and juice have various imperative phenolic compounds that have aptitude to reserve tumor cells and this finding agreed with the results of Sohi *et al*., (2003) who stated that phenolic components as a free radical scavenger and as an inducer of apoptosis in leukemia, lung cancer, and colon cancer cell lines. The obtained data revealed that many polyphenols were detected in pomegranate peels and juice, and the quantities of the detected polyphenols were considerably high, greater quantities of polyphenols compounds were detected in the pomegranate peels aqueous extract.

Table 4. Phenolic compounds content of pomegranate peels extracts and juice (mg/100g)

Phenolic compounds	Test results of phenolic compounds (mg/100g dry weight)		
	peels ethanol extract	peels aqueous extract	juice
Pyrogallol	1002.95	5094.49	115.12
Gallic	80.8941	289.266	1.904
4-Amino-benzoic	38.3268	114.587	1.164
Protocatchuic	88.0764	140.481	6.462
Catechin	654.41	1300.46	42.23
Catechol	12.5549	48.767	1.462
Chlorogenic	37.3761	463.204	14.344
Epicatechin	24.8749	93.256	2.329
P-OH-benzoic	88.1278	359.531	3.991
Caffeine	31.3557	77.855	6.352
Caffeic	11.4951	11.161	0.757
Vanillic	35.8778	205.6	3.379
P-Coumaric	14.2917	38.934	1.084
Ferulic	16.2965	36.882	1.4281
Iso-ferulic	5.3484	14.107	0.627
e-vanillic	475.096	189.593	11.962
Ellagic	--	173.936	3.56624
Alpha-coumaric	103.301	76.3724	--
Benzoic	46.675	42.8574	12.042
3,4,5-methoxy-cinnamic	1.52912	2.50132	0.437
Coumarin	5.37025	3.94398	0.82119
Salycillic	16.0943	41.741	4.46472
Cinnamic	0.34879	0.66587	0.12932

Pyrogallol was predominant in peels aqueous extract, peels ethanol extract and juice 5094.49 mg/100g, 1002.95 mg/100g and 115.12 mg/100g, respectively followed by Catechin 1300.46 mg/100g, 654.41 mg/100g and 42.23 mg/100g, respectively. Other noticeable high amounts phenolic compounds were Chlorogenic, *P*-OH-benzoic and e-vanillic. Other mentioned phenolic substrates with lesser contented than were Iso-ferulic, Coumarin, 3,4,5-methoxy-cinnamic, and Cinnamic which are also functional bioactive phenols that exist pomegranate peels extracts and juice with minor amounts.

Farag *et al*., (2014), who utilized HPLC chromatography for characterization the polyphenolic compounds in pomegranate leaves and peels juices. He found 8 phenolic compounds in pomegranate leaves and thirty polyphenolic compounds were separated from pomegranate peels. The essential compounds found in pomegranate peel and leaves juices were gallic acid, protocatechuic acid, 3-hydroxy tyrosol, respectively.

Flavonoids of pomegranate peels extracts and juice (mg/100g):-

Flavonoids of pomegranate peels and juice under investigation were recognized and quantified by HPLC technique. The results were shown in Table (5). Eighteen flavonoids of pomegranate peel and juice could be identified. Greater quantities of flavonoids were detected in the pomegranate peel aqueous extract. hisperidin was predominant flavonoid 8607.94 , 7446.37 and 122.58 mg/100g for pomegranate peels aqueous extract, pomegranate peels ethanol extract and juice respectively, followed by luteo.6-arbinose 8-glucose 3788.29, 1049.80 and 322.16 mg/100g, naringin768.38, 449.80 and 130.52 mg/100g and acacetin179.39, 130.69 and 66.23 mg/100g for pomegranate peels aqueous extract, pomegranate peels ethanol extract and juice, respectively. Other perceptible high contents flavonoids were quercetin-3-O-glucoside, quercetrin, rutin, naringenin and hespertin. Other detected flavonoids with lesser content than were (kaempferol, quercetin, rhamnetin and apigenin) which are exist in pomegranate peels extracts and juice with minor amounts.

Shadab *et al*., (2017) illustrated that aqueous extract of peels powder showed good antioxidant effect. They also mentioned that phenolic compounds, tannins and flavonoids are the most important phytochemicals present in the pomegranate peels. It appears that there is a positive relationship among the chemical structures of polyphenols and flavonoids moieties in peels and juice of pomegranate fruits and its antioxidant properties. The numbers of OH group and location at the aromatic ring have a deep cause on the antioxidant activity. It is value noting that some researches recognized that chlorogenic acid and flavonoids mainly quercetin and its glycoside derivatives is the main components accountable for the antioxidant activities (Silvia *et al*., 2011).

Table 5. Flavonoids content of pomegranate peels extracts and juice (mg /100g)

Flavonoids	Test results of flavonoids (mg/100g)		
	peels ethanol extract	peels aqueous extract	juice
Luteo.6-arbinose 8-glucose	1049.80	3788.29	322.16
Loteo.6-glucose 8-arbinose	608.20	705.16	17.29
Apig.6-rhamnose 8-glucose	158.20	386.27	54.09
Apig.6-glucose 8-rhamnose	--	386.39	17.74
Naringin	449.80	768.38	130.52
Luteo.7-glucose	396.55	--	--
Hisperidin	7446.37	8607.94	122.58
Rutin	60.49	97.11	13.53
Quercetin-3-O-Glucoside	360.62	398.42	--
Apig.7-O-neohespiroside	167.44	163.70	4.38
Apig.7-glucose	376.19	106.60	11.18
Quercetrin	103.01	192.35	11.02
Quercetin	8.71	8.77	1.42
Kamp.3-(2-p-comaroyl) glucose	--	326.40	11.37
Naringenin	26.75	86.79	4.38
Hespertin	49.82	51.08	13.01
Kaempferol	12.21	26.13	1.48
Rhamnetin	2.98	7.80	1.88
Apigenin	2.23	4.44	1.43
Acacetin	130.69	179.39	66.23

These classes of components have a large variety of biological properties counting radical scavenging properties (Balasnram *et al*., 2006). Overall, this summit needs additional research to explain the effect of individual phenolic compounds and its concentration on the antioxidant activity.

Antimicrobial Activity

Antibacterial activity of pomegranate peels extracts and juice

The antibacterial activities were initially estimated by the agar well-diffusion method. Ampicillin 80µg/disc was used as a positive control. The antibacterial activities of pomegranate peel extracts and juice were assayed against pathogenic bacteria (Gram +ve bacteria (*Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* ATCC 35556), Gram -ve bacteria (*Escherichia coli* ATCC 23282 and *Pseudomonas aeruginosa* ATCC 10145)). The results were citied in Table (6) and Table (7).Table (6) presents diameters of inhibition zones (clear zones around discs) exerted by the various peels extracts (aqueous extract, ethanolic extract) and juice towards the selected microorganisms.

Table 6. Antibacterial activity of pomegranate peels extracts and juice against pathogenic bacteria.

Pomegranate		Conc. (ppm)	Gram +ve bacteria				Gram -ve bacteria			
			Bacillus subtilis		*Staph. aureus*		*Escherichia coli*		*Pseud. aeruginosa*	
			G.D	I%	G.D	I%	G.D	I%	G.D	I%
Peels	Aqueous extract	500	22	21.4	21	25.0	15	44.4	20	31.0
		1000	16	42.8	15	46.4	11	59.2	15	48.2
	Ethanol extract	500	21	25.0	20	28.5	16	40.7	17	41.3
		1000	15	46.4	14	50.0	11	59.2	11	62.0
Juice	Aqueous extract	500	25	10.7	25	10.7	16	40.7	16	44.8
		1000	19	32.1	18	35.7	10	62.9	11	62.0
Control			28		28		27		29	

G.D= growth diameter in mm, I% = Inhibition percentage

As mentioned in Table (6), the pomegranate peels extracts (aqueous and ethanol extracts) and juice exhibited a clear antibacterial activity. It could be seen that, all the examined extracts, at both concentrations (500 and 1000ppm), concealed the mycelial growth of Gram +ve bacteria and Gram -ve bacteria. Predominantly, the peels EtOH extract, at concentration of 1000 ppm, induced the formation of a clear inhibition zone of 15 mm (46.4%), 14 mm (50.0%), 11 mm (59.2%) and 11 mm (62.0%) for *Bacillus subtilis*, *Staph. aureus*, *E. coli* and *Pseud. aeruginosa* respectively. In a different way, the pomegranate juice (aqueous extract), at the same contraction of 1000 ppm, demonstrated a higher antibacterial effect against*E. coli*, forming an inhibition zone of 10 mm (62.9%).

The antibacterial activity of pomegranate peels ethanol extract against *Salmonella* strains was studied by Choi *et al*. 2011 and Wafa Ben Ajmia *et al*., 2017.

In general, these results evidently indicate that the peel extracts and juice of pomegranate are capable, *in vitro*, to efficient irritate the growth both of gram-negative bacteria, for example *E. coli*, and even to a better amount that of gram-positive bacteria, such as B. subtilis (Al-Zoreky 2009). These findings are similar with those freshly obtained in numerous studies on the antimicrobial activity of pomegranate fruits extracts (Braga *et al*., 2005; Priya *et al*., 2012).

The absolute effectiveness of antibacterial activities depends on a number of parameters plus extraction method, seasonality and geographical source of pomegranate fruits (Zografou *et al*., 2013).

Antimicrobial activity of pomegranate peels extracts and juice against pathogenic fungi and yeast:

Using the chemical fungicides is related with some problems such as toxicity, fungal resistance, widespread environmental hazards and high cost (Obagwu and Korsten 2003). Extensive efforts have been intended for screening plants to facilitate extract and separate new natural fungicides

Consequently, the pomegranate peels extracts and juice showed that a significant *in vitro* antifungal activity against *Aspergillus niger* and *Candida albicans*. The antimicrobial activities against pathogenic fungi (*Aspergillus niger)* and yeast (*Candida albicans*) were tested by the agar well diffusion method. The obtained results are mentioned in Table (7).

Table 7. Antimicrobial activity of pomegranate peels extracts and juice against yeast and pathogenicfungi.

Pomegranate		Conc. (ppm)	Yeast		Fungus	
			Candida albicans		*Aspergillus niger*	
			G.D	I%	G.D	I%
Peels	Aqueous extract	500	19	26.9	21	22.2
		1000	14	46.1	16	40.7
	Ethanol extract	500	18	30.7	20	25.9
		1000	13	50.0	15	44.4
Juice	Aqueous extract	500	24	7.69	25	7.40
		1000	18	30.7	19	29.6
Control			26		27	

G.D= growth diameter in mm, I% = Inhibition percentage

As mentioned in Table (7), the pomegranate peels extracts (aqueous and ethanol extracts) and peels-AgNPs exhibited a good antifungal activity at both concentrations (500 and 1000ppm). Principally, pomegranate peels (EtOH extract), at concentration of 1000 ppm, induced the formation of a clear inhibition zone of 13 mm (50.0%) and 15 mm (44.4%) for *Candida albicans and Aspergillus niger* respectively. The extracts, that were rich in tannins, had a wonderful antimicrobial activity against Candida species (Sulaiman *et al*., 2011). Again, we observed that pomegranate peels and juice extracts were more active against moulds and yeasts. These results were similar with the obtained results by Hayouni *et al*., (2011); Anibal *et al*., (2013) and Yang *et al*., (2016). They stated that the ethanol and methanol extracts of *P. granatum* L. were further appropriate to inhibit *Candida* species and *A. niger*. Data herein showed that both pomegranate peels extracts and juice contain an efficient antimicrobial activity, as demonstrated by the inhibitory effect on growth of pathogenic bacteria (Gram +ve bacteria and Gram -ve bacteria) and mycelial growth of *Aspergillusniger and Candida albicans*.

As revealed through the results of the Shan *et al*., (2007) the antimicrobial activities of pomegranate peels and juice are interrelated to the total polyphenol content 61.63 and 15.93 GAE mg/g dry weight respectively, as pointed out in Table(3).

The mechanism accountable for polyphenols activity to microorganisms was corresponded to reaction with sulfhydryl groups of proteins and deficiency of substrates to microorganism (Naz *et al*., 2007). As indicated in the results of antimicrobial activities against pathogenic bacteria, fungi and yeast we noticed that peels ethanol extract produced the great estinhibitionzone compared with juice. So, pomegranate peels extracts and juice have a potent inhibitory effect against gram negative, gram positive bacteria, fungi and yeast.

In vitro Cytotoxicity assay

The cytotoxicity of pomegranate peels extracts and juice on the viability of breast cancer (MCF-7), liver cancer (HEPG-2) and colon cancer (HCT116) cancer cell lines were evaluated using the sulphorhodamine (SRB) bioassay. Cancer cell lines were exposed to different concentration of pomegranate peels extracts and juice (6.25, 12.5, 25, 50, 100 µg/ ml).The results were summarized in Table (8), Table (9) and Table (10).

Cytotoxicity of pomegranate peels extracts and juice on breast cancer MCF-7 Cell lines

Activity of breast cancer MCF-7 cell lines and mortality ratio were expressed as the percentage of endurance cells exposed to pomegranate extracts compared to the activity observed in the negative control for each concentration.

From the mentioned results in Table (8), it was observed that the cytotoxicity of pomegranate peel extracts and juice on breast cancer (MCF-7), varies in a dose-dependent manner.

The minimum inhibiting effect was observed with 6.25µg/ ml concentration and there is steady increase in the inhibition effect against cell viability with the increase in the dose. The highest mortality percent at the concentration of 100µg/ml were shown (88.43%, 81.92%) for pomegranate peels EtOH extract and peels aqueous extract.

Table 8. In vitro Cytotoxicity of pomegranate peels extracts and juice on breast cancer MCF-7 Cell lines, (µg/ ml).

Extract		Concentration µg/ ml	In vitro Cytotoxicity on breast cancer (MCF-7 Cell lines)		
			Viability %	Mortality %	IC_{50} (µg/ml)
	control	0	100	0	
Peels	Aqueous extract	6.25	83.06	16.94	32.98±1.48
		12.5	61.83	38.17	
		25	53.16	46.84	
		50	35.04	64.96	
		100	18.08	81.92	
	EtOH extract	6.25	81.50	18.50	20.61±0.16
		12.5	60.70	39.30	
		25	44.16	55.84	
		50	31.02	68.98	
		100	11.57	88.43	
Juice	Aqueous extract	6.25	89.64	10.36	39.76±1.57
		12.5	68.21	31.79	
		25	58.66	41.34	
		50	37.87	62.13	
		100	19.23	80.77	

The maximum inhibition effects were revealed in IC_{50}= 20.61µg/ ml for the peels EtOH extract. In this study the result of *in vitro* studies, was established that pomegranate peels extracts and juice inhibit cancer cell growth. While the previous reported results by Toi *et al*., (2003) found that polyphenols from fermented pomegranate juice, peels and oil were revealed to effect a obstruct of endogenous active estrogen biosynthesis with successive inhibition of aromatase activity. Also our results were agreement with the finding obtained by Kim *et al*., (2002), who proved that pomegranate seeds oil inhibited propagation of MCF-7 cells, and estrogen receptor unconstructive metastatic human breast cancer cells due to higher content of polyphenols.

Cytotoxicity of pomegranate peels extracts and juice on liver cancer HEPG-2 Cell lines

Accomplish with different concentrations of pomegranate peel extracts and juice showed that a significant changeable cytotoxicity against liver cancer HEPG-2 cell lines compared to negative control cells Table (9).

The capability of cancer cells was negatively associated to test extract concentration. Pomegranate peels extracts and juice showed that superior inhibition effect on cancer cell viability at all tasted concentration which could be because of its superior content of phenolic components that have anti-cancer effect on liver cancer cells during the negative effect on cancer cell. A analogous effect of cytotoxicity was marked at level of (100µg/ml) as mortality percent was (89.67%, 89.44%) for peels EtOH extract and juice (a queous extract), respectivly. The uppermost inhibition effects were shown in IC_{50}=12.52µg/ml for the pomegranate peels EtOH extract followed by juice IC_{50}=14.84µg/ ml.

Interestingly, the results illustrated that pomegranate peels extracts and juice were approximately similar in their effect on mortality percentage against breast cancer (MCF-7) and liver cancer (HEPG-2) cell lines. The effect of pomegranate (peels and juice) expected that antitumor effect on breast cancer (MCF-7) and liver cancer (HEPG-2) cell lines functions by inhibiting the growth of cancer cells.

Table 9. In vitro Cytotoxicity of pomegranate peels extracts and juice on liver cancer HEPG-2 Cell lines,(µg/ ml)

Extract		Concentration µg/ ml	In vitro Cytotoxicity on liver cancer (HEPG-2 Cell lines)		
			Viability %	Mortality %	IC_{50} (µg/ml)
	control	0	100	0	
Peels	Aqueous extract	6.25	80.65	19.35	18.96±1.64
		12.5	57.43	42.57	
		25	43.21	56.79	
		50	27.41	72.59	
		100	11.65	88.35	
	EtOH extract	6.25	77.54	22.46	12.52±1.90
		12.5	53.54	46.46	
		25	37.13	62.87	
		50	24.39	75.61	
		100	10.33	89.67	
Juice	Aqueous extract	6.25	71.72	28.28	14.84±0.26
		12.5	49.35	50.65	
		25	31.99	68.01	
		50	21.19	78.81	
		100	10.56	89.44	

Cytotoxicity of pomegranate peels extracts and juice on colon cancer HCT 116 Cell lines (µg/ ml).

Handling of colon cancer by means of special concentrations of pomegranate peels extracts and juice showed that a considerably variable cytotoxicity against colon cancer cells compared to negative delivery cells. The capability ratio was known as a percentage compared to negative control for every concentration. The deadly consequence was absolutely interrelated with concentration of pomegranate peels extracts and juice. The lowest inhibition was established to be 6.25µg/ml concentration with a regular increase in the inhibition effect against cell viability as the concentration increases, which is evidently illustrated in Table (10).

Table 10. In vitro cytotoxicity of pomegranate peels extracts and juice on colon cancer HCT 116 Cell lines,(µg/ ml).

Extract		Concentration µg/ ml	In vitro Cytotoxicity on colon cancer (HCT 116 Cell lines)		
			Viability %	Mortality %	IC_{50} (µg/ml)
	control	0	100	0	
Peels	Aqueous extract	6.25	76.57	23.43	19.22±0.13
		12.5	57.71	42.29	
		25	47.21	52.79	
		50	25.59	74.41	
		100	15.96	84.41	
	EtOH extract	6.25	64.71	35.29	12.51±1.64
		12.5	48.67	51.33	
		25	37.36	62.64	
		50	24.12	75.88	
		100	9.37	90.63	
Juice	Aqueous extract	6.25	68.21	31.79	16.12±1.28
		12.5	54.75	45.25	
		25	45.86	54.14	
		50	25.41	74.59	
		100	11.95	88.05	

The cytotoxicity of pomegranate peels EtOH and juice (aqueous extract) at concentration of (50µg/ml) leads to mortality percentage of 75.88% and 74.59% respectively, at the same time as concentration of 100µg/ml leads to mortality percentage 90.63% and 88.05% respectively, on colon cancer HCT 116 Cell lines.

For the majority part energetic concentration of pomegranate peels EtOH in reducing activity of cancer cells (HCT 116 Cells) IC_{50}= 12.51µg/ml for pomegranate peels EtOH extract followed by 16.12µg/ml for pomegranate juice respectively.

Generally, we report that pomegranate extracts (peels and juice) have been showing to inhibit cell growth in a multiplicity of human cancer cell lines. Our findings were in agreement with results obtained by Malik *et al*., (2005). He established that pomegranate fruits extract inhibits cell growth in PC3 cell lines in a dose-dependent manner. Lately, Kahn *et al*., (2007) also incorrigible that pomegranate fruits extract inhibits cell growth in A549 human lung carcinoma cells and HCT116 human colon cancer cells. In addition, oral eating of pomegranate fruit extract inhibits the growth and sequence of lung carcinoma in mice after chemically-induced tumor initiation (Khan *et al*., 2007, Seeram *et al*., 2005), these data also propose that mixture therapy of pomegranate extracts with other therapeutics may have beneficial effects in the treatment of tumors.

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التركيب الكيميائي لفاكهة الرمان وتأثيرها كمضاد للبكتيريا ومضاد للسرطان

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للرمان العديد من الفوائد الصحية المختلفة. وقد أوضحت الدراسات السابقة بأنه غني بالمواد الكيميائية الفعالة مثل الفلافونيدات والمركبات الفينولية. هذه الدراسة تهدف الي التعرف على المركبات الفعالة في قشور الرمان والعصير وتأثيرها كمضادات للبكتيريا والسرطان. تمت الدراسة على قشورالرمان والعصير وتم التقدير الكمي للفينولات والفلافونيدات الكلية والتعرف عليها باستخدام التحليل الكروماتوجرافي عالي الكفاءة (HPLC). وتم كذلك تقدير النشاط المضاد للسرطان وأيضاً النشاط المضاد للبكتيريا وتقدير نشاط مضادات الأكسدة. وأوضحت نتائج الدراسة أن كلا من المستخلص الإيثانولي والمائي لعصير وقشورالرمان لهما نشاط مضاد للميكروبات وكذلك نشاط مضاد للسرطان.ومن نتائج التحليل الكيميائي يتضح أن قشورالرمان أفضل من العصيرفي محتواه من المركبات الفينولية بنسبة ٦١.٦٣ مجم/جم قدرت في العصير بنسبة ١٥.٩٣ مجم/جم ، الفينولات بنسبة ٤٩.٣ مجم/جم بينما العصير يحتوي على الفينولات بنسبة ٢٥.٨٥ مجم/جم. كذلك أوضحت النتائج أيضاً ان مستخلص القشور له نشاط مضاد للبكتيريا أفضل من المستخلص المائي للعصيرعند تركيز ١٠٠٠ جزءفي المليون ppm. وأثبت ايضا ان مستخلص قشور الرمان والعصير لهم نشاط عالي كمضادات للبكتريا والفطريات والخمائر ونشاط ضد الخلايا السرطانية. وتوصي الدراسة بأن ثمار ومستخلص الرمان يمكن الإستفادة منها في علاج الأورام السرطانية وضد النموات البكتيرية والفطرية وكذلك تستخدم كمضادات للأكسدة لإحتواء الرمان على المواد الطبيعية الفعالة مثل الفينولات والفلافونيدات.