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ANTIOXIDANT ACTIVITY OF SOME EXTRACTS FROM GAMMA IRRADIATED POMEGRANATE (*Punica granatum* L.) PEEL AND SEED

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ABSTRACT

The present study aims to evaluate the antioxidant activity of pomegranate *(Punica granatum* L.) peel and seed (obtained as waste from juice extraction) using different solvents as diethyl ether, ethyl acetate, ethanol 50%, ethanol 80%, methanol 50%, methanol 80% and distilled water. The measurements of the antioxidant activity of all extracts were carried out using a radical scavenging activity against 2,2′-diphenyl-1-picrylhydrazyl (DPPH), β-Carotene/linoleic acid bleaching and ferric reducing antioxidant power (FRAP). Moreover, the effect of gamma irradiation at dose levels of 3, 6 and 9 kGy on antioxidant activity of the best pomegranate peel and seed samples that possessed highest antioxidant activity was investigated. Results showed that ethanolic 50% peel extract had a higher total phenolic contents(TPC) and total flavonoid contents (TFC) in both peel and seed, (9323.17 mg gallic acid equivalent (GAE) 100 g^{-1}, 2998.05 mg quercetin equivalent (QE) 100 g^{-1} and 352.09 mgGAE100 g^{-1},106.78 mg QE 100 g^{-1}dry weight (DW), respectively than other extracts. Ethanolic 50% extracts showed higher antioxidant activity than other peel and seed extracts. In addition, ethanolic 50% extract of irradiated pomegranate peel and seed at dose level of 6 kGy extract had higher TPC, TFC and antioxidant activity compared to other doses. Thus, ethanolic 50% extract of irradiated pomegranate peel and seed at 6 kGy may be considered as a good source of natural compounds with antioxidant activity which could be suitable as potential ingredient for food products.

Key words: Pomegranate, peel, seed, phenolic compound, antioxidant activity.

INTRODUCTION

The pomegranate (*Punic granatum* L.) belongs to the family Punicaceae. The edible part of the pomegranate fruit (50%) consists of 40% arils and 10% seeds. Arils contain 85% water, 10% total sugars, mainly fructose and glucose, and 1.5% pectin, organic acids such as ascorbic acid, citric acid, and malic acid, and bioactive compounds such as phenolics and anthocyanins (Viuda-Martos *et al.*, 2010).

Once the juice has been extracted, the wastes that remain are composed mainly of pulp and bagasses. Uses for these by-products are scarce and their disposal represents a problem. One way of avoiding this problem would be to re-use the pomegranate bagasses obtained from the pomegranate industries to take advantage of the large quantity of potentially beneficial compounds such as dietary fibre or bioactive compounds mainly phenolic acids and flavonoids which could be used as ingredient in food processing.

Pomegranate husk, a major by-product of the pomegranate juice industry, is rich in ellagitannins which are endowed with potent anti-inflammatory, anti-cancer and anti-atherosclerotic biological properties (Lee *et al.*, 2010). Hence the utilization of pomegranate husk is quite necessary. Hydro alcoholic extracts of pomegranate peels showed anti-diabetic activities by significantly reducing blood glucose level of normal and diabetic rats (Gautam and Sharma, 2012). Synthetic

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antioxidants such as butylated hydroxyl anisole (BHA), butylated hydroxyl toluene (BHT) and tert-buty hydro-quinone (TBHQ) are widely used in the food industry as potential inhibitors of lipid oxidation (Scherer and Teixeira-Godoy, 2009). Nevertheless, toxicological effects together with consumer preference for natural products have resulted in increased interest in the search and use of natural antioxidants in fruits and vegetables that might help attenuate oxidative processes (Viuda-Martos *et al.*, 2010).

Pomegranate fruit and its derivate products have been shown to be effective in retarding the process of lipid oxidation in both *in vitro* and *in vivo* assays (Devatkal and Naveena, 2010). The antioxidant activity of pomegranates depend on the part of the fruit used. Tzulker *et al.* (2007), reported that the homogenates prepared from the whole fruit exhibited an approximately 20-fold higher antioxidant activity than the level found in the aril juice. Li *et al.* (2006) reported that pomegranate peel had the highest antioxidant activity among the peel, pulp and seed fractions of 28 kinds of fruits commonly consumed in China.

On the other hand, gamma irradiation (10 kGy) increased phenolic acid content in cinnamon and clove while phenolic content in nutmeg remained unaltered (Variyar *et al.*, 1998). Although, some studies report that, gamma irradiation does maintain or enhance the antioxidant properties, there are a few examples wherein the antioxidant properties of the plant material were decreased (Alothman *et al.*, 2009).

Therefore, the aim of this work was to determine the total phenol (TPF), the total flavonoid (TFC) and antioxidant activity of pomegranate peel and seed extracts by using different solvents. Meanwhile, use of different test measurements to investigate the antioxidant activity of samples under investigation. Also, investigate the effect of gamma irradiation on antioxidant activity of pomegranate peel and seed samples which showed best solvent extract.

MATERIALS AND METHODS

Materials

Mature pomegranate fruits having no visible external cuts or spoilage were purchased from the local market, Zagazig, Egypt. The pomegranate fruits were peeled manually into two parts: the arils was the first one introduced in a blender to obtain juice and seeds were separated. The second part was the peel. Both of two parts were rinsed by distilled water then dried in oven at 45°C for 72 hr then ground to a fine powder in a coffee grinder for 2 min., at 15 sec. intervals, the process was stopped for 15 sec. to avoid heating of sample .

Tert-butyl hydroquinone (TBHQ), butylated hydroxyl tuluen (BHT) 1,1-Diphenyl-2-picrylhydrazyl (DPPH), β-carotene, gallic acid, quercetin, iron (III) chloride and aluminium chloride were purchased from Sigma (St. Louis, MO, USA). All other used chemicals were analytical grade.

Preparation of Plant Extract

Dry peel powder (20 g)were extracted individually into200 ml of diethyl ether, ethyl acetate, ethanol 50%, ethanol 80%, methanol 50%, methanol 80% and distilled water after soaking in hexane to remove fatty materials. The seed powder was extracted in a Soxhlet apparatus with hexane (100 mL) for 8 hr for the removal of fatty matter. The hexane extract was discarded, and residues were successively extracted individually into 200 ml of diethyl ether, ethyl acetate, ethanol 50%, ethanol 80%, methanol 50%, methanol 80% and distilled water. All extracts were shaked at room temperature overnight at a speed of 1000 vibration/min. The extracts were filtered through Whatman filter paper No. 42 and residue was extracted again with 100 ml of respective solvent to ensure the complete extraction of phenolic compounds. Filtrate was subjected to rotary evaporator at 45°C under reduced pressure to remove solvent. The extracts were stored at -18 °C to further analyses.

Determination of Total Phenolics Content (TPC)

Total phenolic content was determined according to Saeedeh and Asna (2007).Aliquot of extract solution (20 µL)was mixed with 1.16 mL of distilled water and 100 µL of Folin–Ciocalteu's reagent followed by 300 µL of200 g L^{-1} Na_2CO_3 solution. The mixture was incubated in a shaking incubator at 40 °C for30min and its absorbance was measured at 760 nm. Gallic acid

was used as standard for the calibration curve. Total phenolic content expressed as gallic acid equivalent (GAE) was calculated using the following linear equation based on the calibration curve:

$$y = 0.0045x + 0.0743 \ (R^2 = 0.9944)$$

Where:

y is the absorbance and x is the concentration (mg GAE g^{-1} extract) R^2=Correlation Coefficient.

Determination of Total Flavonoids Content (TFC)

Total flavonoid content was determined by the method of Ordon *et al.* (2006). A aliquot (0.5mL) of 20 gL^{-1} $AlCl_3$ ethanolic solution was added to 0.5 mL of extracted solution. After 1 hr at room temperature. The absorbance was measured at 420 nm. A yellow colour indicated the presence of flavonoids. Extracted samples were evaluated at a final concentration of 0.1 mg mL^{-1}. Total flavonoid content was expressed as quercetin equivalent (QE) and calculated using the following equation based on the calibration curve:

$$y = 0.0072 \ x \ (R^2 = 0.9853)$$

Where:

y is the concentration (mg QE g^{-1} extract), x is the absorbance and R^2=Correlation Coefficient.

Antioxidant Activity of Extracts

Because of the differences among the various test systems available, the results of a single method can provide only a limited assessment of the antioxidant properties of a substance. For this reason, the antioxidant capacity of each extract was determined through three complementary assay procedures.

DPPH Radical-scavenging Activity

The electron donation ability of the obtained extracts was measured by bleaching of the purple colored solution of DPPH according to the method of Hanato *et al.* (1988). One hundred µL of each extracts was added to 3 mL of 0.1 mM DPPH dissolved in toluene, ethyl acetate, ethanol and methanol according to the solvent used for extraction. After incubation period of 0, 30 and 60 min at room temperature, the absorbance was determined against a control at 517 nm (Gulcin *et al.*, 2004). Percentage of antioxidant activity of free radical DPPH was calculated as follow:

Antioxidant activity (Inhibition) % = $[(A_{control} - A_{sample})/A_{control}] \times 100$

Where:

$A_{control}$ is the absorbance of the control reaction and A_{sample} is the absorbance in the presence of plant extract. BHT was used as a positive control. Samples were analyzed in triplicate.

β-Carotene/linoleic Acid Bleaching(βCB)

The ability of extracts and synthetic antioxidants to prevent the bleaching of β-carotene was assessed as described by Keyvan *et al.* (2007). In brief, 0.2 mg of β-carotene in 1 mL of chloroform, 20 mg of linoleic acid and 200 mg of Tween 20 were placed in a round-bottom flask. After removal of the chloroform, 50 mL of distilled water were added and the resulting mixture was stirred vigorously. Aliquots (3 mL) of the emulsion were transferred to test tubes containing extract or synthetic antioxidant. Immediately after mixing 0.5 mL of extract solution, an aliquot from each tube was transferred to a cuvette and the absorbance at 470 nm was recorded (Abs^0). The remaining samples were placed in a water bath at 50 °C for 2 hr, then the absorbance at 470 nm was recorded (Abs^{120}). A control with no added extract was also analyzed. Antioxidant activity was calculated as follows:

Antioxidant activity (%) = $[1 - (Abs^0_{sample} - Abs^{120}_{sample})/(Abs^0_{control} - Abs^{120}_{control})] \times 100$

Where:

Abs^0_{sample} is the absorbance of sample at 0-time, Abs^{120}_{sample} is the absorbance after 120 min, $Abs^0_{control}$ is absorbance of control at 0-time and $Abs^{120}_{control}$ is the absorbance of control after 120 min.

Ferric Reducing Antioxidant Power (FRAP)

Reducing power of all extracts was measured by method of Oyaizu (1986) and modified by (Gülçin *et al.*, 2010). The reduction of $Fe^{+3}$to Fe^{+2}wasdetermined by measuring absorbance of the Perl's Prussian blue complex. This method is based on the reduction of (Fe^{+3}) ferricyanide in stoichiometric excess relative to the antioxidants. For this purpose, 0.1 mL of each

extracts mixed with 1 ml of 0.2 M sodium phosphate buffer (pH6.6) and 1 ml (1%) of potassium ferricyanide [$K_3Fe(CN_6)$]. The mixture was incubated at 50°C. After 20 min of incubation, the reaction mixture was acidified with 1 ml of trichloroacetic acid (10%). Finally, 0.25 ml of $FeCl_3$ (0.1%) was added to this solution. Distilled water was used as blank and for control. The absorbance of the mixture was measured at 700 nm using a UV spectrophotometer. Decreased absorbance indicates ferric reducing power capability of sample.

Irradiation Treatment

Pomegranate peel and seed powder were packed in polyethylene bags (25g peel or seed/ bag) then irradiated using a ^{60}Co Russian gamma chamber, (dose rate 1.3 kGy/hr), belonging to Cyclotron Project, Nuclear Research Center, Atomic Energy Authority, Cairo, Egypt. The applied radiation doses were 3, 6 and 9 kGy.

Characterization of Phenolic Compounds by HPLC

The liquid chromatographic system consisted of a Shimadzu LC-6A model, fitted with a Waters-Bondapack C_{18}column (250 x 4.6 mm i.d.) and an SCL-6A system controller. The injection system used wasa20µL sample loop. Detection was done by a UV-visible spectrophotometer SPD- 6AV set at a sensitivity of 0.04 AUFS and a wavelength of 280 nm. Elution was carried out at a flow rate of 0.7 mL/min under a linear gradient of acetonitrile (solvent A) and 0.3% phosphoric acid (solvent B) from 10% A to 20% A in 45 min and then to 60% A in 20 min. The pomegranate peel extracts were dissolved in a mixture of methanol and water (6:4 v/v), and 20 µL was injected into the HPLC. The compounds were quantified using a Shimadzu C-R4A Chromatopak data processor at a chart speed of 2.5 mm/min (Singh *et al.,* 2002).

RESULTS AND DISCUSSION

Total Phenolic Content (TPC) as Affected by Used Solvent

The results in Table 1 presented that the amount of total phenolic compounds varied in the different extracts, ranging from 1408.00 to 9323.17 mg GAE 100 g^{-1}DW for pomegranate peel and 030.70 to352.09 mg GAE 100 g^{-1} DW in pomegranate seed extracts.

TPC of peel was higher than those of seed in all solvent extracts because peel tissues usually contain larger amounts of phenolic compound than seeds, Li *et al.* (2006) revealed that peel tissues usually contain larger amounts of phenolics than pulp. Moreover, ethanol 50% was better than the other solvents. In both of peel and seed at extracting phenolic compounds owing to their higher polarity and good solubility for phenolic components from plant materials (Wieland *et al.*, 2006). The lower-polarity solvents, particularly diethyl ether, ethyl acetate showed much lower ability to extract phenolic compounds compared with the higher-polarity solvents. The phenolic and flavonoids contents depend on the cultivar, growing region, climate, maturity, cultivation practice, storage conditions and method used to obtain the juice (Poyrazoglu *et al.*, 2002). These compounds are known for their properties to scavenge free radicals and to inhibit lipid oxidation *in vitro* (Noda *et al.*, 2002).

Total Flavonoids Content (TFC)

Table 1 shows the flavonoid content of peel and seed extracts. Because antioxidant activity does not always correlate with the presence of large quantities of polyphenolic compounds, the flavonoids data need to be examined. Flavonoids possess a broad spectrum of chemical and biological activities, including radical-scavenging properties. Peel extracts had the highest total flavonoid content, with values ranging from 0041.06 (diethyl ether extract) to 2998.05 mg QE $100g^{-1}$ DW (ethanol 50%) followed by seed from 012.61 (ethyl acetate) to 106.78 mg QE 100 g^{-1} DW (ethanol 50%), depending on the solvent. The highest flavonoid contents in both peel and seed were observed in extracts with ethanol 50%, while the lowest level were found in extracts with diethyl ether in peel and ethyl acetate in seed. A previous report showed that flavonoids represent the main group of phenolic compounds in white onion (Yang *et al.*, 2004).

Table 1. Total phenolic (TPC) and flavonoid (TFC) contents of pomegranate peel and seed extracted using different solvents

Solvent extracts	TPC (mg GAE 100 g^{-1} DW)		TFC (mg QE 100 g^{-1} DW)	
	Peel	Seed	Peel	Seed
Diethyl ether	1408.00	090.44	041.06	037.85
Ethyl acetate	8486.85	030.70	2161.73	012.61
Ethanol 50%	9323.17	352.09	2998.05	106.78
Ethanol 80%	5529.86	309.08	1661.44	092.21
Methanol 50%	8534.64	252.92	2467.89	080.57
Methanol 80%	7459.37	233.81	1926.52	048.53
Distilled water	3062.72	144.20	1523.29	040.77

Antioxidant Activity of Extracts

There are substantial differences in sample preparation techniques, antioxidants extraction methods (solvent, temperature, *etc.*), the selection of end-points and the expression of results even within the same method, so that comparison between the values reported by different laboratories can be quite difficult (Pérez-Jiménez *et al.*, 2008). The antioxidant activity of pomegranate components has been the subject of many studies, most conducted *in vitro* and *in vivo* (Cam *et al.*, 2009). All these activities may be related to the diverse phenolic compounds present in pomegranate, including punicalagin isomers, ellagic acid derivatives, and anthocyanins (delphinidin, cyaniding, pelargonidin 3-glucosides and 3,5-diglucosides). These compounds are known for their properties to scavenge free radicals and to inhibit lipid oxidation *in vitro* (Noda *et al.*, 2002). As mentioned by Huang *et al.* (2005) no single method is adequate for evaluating the antioxidant capacity of foods or extracts, since different methods can yield widely diverging results. Thus, several methods based on different mechanisms should be used.

DPPH Radical-Scavenging Activity

The DPPH radical is considered to be a model for a lipophilic radical. A chain in lipophilic radicals is initiated by the lipid autoxidation. DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al.*, 1997). The reduction capability of DPPH is determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. Positive DPPH test suggests that samples are free radical scavengers. The scavenging effect of peel, seed extracts and BHT on DPPH radical was compared (Table 2). All peel and seed extracts exhibited antioxidant activity. All extracts that contained high amount of total phenolic compounds particularly all peel extracts showed relatively high antioxidant activity when compared with that of BHT. In contrast, the ethanolic extract of seed possessed higher scavenging capacity on DPPH radical between all seed extracts. It showed lower scavenging effect compared with BHT. In general, peel ethanolic extract had the highest antioxidant activity (91.40% after 60 min.) compared with other peel extracts. While, all seed extracts which ranged from 29.18% (diethyl ether) to 57.22% (ethanol 50%) and BHT (61.97%) after 60 minute. It has been proven that the antioxidant activity of peel and seed extracts is mainly ascribable to the concentration of phenolic compounds (Heim *et al.*, 2002).

Components within the extracts are capable of scavenging free radicals via electron- or hydrogen-donating mechanisms and thus should be able to prevent the initiation of deleterious free radical-mediated chain reactions in susceptible matrices, *e.g.* biological membranes.

Table 2. DPPH radical scavenging activity of pomegranate peel and seed extracts by using of different solvents

Solvent extracts	(%) of DPPH radical scavenging activity					
	Peel			Seed		
	Time (min.)			Time (min.)		
	0	30	60	0	30	60
Diethyl ether	7.50	10.64	15.55	16.82	22.99	29.18
Ethyl acetate	86.48	88.82	90.78	07.97	12.02	52.09
Ethanol 50%	88.02	91.13	91.40	29.96	42.36	57.22
Ethanol 80%	77.71	89.71	91.05	23.05	41.88	43.91
Methanol 50%	86.21	89.98	90.87	18.96	36.73	38.30
Methanol 80%	86.84	87.94	90.78	13.42	29.48	31.08
Distilled water	70.38	89.27	90.96	9.14	19.08	35.26
BHT(200ppm)	25.09	48.56	61.97	25.09	48.56	61.97

This further shows the capability of the extracts to scavenge different free radicals in different systems, indicating that they may be useful therapeutic agents for treating radical-related pathological damage. The extract is capable of scavenging free radicals, thus preventing the initiation and propagation of free-radical-mediated chain reactions. This can be beneficial in the preservation of foodstuffs, drug products and cosmetics, where free radical-mediated chain reactions result in lipid oxidation and subsequent deterioration of the products (Dastmalchi *et al.*, 2008).

β-Carotene/Linoleic Acid Bleaching (βCB) Assay

In this assay, oxidation of linoleic acid produces hydroperoxide derived free radicals that attack the chromophore of β-carotene, resulting in bleaching of the reaction emulsion. An extract capable of retarding/inhibiting the oxidation of β-carotene may be described as a free radical scavenger and primary antioxidant (Liyana-Pathirana and Shahidi 2006). Effect of peel and seed extracts on oxidation of β-carotene/ linoleic acid at 50°C is shown in Table 3. The presence of antioxidants in the peel and seed extracts reduced the oxidation of β-carotene. There were differences between extracts and BHT. Thus, the degradation rate of β-carotene depends on the antioxidant activity of the extracts and the phenolic compound rich extracts of peel and seed exhibited antioxidant activity in a β-carotene/linoleate model system. Table 3 reveals that there were differences between the antioxidant activities of these extracts.

It is clear that ethanolic 50% extract performed better in reducing the oxidation of β-carotene than other extracts. All peel and seed extracts were capable for inhibiting the bleaching of β-carotene by scavenging linoleate-derived free radicals. Variations between extraction were observed which showed that ethanolic 50% extract was the highest one (70.01% and 48.00%) in both of peel and seed extracts, respectively. In addition, ethanolic 50% extract of peel showed bleaching of β-carotene more than those of all seed extracts and BHT which have bleaching of β-carotene more than all seed extracts. These observation agree with those of Singh *et al.* (2002). There was no correlation between the degradation rate and the bleaching of β-carotene, in other words, no correlation between TPC, and βCB. This may be due to the different types of antioxidants that were assayed by the two methods. Where TPC gave an indication of the levels of both lipophilic and hydrophilic compounds. In contrast βCB, only gives an indication of the levels of lipophilic compounds (Chew *et al.*, 2008). Many studies showed that there were no correlation between TPC and βCB (Mariod *et al.*, 2006).

Table 3. Antioxidant activity of pomegranate peel and seed extracts using different solvents in β- carotene/ linoleic acid system

Solvent extracts	Antioxidant activity (%)	
	Peel	Seed
Diethyl ether	20.96	32.99
Ethyl acetate	63.19	23.46
Ethanol 50%	70.01	48.00
Ethanol 80%	47.80	38.41
Methanol 50%	54.69	40.02
Methanol 80%	59.09	43.98
Distilled water	47.50	34.75
BHT(200ppm)	60.85	60.85

Ferric Reducing Antioxidant Power (FRAP)

Antioxidant compounds cause the reduction of ferric (Fe^{+3}) form to the ferrous (Fe^{+2}) form because of their reductive capabilities. Prussian blue-colored complex is formed by adding to the ferrous (Fe^{+2}) form. Therefore, reduction can be determined by measuring the formation of Perl's Prussian blue at 700 nm (Chang *et al.*, 2002).

In this assay, yellow colour of the test solution changes to green or blue colour depending on the reducing power of antioxidant samples. A higher absorbance indicates a higher ferric reducing power.

As shown in Table 4 ethanolic peel extracts showed as increase in ferric reducing power compared with other extracts. It reached 2.676 while it was 1.033 in methanolic 50% extract. All peel extracts were higher than BHT (1.019). On the other hand, seed ethanolic 50% extract was the highest (1.681) compared to the lowest which was ethyl acetate extract (1.046). BHT was lower than all seed extractions of ferric reducing power (1.019) (Table 4).

According to the results of the present study, both ferric reducing power and total phenolic content of peel ethanolic 50% extract were higher than those of all extracts. Total phenolic content and ferric reducing power were related with each other. Fe (III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant action (Dorman *et al.*, 2003). The extracts that contained high amount of total phenolic compounds showed relatively high antioxidant activity (Table 1). It has been proven that ferric reducing power of plant extracts is mainly ascribable to the concentration of phenolic compounds in the plant (Heim *et al.*, 2002).

In all previous used methods for the determination of antioxidant activity, peel extract samples showed higher antioxidant activity than seed extract samples particularly ethanolic 50% extract in both of peel and seed. This may be due to that peel samples containing greater concentration of phenols, flavonoids and tannins than seed extract samples. The phenolic, flavonoid and tannins contents peel and seed extracts are strongly dependent on the processing steps to obtain the juice. As reported by Tzulker *et al.* (2007), juices with higher contents of peel residues have from 2-fold (fruits squeezed in juice extractors) to 20-fold (homogenates of the whole fruit) higher antioxidant activity than the juices prepared only with the arils. The action mechanism set in motion by the antioxidant activity of these compounds is still not clearly understood. There are many different methods for determining antioxidant function each of which depends on a

Table 4. Absorbance of ferric reducing power (FRAP) at 700 nm of pomegranate waste extracted using different solvents

Solvent extracts	Absorbance of ferric reducing power	
	Pomegranate peel	Pomegranate seed
Diethyl ether	1.113	1.106
Ethyl acetate	2.530	1.046
Ethanol 50%	2.676	1.681
Ethanol 80%	2.459	1.590
Methanol 50%	1.033	1.400
Methanol 80%	2.610	1.290
Distilled water	1.970	1.223
BHT (200ppm)	1.019	1.019

particular generator of free radicals, acting by different mechanisms (Huang *et al.*, 2005). Antioxidants may act in various ways such as scavenging the radicals, decomposing the peroxides and chelating the metal ions (Cam *et al.*, 2009).

Effect of Gamma Irradiation

The ethanolic 50% extract was the best extract in both peel and seed fractions which was higher in each of total phenolic content, total flavonoid and antioxidant activity determined using (DPPH) radical scavenging method, β-Carotene/linoleic acid bleaching and ferric reducing antioxidant power (FRAP).

Data presented in Table 5 revealed that the ethanolic 50% extract of irradiated peel and seed samples at 6 kGy had an increaments in TPC and TFC compared to 3 and 9 kGy which had reduction in TPC and TFC compared to the ethanolic 50% extract of non-irradiated peel and seed samples.

This increase in TPC and TFC could be attributed to the degradation of tannins present in pomegranate peel and seed powder having higher molecular weight into the release of simple phenolic compounds like gallic acid, and tannic acid. Irradiation may break this complex to facilitate release of active ingredients, which were contributed to increase the total phenolic content (Kumari *et al.*, 2009). The enhanced antioxidant capacity/activity of a plant after irradiation is mainly attributed either to enzyme activity increase (*e.g.* phenylalanine ammonialyase and peroxidase activity) or to the extractability increase from the tissues extractability by depolymerization and dissolution of cell wall polysaccharides by irradiation (Alothman *et al.*, 2009). Bhat *et al.* (2007) observed that, except for 2.5 kGy, rest of the doses showed a significant dose-dependent increase in total phenolics to higher extractability by depolymerization and dissolution of cell wall polysaccharides by irradiation, which was known to increase the activity of phenylalanine ammonialyase, responsible for the synthesis of phenolic compounds.

Table 5 also shows increasing in the three parameters of antioxidant activity evaluation (DPPH, βCB and FRAP) at 6 kGy in both ethanolic 50% extracts of peel and seed which were higher antioxidant activity dose compared to control, 3 and 9 kGy. This increase could be attributed to enhanced free phenolics of the irradiated samples. The antioxidant activity is due to the presence of polyphenols or gallic acid. As mentioned earlier, this increase in total phenolic and flavonoid content was thus suggestive of their enhanced antioxidant properties. Kumari *et al.* (2009) also showed similar results with triphala, wherein they have found out an increase in gallic acid concentration and total phenolics in the water extract due to irradiation that leads to increase in antioxidant property.

Table 5. Total phenolic compound (TPC), total flavonoid content (TFC), DPPH radical scavenging activity, β-carotene/ linoleic acid bleaching (βCB) system and absorbance of ferric reducing power (FRAP) of the ethanolic 50% extract of γ irradiated pomegranate peel and seed samples

γ dose (kGy)	TPC (mg GAE 100 g^{-1} DW)		TFC (mg QE 100 g^{-1} DW)		(%) of DPPH radical scavenging activity						β- carotene/ linoleic acid bleaching system (% activity)		Absorbance of ferric reducing power	
					Peel			Seed						
	Peel	Seed	Peel	Seed	Time (min)			Time (min)			Peel	Seed	Peel	Seed
					0	30	60	0	30	60				
0	9323.17	352.09	2998.05	106.78	88.02	91.13	91.40	29.96	42.36	54.01	70.01	48.00	2.676	1.681
3	9196.05	321.35	3005.52	114.99	89.35	90.33	91.67	35.97	44.16	56.00	70.31	48.30	2.794	1.722
6	9411.11	409.16	3067.35	144.86	89.91	90.61	92.41	39.44	45.14	58.10	70.60	49.33	2.868	1.923
9	8988.17	264.90	1717.44	103.04	87.46	89.68	90.47	28.44	36.89	42.34	68.83	46.97	2.571	1.568
BHT (200 ppm)	----	----	----	----	25.09	48.56	61.97	25.09	48.56	61.97	60.85	60.85	1.019	1.019

On the other hand the decrease in antioxidants caused in ethanolic 50% extracts of irradiated peel and seed at 3 and 9 kGy compared to 6 kGy could be attributed in general, to the formation of radiation-induced degradation products or the formation of free radicals (Sajilata and Singhal, 2006). Breitfellner *et al.* (2002) have reported that γ-irradiation (1-10 kGy) of strawberries lead to the degradation of phenolic acids like cinnamic, p-coumaric, gallic, and hydroxybenzoic acids. The hydroxylation (decomposition) of these phenolic acids has been attributed to the formation of free hydroxyl (OH^-) radicals during the treatment.

Characterization of Phenolic Compounds

This analysis was carried out on the best treatment of investigated extracts (ethanolic 50% extract) and (6 kGy) of gamma irradiation dose for both peel and seed. Table 6 shows a large number of compounds of which fourteen phenolic acids were identified. The phenolic acids were identified according to their retention time in comparison with authentic samples. The identified phenolic acids were pyrogallol (5.98 min), gallic (6.39 min), protocatechuic (7.57 min), catechein (7.91 min), catechol (7.94 min), cholorogenic (8.53 min), P.H. benzoic (8.81 min), caffeic (9.30 min), vanillic (10.18 min), ferulic (11.02 min), salicylic (12.53 min), ellagic (12.67 min), coumarin (13 min) and caffiene (10.59 min). As mentioned above the irradiation of both peel and seed led to phenolics increasing. The results obtained from HPLC analysis revealed that not only quantitative phenolic compound was increased but there were also an qualitative increasing was observed as a result of irradiation treatment. Catechol and salycilic were observed in ethanolic 50% peel extract by 6 kGy irradiation treatment although they were not found before irradiation treatment. In addition catechol, salicylic, catechein, vanillic, were observed in ethanolic 50% extract of irradiated seed. On the other side, some phenolic compounds were absent by irradiation treatment in peel and seed such as catechein and coumarin in peel and protocatechuic, p.h.benzoic, ellagic and cinnamic in seed. The increasing in phenolic contents were associated with the degradation of tannins (Variyar *et al.*, 1998) and changes in the conformation of the molecules (Topuz and Ozdemir, 2004), as a result of the irradiation treatment. In contrast, Koseki *et al.* (2002) reported a decrease in the amount of total phenolic compounds in dehydrated rosemary

Table 6. Identified phenolic compounds (mg/100gm) in ethanolic 50% extract of non-irradiated and irradiated peel and seed samples at dose level of 6 kGy

Test items	Phenolic compounds (mg /100gm)			
	Non irradiated		Irradiated at 6 kGy	
	Peel	Seed	Peel	Seed
Pyrogallol	-	-	718.62	82.69
Gallic acid	152.03	-	2996.14	05.90
Protocatechuic	311.74	04.76	0996.40	-
Catechein	185.86	-	-	150.25
Catechol	-	18.24	2905.99	182.79
Chlorogenic	141.28	14.41	1262.59	403.39
P.H.benzoic	118.66	20.18	1129.87	-
Caffeic	016.33	02.8	0491.19	072.12
Vanillic	022.97	-	1331.93	05.15
Caffiene	028.80	06.07	0236.28	060.97
Ferulic	006.92	03.53	0152.80	025.70
Salicylic	-	-	0475.79	32.52
Ellagic	148.53	10.24	0247.97	-
Coumarin	014.98	01.55	-	025.08
Cinnamic	-	01.14	-	-

after irradiation doses between 10 and 30 kGy, with respect to control. The difference in the effect of radiation on total phenolic content may be due to plant type, geographical and environmental conditions, state of the sample (solid or dry), phenolic content composition, extraction solvent, extraction procedures, temperature, dose of gamma irradiation, etc.

Conclusion

Generally, the results shown above indicate that the extraction with ethanol 50% showed high antioxidant activity, which was confirmed by various methods used for the antioxidant assay. Thus, the results of the present work indicate that the selective extraction of antioxidant from natural sources by appropriate solvent was very important in obtaining fractions with high antioxidant activity. Also, it indicate the presence of compounds possessing antioxidant activity from peel and seeds of pomegranate. Peel could be consider as an enriched source of the antioxidants exhibiting higher activity as compared to seeds. Although the random effect of irradiation treatment but can be useful to enhance antioxidant activity of pomegranate peel and seed.

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النشـــاط المضـــاد للأكســـدة لبعض مستخلصـــات قشـــور وبـــذور الرمـــان المعاملة بأشعة جاما

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تهدف هذه الدراسة لتقييم النشاط المضاد للأكسدة لمستخلصات مخلف قشور وبذور الرمان باستخدام مذيبات مختلفة مثل داي إيثايل إيثر، إيثايل أسيتات، إيثانول ٥٠%، إيثانول ٨٠%، ميثانول ٥٠%، ميثانول ٨٠% وماء مقطر. وقد استخدم فى تقدير النشاط المضاد للأكسدة ثلاثة طرق هي (DPPH) radical scavenging, β-Carotene/linoleic acid bleaching and the ferric reducing antioxidant power (FRAP). كما تم دراسة تأثير أشعة جاما بجرعات ٣، ٦ و٩ كيلو جراى على النشاط المضاد للأكسدة لأفضل مستخلص من عينات قشور وبذور الرمان يحتوى على أعلى نشاط مضاد للأكسدة، وقد أظهرت النتائج أن المستخلص الإيثانولى بتركيز ٥٠ % لكل من مخلف القشرة والبذرة كان الأعلى فى المحتوى من الفينولات الكلية والفلافونيدات حيث بلغ محتواه من الفينولات الكلية مقدرة كحامض جاليك حوالى ٩٣٢٣,١٧ ملجم /١٠٠جم عينة مقدرة كوزن جاف بالنسبة للقشرة و ٣٥٢,٠٩ ملجم/١٠٠ جم عينة مقدرة كوزن جاف بالنسبة للبذرة كما بلغ محتواه من الفلافونيدات منسوبة للكيورستين حوالى ٢٩٩٨,٠٥ ملجم/١٠٠ جم قشرة مقدرة كوزن جاف و ١٠٦,٧٨ ملجم/ ١٠٠ جم عينة بذرة مقدرة كوزن جاف، كما أظهرت النتائج أن المستخلص الإيثانولي تركيز ٥٠% كان هو الأعلى فى النشاط المضاد للأكسدة باستخدام الطرق المذكورة أعلاه في كل من مستخلصات القشرة والبذرة كما أنه كان الأعلى فى القشرة منه فى البذرة، بالإضافة إلى ذلك فقد أوضحت النتائج المتحصل عليها أن المستخلص الإيثانولي لكل من مخلف القشرة والبذرة المعامل بجرعة إشعاعية ٦ كيلوجراى أعلى فى المحتوى من الفينولات الكلية والفلافونيدات وأعلى فى الفاعلية المضادة للأكسدة مقارنة بمستخلصات القشرة والبذرة المعاملة بجرعات ٣، ٩ كيلو جراي، ومن خلال النتائج السابقة يتضح أنه يمكن استخدام المستخلص الإيثانولي بتركيز ٥٠% لقشور وبذور الرمان المعاملة بجرعة ٦ كيلو جراى كأفضل معاملة للحصول على مركبات ذات نشاط مضاد للأكسدة يمكن تطبيقها فى مجال الصناعات الغذائية.

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