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Journal of Medical and Biological Science Research
Vol. 1 (7), pp. 92-99, September, 2015
ISSN: 2449-1810
Research Paper
http://pearlresearchjournals.org/journals/jmbsr/index.html

Antioxidant Activities of Pomegranate Peels and Leave Crude Juices on Sunflower Oil Oxidative Rancidity

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Accepted 15 September, 2015

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ABSTRACT

The leave and peels of pomegranate plant, wonderful variety, were mechanically pressed to obtain crude juices. The latter materials were subjected for the determination of total polyphenols and flavonoids. The antioxidant activity of leave and peels crude juices on sunflower oil oxidation was assessed by three methods that is, 2, 2-diphenyl-1-picryl-hydrazyl (DPPH), reducing power and designation of the induction period by rancimat apparatus. The results indicated the amounts of polyphenols and flavonoids in crude peels juice were markedly higher than the crude leave juice. The antioxidant activity of crude peels juice was higher than that of crude leave juice. Statistical analysis demonstrated that there is a positive correlation between polyphenolic content and the antioxidant activity of pomegranate crude juices. The present results stressed on the use of pomegranate crude juices as a natural antioxidant since it is nearly priceless, caused no harmful effect on human being health and induced powerful antioxidant effect compared to the well known BHT, the synthetic antioxidant.

Key words: Pomegranate Peels and Leave Crude Juices, Polyphenols, Sunflower Oil Stability and Rancimat Apparatus.

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INTRODUCTION

Pomegranate fruits have been widely used in many different cultures and countries for thousand of years. Pomegranate fruit has gained a great deal of popularity over years. The pomegranate fruits have commonly been linked to improve heart health and other varied claims including protecting against prostate cancer and slowing cartilage loss in arthritis. The majority of researches have focused on the pulp and juice of fruits (Ali et al., 2014; Sayed et al., 2012). However, some scientists reported that the peel offers high yields of phenolic, flavonoid and pro-anthocyanidin than the pulp. Pomegranate juice and peel contained substantial amounts of polyphenols such as ellagic tannins, ellagic acid and gallic acid (Loren et al., 2005). Kaneria et al. (2012) mentioned that the pomegranate leaf stands out as a polyphenol-rich source, exhibiting high levels of flavonoids and tannins, such as punicalin, pedunculagan, gallagic acid, ellagic acid, and its esters of glucose.

The phytochemical screening of the methanolic extract of the leave revealed the presence of carbohydrates, reducing sugars, sterols, glycosides, phenolics, tannins, flavonoids, proteins and saponins, whereas, gums were not detected. Total antioxidant potential of the methanolic and aqueous extracts were found as 2.26 and 1.06 mg of ascorbic acid equivalent per ml of the extract, respectively (Hegde et al., 2012). Pomegranate is rich in antioxidant of polyphenolic class which includes tannins and anthocynins and flavonoids (Ricci et al., 2006; De Nigris et al., 2007). Pomegranate is useful for cases of high fever, chronic diarrhea and dysentery alombep and

expelling intestinal worms especially tapeworms and treatment of hemorrhoids, as it is beneficial to cold and treatment of skin disease, scabies and a mix powder peel with honey and used daily in the form of paint.
Several scientists have conducted researches on various pomegranate botanical parts extracts using solvents of different polarities. To the best of our knowledge, no one has tried to do researches on the internal sap of pomegranate leave and peels without recourse to solvents. One has to recall that some solvents might have deleterious effects on human being health. Therefore, the main objectives of the present work were to estimate the contents of polyphenols and flavonoids as well as the antioxidant activities of peels and leave crude juices of pomegranate plant on sunflower oil stability by determining the 2, 2-diphenyl-1-picryl-hydrazyl (DPPH), reducing power and designing the induction period by rancimat apparatus.

MATERIALS AND METHODS

Plant Samples

Ripe pomegranate fruits were collected in October, 2014 from pomegranate trees in El- Menia governorate, Egypt. Samples of ripe pomegranate fruits were handpicked from different trees of Wonderful cultivar. The plant was authenticated by Dr. Abdalatif, A. M. Associate Prof. of Horticulture Department, Faculty of Agriculture, Cairo University. The English, scientific and family names of the plant under study are: Pomegranate*, Punica granatum L* and Lythraceae, respectively.

Preparation of Pomegranate Peels and Leave Crude Juices

Leave and peels of ripe pomegranate fruits were manually separated, cleaned from dust followed by seed removal then mechanically pressed by a Carver hydraulic laboratory press (Carver model C S/N 37000- 156; Fred S. Carver nc, Menomonee Falls, WI, USA). The resultant crude juices were centrifuged at 6000 xg for 0.5 h and kept in the dark at -5ºC prior to use. Total soluble solids (TSS) values were 13.5 and 3% (by a refractometer) for peels and leave pomegranate crude juices, respectively.

Sunflower Oil

Refined sunflower oil was obtained from Cairo Oil and Soap Co. (El-Ayat, Giza, Egypt). The oil peroxide and acid values were 0.94 meq kg^{-1} and 0.36 mg KOH g^{-1} oil, respectively. This means that the oil used in the present work was of good quality.

Chemicals

Gallic acid, Folin-Ciocalteau phenol reagent, DPPH and BHT were purchased from Sigma Chemical Co. (St Louis, MO, USA). Quercetin and ascorbic acid were purchased from Aldrich, Milwaukee, WI, USA. All solvents were of analytical reagent grade and redistilled before use.

Total Polyphenolic Content (TPP)

The total phenolic compounds in the crude juices were determined by the Folin- Ciocalteau method (El-falleh et al., 2012). An aliquot of crude juice sample (0.2 ml) was mixed with 0.5 ml Folin- Ciocalteau reagent then 4 ml of sodium carbonate (1M) and allowed to stand for 30 min at room temperature. The absorbance was measured at 750 nm using a spectrophotometer (Beckman, DU 7400 USA). TPP content in the juice was calculated and expressed as gallic acid equivalent per g dry weight (mg GAE/g DW) by reference to regression equation of standard curve ($Y=0.0184x – 0.0392$, $R^2=0.9863$).

Flavonoid Content

The colorimetric aluminum chloride method (El-falleh et al., 2012) was used for the determination of the total flavonoid content of the crude juices. An aliquot of the crude juice (0.5 ml) was mixed with sodium nitrite (0.3 ml, 0.5%) for 5 min then aluminum chloride (0.3 ml, 10%) was added. After 6 min the reaction was stopped by adding sodium hydroxide (2 ml, 4%). The total volume was made up to 10 ml with distilled water. The absorbance was recorded at 510 nm using known concentrations of quercetin. The concentration of flavonoids in the juice samples were calculated from the regression equation of calibration plot ($Y=0.010-0.143$, $R^2=0.989$) and expressed as mg quercetin equivalent /g of dry weight sample.

Antioxidant Activity

2, 2-diphenyl-1-picryl-hydrazyl (DPPH) Assay

The scavenging activity on DPPH radical of pomegranate leave and peels crude juices was determined following the method of Rajan et al. (2011). The crude juice of different concentrations was mixed with an aliquot of DPPH (1 ml, 0.004% w/v). The mixture was vigorously shaken and left to stand for 30 min in the dark at room temperature. The absorbance at 517 nm was recorded to determine the concentration of remaining DPPH. The radical- scavenging activity was calculated as % inhibition by the following formula:
Inhibition (%) = (A control – A test) / A control X 100. Where; A control = the absorbance of the control reaction. A test = the absorbance of the pomegranate leave and peels crude juices. Ascorbic acid was used as a reference compound. Effective concentrations at 50%

(EC_{50}) were calculated from regression equations of calibration plots (Y= 1.0445x + 28.826, R^2 = 0.9658 and Y = 2.8301x + 41.276, R^2 = 0.9667 for peels and leave crude juices, respectively) to denote the effective concentration of a sample required to decrease the absorbance at 517 nm by 50%.

Reducing Power Assay

The reducing powers of pomegranate peels and leave crude juices were carried out as described by Rajan et al. (2011). An aliquot of the crude juice (1 ml) was mixed with 2.5 ml phosphate buffer (0.2 M, pH 6.6) and 2.5 ml potassium ferricyanide (10 g/L), then the mixture was incubated at 50ºC for 20 min. Trichloroacetic acid (2.5ml, 10%) was added to the mixture and centrifuged at 1000 xg for 10 min. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water then 0.5 ml $FeCl_3$ (1g/L) and the absorbance was measured at 700 nm using a spectrophotometer (Beckman, DU 7400 USA). Ascorbic acid was used as a standard and phosphate buffer as a blank solution. The absorbance of the final reaction mixture of two parallel experiments was expressed as mean ± standard deviation. The antioxidant activity of the juice was expressed as IC50 and compared with standard. The equations of calibration plots of the ascorbic acid was (Y = 0.0631x + 0.05, R^2 = 0.9843).

Designation of Induction Period of Sunflower Oil by Rancimat Apparatus

Rancimat 679 (Metrohm Ltd., CH-9100 Herisau, Switzerland) was used for the determination of oxidative stabilities of model system compounds of sunflower oil mixed with peels and leave juices at various levels (100, 200 and 400 ppm) obtained from pomegranate plant organs. Another model system consists of BHT (200 ppm) and sunflower oil was conducted to compare the effectiveness of crude juices and BHT on sunflower oil stability. Oil sample (5 g each) were exposed to a stream of atmospheric oxygen at 100 ± 2ºC. The volatile decomposition products were detected with a conductivity cell (Mendez et al., 1996). The designation of an induction period, measured by using rancimat instrument, was taken as a tool to compare the effectiveness of the plant crude juices on sunflower oil stability. The induction period for each model system was assessed in triplicate and the mean values are shown in Table 1 and Figure 3. All measurements were performed in triplicate.

Statistical Analysis

The least significant difference (L.S.D) test was applied to compare the difference between treatments. The letters a, b, c, d were used to indicate statistical significant differences between the data of the present work. All the measurements were performed in triplicate and data reported as mean values ± standard error (SE). Data were subjected to analysis of variance (ANOVA). The confidence limits in this study were based on ($P < 0.01$). Analysis of variance and LSD tests were used to compare the mean values of the studied parameters using SPSS (Statistical Program for Social Sciences, SPSS Corporation, Chicago, IL, USA) version 17.0 for windows and ASSISTAT Version 7.7 beta (2014).

RESULTS AND DISCUSSION

Several researchers have studied the constituents and characteristics of internal sap of pomegranate plant parts through extraction with different solvents of varied polarities (Bhandary et al., 2012; Tiwari et al., 2011; Miguel et al., 2004). In the present work, the internal pomegranate plant sap was obtained by mechanical press without recourse to solvents. One has to point out that the pomegranate botanical parts are safe natural organs and obtained from annual pruning of pomegranate trees and are regarded as waste materials. It is well known that some solvents might possess side deleterious effects on human being organs. Therefore, the main target of the present work was to obtain the pomegranate internal plant sap in its native form to determine the amounts of polyphenols, flavonoids and reducing substances. Also, the work was extended to evaluate the activity of pomegranate peels and leave crude juices as natural antioxidants.

Total Phenolics and Flavonoids of Pomegranate Leave and Peels Crude Juices

Phenolic compounds are widely distributed in the plant kingdom. These compounds serve as important antioxidants because of their ability to donate hydrogen atom or an electron in order to form stable radical intermediates. Hence, they prevent the oxidation of various biological molecules (Cuvelier et al., 1992). Figure 1 presents the quantities of total polyphenols and flavonoids of pomegranate leave and peels juices. The data demonstrated that the levels of polyphenols and flavonoids varied according to the pomegranate botanical part. Crude peels juice contained higher amounts of total polyphenols and flavonoids, being about 1.22 and 1.43 times as great as that in leave crude juice, respectively. Similar results were obtained by El-falleh et al. (2012).

Antioxidant Activity

Edible oils with higher contents of unsaturated fatty acids, especially polyunsaturated fatty acids are more susceptible to oxidation. Lipid oxidation can not only

Table 1. Effect of peels and leave crude juices at different concentrations on sunflower oil oxidative rancidity.

System	Induction period (h)[1]	Antioxidant activity [2]
Sunflower oil (Control, C)	11.18 [a]	1.00
C + BHT (200 ppm)	13.91 [b]	1.24
	Peels juice	
C+ Peel juice (100 ppm)	13.23 [b]	1.18
C+ Peel juice (200 ppm)	15.12 [c]	1.42
C+ Peel juice (400 ppm)	16.99 [c]	1.52
	Leave juice	
C+ leave juice (100 ppm)	12.98 [b]	1.16
C+ leave juice (200 ppm)	13.29 [b]	1.89
C+ leave juice (400 ppm)	15.14 [c]	1.35

[1]Induction period refers to the time (h) at the break point of the two extrapolated straight parts of the curve obtained by rancimat apparatus.
[2]The antioxidant activity (AA) was calculated from the following equation: AA = induction period of sample / induction period of control.
The letters: a, b and c refer to significant difference at a level of 1% probability (L.S.D =1.73).

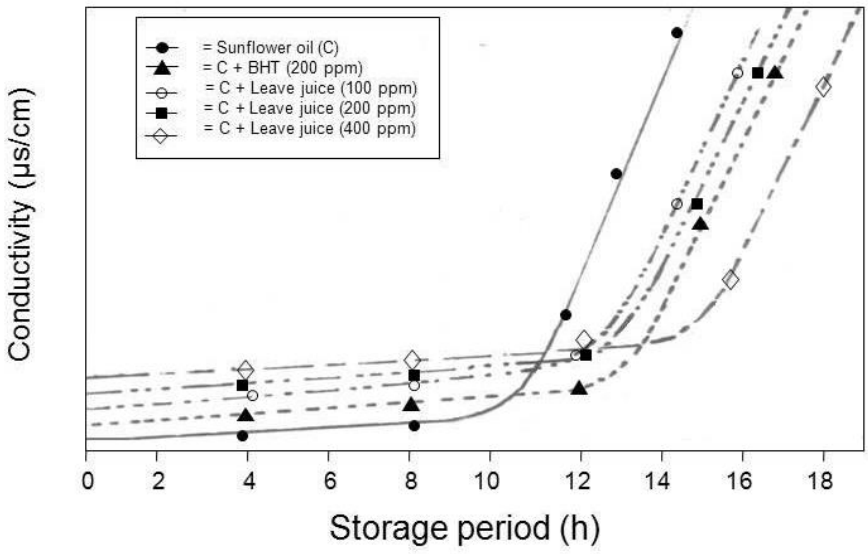

Figure 3. Relationship between various concentrations of pomegranate peels **(A)** and leave **(B)** crude juices and induction periods of sunflower oil oxidative rancidity.

produce rancid odors, unpleasant flavors and discoloration, but can also decrease the nutritional quality and safety due to degradation products, resulting in harmful effects on health (Lercker and Rodriguez-Estrada, 2000). There is currently great interest world-wide in finding new and safe antioxidants from natural sources to prevent oxidative rancidity of food. Hence, the present study focused on pomegranate peels and leave crude juices containing polyphenols and flavonoids, which do not have an undesirable odor when inhaled through the nose or undesirable tongue taste.

As stressed by Huang et al. (2005) no single method is adequate for evaluating the antioxidant capacity of foods. Therefore, the DPPH, reducing power assay and O_2 uptake by pomegranate crude juices were applied to follow up the course of sunflower oil oxidation.

2, 2-diphenyl-1-picryl-hydrazyl (DPPH) Assay

The free radical scavenging activity determined by DPPH was expressed as the EC_{50} value (the effective concentration of the juice required to inhibit 50% of the initial DPPH free radical). The EC_{50} values of peels and leave crude juices are shown in Figure 2. Crude peels juice possessed powerful antioxidant activity than leave crude juice, being approximately 6.59 times as great as

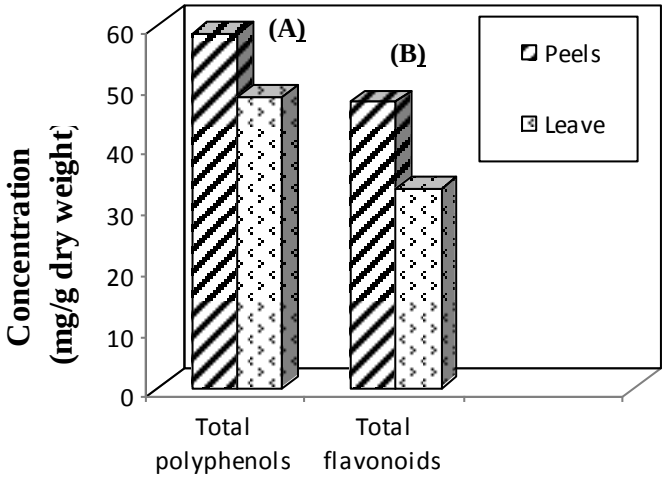

Figure 1. Total polyphenols (A) and total flavonoids (B) contents of pomegranate peels and leave crude juices.

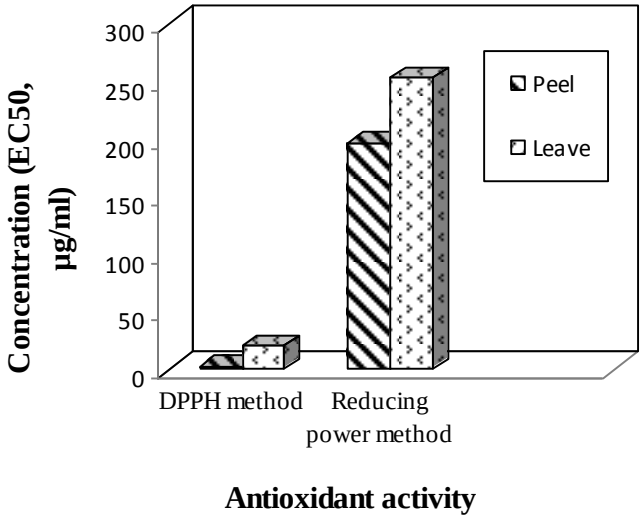

Figure 2. The antioxidant activity of pomegranate peels and leave crude juices.

the induced by leave juice. On the contrary, El-falleh et al. (2012) data indicated that the water extract of pomegranate leave exhibit higher antioxidant activity than that of peel extract. On the other hand, Singh et al. (2001) reported that peel is a good source of antioxidants. Furthermore, Ardekani et al. (2011) found that the antioxidant capacity of pomegranate peel extract was 10 times higher than the pulp extract. These results add weight to the findings of the present study.

Reducing Power Assay

The reducing powers of pomegranate peels and leave crude juices are presented in Figure 2. The peels crude juice exhibited stronger reducing power than did the leave juice. In contrast, El-falleh et al. (2012) reported that pomegranate leaf had higher reducing powers than did peel extract. One can interpret the discrepancy in reducing power to the way of extracting the pomegranate botanical parts. In the present work, the crude juices of pomegranate peels and leave were obtained by pressing unlike the method used in El-falleh et al. (2012) was dependent in extraction with methanol.

Designation of Induction Period by Rancimat

The antioxidant activities of pomegranate peels and leave crude juices were also assessed by rancimat apparatus. This method assigned the induction period for the onset

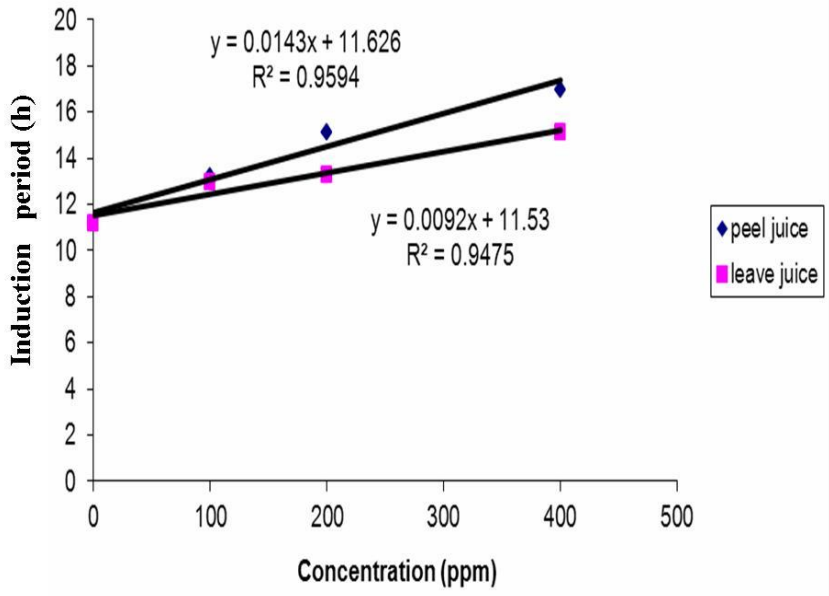

Figure 4. Relationship between various concentrations of pomegranate peels and leave crude juices and induction periods of sunflower oil oxidative rancidity.

the oxidative rancidity of sunflower oil at 100°C. In the present study, simple model systems comprising sunflower oil mixed with pomegranate peels and leave crude juices were used to assess oxidation behavior. An experiment was performed with sunflower oil and BHT (200 ppm) to compare the antioxidant efficiency of the pomegranate peels and leave crude juices with the most commonly used synthetic antioxidant material. It has been reported that synthetic antioxidants (BHT, BHA and PG, Propyl gallate) are added at concentrations of 100 to 400 ppm to fats and oils to suppress the development of peroxides during food storage (Allen and Hamilton, 1983). Therefore, the pomegranate peels and leave crude juices were added to sunflower oil at concentrations of 100, 200 and 400 ppm. Table 1 shows the effect of the pomegranate peels and leave crude juices on oxidative rancidity of sunflower oil.

The results illustrated that both crude juices of pomegranate peels and leave added at various concentrations to the test system, exhibited antioxidant effect on sunflower oil stability. Using data in Table 1 when relative concentrations of the pomegranate peels and leave crude juices are plotted against the induction periods, according to the method of Beddows et al. (2000) (Figure 4) a linear relationship results were existed. It means that the antioxidant activities of pomegranate peels and leave crude juices had direct relationship with it is concentration of polyphenolic compounds. Similar results were achieved by Li et al. (2011) and Kaneria et al. (2012) who showed high correlations between phenolic composition and antioxidant activity of pomegranate. In addition, crude peels juice was far more active in retarding sunflower oil oxidation than leave juice.

The levels of 200 and 400 ppm for pomegranate juice induced antioxidation activity either similar or superior to that of a system comprising sunflower oil and BHT (200 ppm), respectively. The aforementioned data illustrate that crude peels juice when added to food products especially to lipids and lipid-containing foods, would increase the shelf-life by retarding lipid peroxidation.

Synthetic antioxidants BHA, BHT and gallic esters have been suspected to be carcinogenic. In addition, BHT at 200 ppm induced significant increase in the enzyme activities of rat's liver and kidney and severely altered the features of these organ tissues (Farag et al., 2006). Furthermore, WHO recommends the use of natural antioxidants that can delay or inhibit the lipids or other molecules by inhibiting the initiation or propagation steps of oxidative chain reaction (Velioglu et al., 1998).

Consequently, strong limitations have been placed on the use of synthetic antioxidants and the trend nowadays is to replace them with naturally occurring antioxidants. Hence, the data of the present work suggest that pomegranate peels juice can be used adequately as food supplement to retard or prevent lipid oxidation and also to cure from some diseases that induce through free radicals. It is worth noting that the effects of some phenolics are related to the increase in the activity of antioxidant enzymes (Chiang et al., 2006) and the induction of the synthesis of antioxidant proteins (Chung et al., 2006). It appears that there is a relationship between the antioxidant efficiency and the chemical composition of phenolic compounds. The main structural

feature required for antioxidant activity is a phenolic ring containing hydroxyl groups. The evidence for these structural requirements are supported by the powerful antioxidant activities of the well-known synthetic BHT and the natural antioxidant thymol (Farag et al., 1989; and Topallar et al., 1997). In this context, Amjad and Shafighi (2013) reported that the chemical structure of phenolics plays a role in the free radical-scavenging activity, which is mainly depending on the number and position of hydrogen donating hydroxyl groups on the aromatic rings of the phenolic molecules.

Furthermore, Balasundram et al. (2006) mentioned that the antioxidant activity of phenolic compounds depends on the structure, in particular the number and position of hydroxyl groups and the nature of substitutions on the aromatic ring.

One would relate the antioxidant activity of BHT or thymol to the inhibition of hydroperoxide formation. The first step in lipid oxidation is the abstraction of hydrogen atom from unsaturated fatty acid and subsequent oxygen involvement gives a peroxy radical. Generally, the antioxidants suppress the hydrogen atom abstraction from unsaturated fatty acid which leads to the decrease of hydroperoxide formation. It is well known that phenolic compounds act as hydrogen donors in the reaction mixture and therefore, the formation of hydroperoxides is decreased. The results of the present work are in line with this theory. One would also has to mention that crude peels juice induced more powerful antioxidant than crude leave juice since the former extract contains 1.22 times as much total polyphenols as leave crude juice. It is worth noting that some researches established that chlorogenic acid and flavonoids particularly quercetin and its glycoside derivatives are the main compounds responsible for the antioxidant properties (Silvia et al., 2011). These classes of compounds possess a broad spectrum of biological activities including radical scavenging properties (Balasundram et al., 2006).

It is worth mentioning that HPLC data (Farag *et al.,* 2015) demonstrated that chlorogenic acid was present in both peels and leave crude juices as minor constituent (< 10% - > 1%) and hence add weight to our findings. Furthermore, Amjad and Shafighi (2012) mentioned that ellagic acid, as a member of phenolics, is considered to play an important role in antioxidant activity.

This acid can react with free radicals due to its ability to chelate with metal cations, a potent oxidant against lipid peroxidation in mitochondrion and micosome. From the aforementioned data, one can interpret the powerful antioxidant effect of pomegranate peels crude juice components to two main basic factors that is, scavenging the free radicals and chelate the mineral cations.

The outcome from the present study suggests using pomegranate peels crude juice as a natural antioxidant since it is nearly priceless, safe and induced powerful antioxidant effect compared to the well known BHT, the synthetic antioxidant.

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