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Research Article

HPLC-Analysis of Polyphenolic Compounds and Free Radical Scavenging Activity of Pomegranate Fruit (*Punica granatum* L.)

*Sami I. Ali[1], Farouk K. El-Baz[1], Gehan A.E. El-Emary[2], Ekhlaque A. Khan[3], Amal A. Mohamed[1]

[1]*Plant Biochemistry Dept., National Research Centre, Dokki- Cairo, Egypt.*
[2] *Faculty of Technology and Development, Zagazig University, Egypt.*
[3]*Centre for Life Sciences, Central University of Jharkhand, Ranchi, India.*

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ABSTRACT
Pomegranate fruits have been commonly used in herbal remedies by local healers in many countries. In this work, total phenolic, flavonoid and anthocyanin contents of different parts of pomegranate fruit (peel, flesh, seeds, and whole fruit) were evaluated using two different solvents (methanol and ethyl acetate). The highest total phenolic and flavonoid contents were detected in peel methanol extract (103.2 mg/g dw and 132.4 mg/g dw) respectively. Also, the highest anthocyanin content was detected in peel extract (3.86 mg/g fw). HPLC coupled with diode-array detection was used to investigate the major phenolic compounds in peel methanolic extract. Among the phenolic compounds, chlorogenic acid, rutin and coumaric acid are present predominantly in the peel extract. Furthermore, the peel methanol extract exhibited the highest antioxidant activities using 2,2-diphenyl-1-picrylhydrazyl radical scavenging activity (DPPH) and the ferric reducing antioxidant power (FRAP) assays. The high antioxidant activities of peel extract may be attributed to its high contents of total phenolic and total flavonoid. The results clearly indicate that pomegranate peel extract appeared to have more potential effects as a health supplement rich in natural antioxidants than other parts.

Keywords: Antioxidant activity, anthocyanin, DPPH, pomegranate, total phenolic.

INTRODUCTION

Recently, interest has been increased considerably in finding naturally occurring antioxidants for use in foods or medicine to replace synthetic compounds which are being restricted due to their carcinogenicity. Natural antioxidants can protect the human body from free radicals and arrest the progress of many chronic diseases as well as retard lipid oxidative rancidity in food[1]. The protective action of fruits and vegetables has been attributed to the presence of antioxidants, especially antioxidant vitamins including ascorbic acid, α-tocopherol and β-carotene[2]. Phenolic or polyphenols including flavonoid, commonly found in edible plants, have received considerable attention because of their biological functions such as antioxidant, antimutagenic and antitumor activities[3]. The antioxidant capacity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers or metal chelators[4]. The pomegranate (*Punica granatum* L.) is widely grown in many tropical and subtropical countries[5]. It has known as a source of antimicrobial[6], anticancer[7], antivirus, antioxidant, and antiproliferative[8] substances which has led to being the center of attention in many studies. Pomegranate is known to contain considerable amount of phenolic compounds, including anthocyanins, ellagic acid, punicalin, punicalagin, pedunculagin and other flavanols[9]. The critical point in studying polyphenols in plant materials is the extraction procedure used since it dictates the nature and quantity of polyphenols that will be transferred to the extract and further characterized. Solvent extraction is frequently used technique for isolation of plant antioxidant compounds[10]. However, polyphenolic content and antioxidant activities of the plant materials are strongly dependent on the nature of extracting solvents due to the presence of varied chemical characteristics and polarities that may or may not be soluble in a particular solvent[11]. Several studies revealed that phenolic content differ with solvent polarities. In this concern, Mohamed et al.[12] reported that methanol was more effective than methylene chloride for the extraction of total phenolic and total flavonoid from *Syzygium cumini* leaves. Also, Zhou[13] showed that 50% acetone was more effective than water for the extraction of wheat total phenolic. Therefore, the present study was aimed to evaluate the effect of two different polar solvents (methanol and ethyl acetate) on the contents of total phenolic, total flavonoid, total anthocyanin and the antioxidant activities of different fruit parts (peel, flesh, seeds, and whole fruit) of pomegranate (*Punica granatum* L.). As well as, to investigate the major phenolic compounds in peel methanolic extract by using HPLC-diode-array detection system.

* *Author for correspondence: E-mail: samiali.nrc@gmail.com*

Table 1: Total phenolic (mg /g dw) and total flavonoid (mg /g dw) contents in fruit parts of pomegranate extracted by methanol and ethyl acetate solvents.

Fruit parts	Total phenolic		Total flavonoid	
	Methanol	Ethyl acetate	Methanol	Ethyl acetate
Peel	103.2± 2.77	1.9±0.13	132.4±2.69	8.7±0.29
Flesh	101.3±2.35	1.5±0.08	128.3±1.92	8.6±0.02
Seeds	14.9±1.61	0.9±0.05	64.6±0.68	7.6±0.25
Whole fruit	81.8±1.44	1.4±0.08	124.1±3.82	9.7±0.19

Each value is represented as mean ± SD (n = 3).

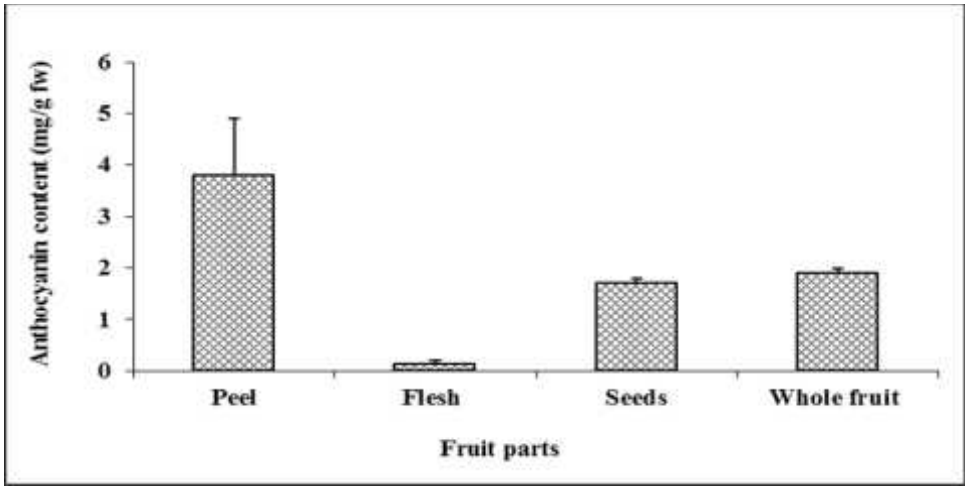

Fig. 1: Total anthocyanin content (mg/g fw) of pomegranate peel, flesh, seeds, and whole fruit parts. Each value is represented as ± SD (n = 3).

MATERIALS AND METHODS

Chemicals and reagents: 2,2-Diphenyl-1-picrylhydrazyl (DPPH), pyrogallol, chlorogenic acid, coumaric acid, ferulic acid, cinnamic acid, benzoic acid, catechin, rutin, acacetin, genistein, kaempferol, quercetin and BHA were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Folin-Ciocalteus's phenol reagent and sodium carbonate were obtained from Merck Chemical Supplies (Damstadt, Germany). All other solvents and chemicals were of analytical grade.

Plant materials: Fresh ripe fruits of pomegranate (Balady cultivar) were purchased from local market. Then washed firstly with tap water followed by distilled water, some fruits were separated into three parts (peel, flesh and seeds) and the others were used as whole fruit. The small pieces of each part and of whole fruit were shade dried at room temperature until constant weight.

Preparation of crude extract: Dried fruit parts were ground into a powdery form and 5.0 g from each material was extracted by soaking in 100 ml of methanol (relative polarity 0.762) and ethyl acetate (relative polarity 0.228) on an orbital shaker (Heidolph, Unimax 2010) at 160 rpm for 48 h. Then, after 48 hours, the sample was filtered with Whatman filter paper no. 1. Residues were re-extracted twice with fresh aliquots of the same solvent. The filtrates were combined and the solvents were removed by using a rotary evaporator (Büch Rota-vapor) under reduced pressure at 40°C. Each dried extract was re-suspended in methanol at concentration (1mg/ml) as stock solution and used for further analysis[10].

Determination of total phenolic content: Total phenolic (TP) content in all fruit parts was determined by Folin-Ciocalteu method[14]. An aliquot of each crude extract was completed to 3 ml with distilled water then mixed thoroughly with 0.5 ml of Folin-Ciocalteu reagent. After mixing for 3 min, 2 ml of 7% (w/v) sodium carbonate was added and allowed to stand for a further 60 min in the dark. The absorbance of the reaction mixtures was measured at 650 nm using the Thermo model Unicom UV 300 UV-VIS spectrophotometer. Gallic acid was used as the standard to produce the calibration curve. The results were expressed as milligram of gallic acid equivalents (GAE) per gram of dried sample.

Determination of total flavonoid content: Total flavonoid (TF) content was determined using colorimetric method as described previously by Dewanto et al.[15]. In brief, 50 µl of each crude extract were made up to 1 ml with methanol then mixed with 4 ml of distilled water and subsequently with 0.3 ml of 5% $NaNO_2$ solution. After 5 min of incubation, 0.3 ml of 10% $AlCl_3$ solution was added and allowed to stand for 6 min, followed by adding 2 ml of 1 M NaOH solution to the mixture. Then water was added to the mixture to bring the final volume up to 10 ml and the mixture was allowed to stand for 15 min. The absorbance was measured at 510 nm. Rutine (RU) was used as standard to produce the calibration curve. Total flavonoid content was expressed as mg rutine (RU) equivalents per gram of dried sample.

Total anthocyanin content: Total anthocyanin content was determined according to the method of Martinez and Favret[16]. Anthocyanin was extracted from fresh tissues of each fruit part and from whole fruit by maceration in methanol:HCl (99:1, v/v) solution for 24 h. The extracts were clarified by cooling centrifugation at 10000 rpm for 10 min at 4°C. The obtained supernatant was used for the

Table 2: Major phenolic compounds (%) identified in pomegranate peel methanolic extract by HPLC.

Compounds	R_t^* (min)	Area (%)
Pyrogallol	5.527	0.529
Chlorogenic acid	8.891	11.915
Catechin	11.911	0.019
Rutin	15.262	4.828
Coumaric acid	15.580	1.297
Ferulic acid	16.617	0.077
Benzoic acid	18.739	0.025
Acacetin	19.448	0.024
Cinnamic acid	23.216	0.018
Genistein	23.966	0.013
Kaempferol	24.513	0.024

R_t: retention time (min)

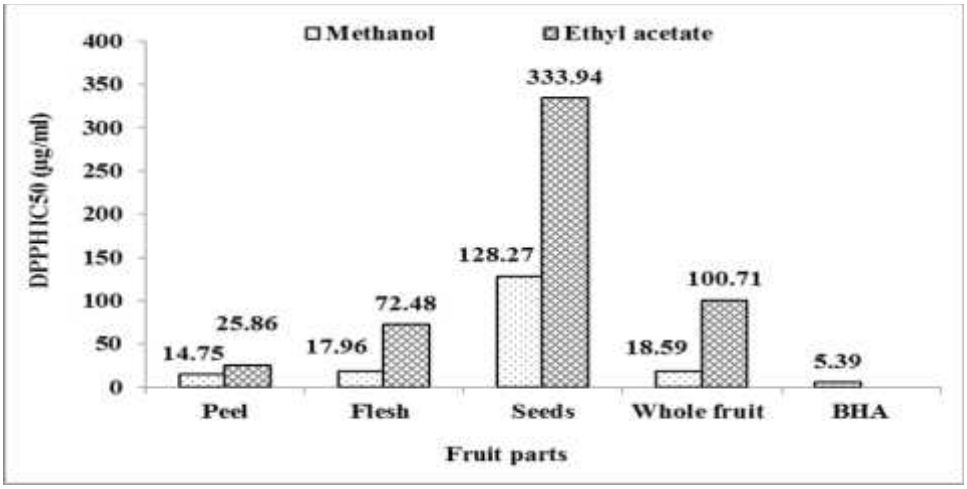

Fig. 2: DPPH IC_{50} (µg/ml) of methanol and ethyl acetate extracts of pomegranate parts (peel, flesh and seed and whole fruit), BHA used as positive control.

assay of anthocyanin content. Quantification of anthocyanin was done spectrophotometrically at 527 nm.

Absorbance readings were converted to total amount of anthocyanin as a cyanidin-3-glucoside equivalent using a molar extinction coefficient (Ɛ) of 2.96×10^4 which mentioned by Cheng and Breen[17]. Results were expressed as milligram of cyanidin-3-glucoside equivalents per gram of fresh weight.

High Performance Liquid Chromatography (HPLC) analysis of phenolic compounds in peel methanolic extract

Sample preparation and standard compounds: For sample preparation, 10 mg of the dried methanolic crude extract of peel part were re-dissolved in 1 ml of methanol HPLC spectral grade by vortex mixing for 10 min. For standard compounds, stock solutions (10µg/ml) of available pure known compounds such as pyrogallol, chlorogenic acid, coumaric acid, ferulic acid, cinnamic acid, benzoic acid, catechin, rutin, acacetin, genistein and kaempferol were chromatographed as external standards. All standards were dissolved in methanol HPLC spectral grade before injection in the analytical HPLC system. Prior to HPLC analysis, all solutions (sample and standards) were filtered through 0.2 µm nylon syringe filter (Axiva, India).

HPLC system: Chromatographic analysis were carried out using Agilent 1260 Infinity Quaternary LC, equipped with Agilent 1260 Infinity; Quaternary Pump (G1311B), Autosampler (G1329B, ALS) and Diode Array Detector (G1315D, VL) coupled to Agilent OpenLAB ChemStation B.04.03 software. Phenolic compounds were separated on Agilent ZORBAX Eclipse Plus-C18 reversed-phase column (4.6 × 250mm, 5 µm).

Chromatographic conditions: The chromatographic conditions for analytical HPLC followed the previous published method of Khalil et al.[18]. The mobile phase consisted of 0.5% acetic acid in distilled water at pH 2.65 (solvent A), 0.5% acetic acid in 99.5% acetonitrile (solvent B). The elution gradient was linear starting with (A) and ending with (B) over 50 min, using an UV detector set at wavelength 254 nm. The concentration of an individual compound was calculated on the basis of peak area measurements. The flow rate was kept constant throughout the analysis at 1 ml/min and the injection volume was 5 µl.

Identification of compounds: The phenolic compounds of peel methanol extract were identified by comparing their retention times (R_t) with those of pure standards. The results were expressed as area % of each identified compound from the total area.

Table 3. Ferric reducing antioxidant power of different parts of pomegranate fruit at concentration (100 µg/ml) extracted by methanol and ethyl acetate.

Fruit parts	Ferric reducing power (mg /g)	
	Methanol	Ethyl acetate
Peel	166.9±2.86	2.1±0.39
Flesh	159.6±1.38	1.5±0.15
Seeds	20.4±1.89	1.2±0.17
Whole fruit	143.9±2.91	1.6±0.22

Each value is presented as mean ±SD (n = 3).

Determination of antioxidant activities

DPPH radical scavenging assay: Scavenging activity of different extract against DPPH radicals was assessed according to the method of Tenpe et al.[19]. One milliliter of DPPH radical solution (0.1 mM) in methanol was added to 3 ml of both methanolic and ethyl acetate extracts of each fruit part and whole fruit at different concentrations (10-150 µg/ml). The absorbance values were measured at 517 nm after incubation for 30 min at room temperature in the dark. The experiment was conducted in triplicate. The percentage of DPPH scavenging effect (%) was calculated using the following equation**:**

$(\%) = [A_{DPPH} - A_S / A_{DPPH}] \times 100$

Where, A_{DPPH} is the absorbance of the DPPH solution without the extracts and A_S is the absorbance of the solution when the sample extract was added.

Similar process was repeated for BHA which used as a positive control.

The results were expressed as IC_{50} values (µg/ml). The IC_{50} values were calculated by sigmoid non-linear regression model using plots, where the abscissa represented the concentration of tested plant extracts and the ordinate represent the average percent of scavenging capacity (excel program). IC_{50} values denote the concentration of sample required to scavenge 50% of DPPH radical.

Ferric reducing antioxidant power (FRAP) assay: Ferric reducing power assay was determined following the method reported by Zhao et al.[20]. The diluted methanol and ethyl acetate extracts (100 µg/ml) was mixed with phosphate buffer (2.5 ml, 200 mM, pH 6.6) and 1% potassium ferricyanide (2.5 ml). The mixtures were incubated at 50°C for 20 min. Then, 2.5ml of 10% trichloroacetic acid was added and the mixture was centrifuged at 10000 rpm for 10 min. The upper layer of the solution (5 ml) was mixed with distilled water (5 ml) and 0.1% ferric chloride (1 ml). The absorbance of the reaction mixtures were measured at 700 nm.

Standardization of the assay was done by preparing standard curve using 10-100 µg/ml solutions of BHA in water. The resultant values were expressed as mg BHA equivalents per gram of dried sample.

Statistical analysis: Statistical analysis was carried out using Microsoft corporation excel program. All experiments were performed in triplicate. Results are presented as a value ± standard division of mean (SD).

RESULTS AND DISCUSSION

Total phenolic (TP) and total flavonoid (TF) contents: The levels of total phenolic and total flavonoid compounds in different fruit parts (peel, flesh, seeds and whole fruit) of pomegranate are shown in Table (1). With increasing in solvent polarity, TP and TF contents also increased in the extracts. The highest content of TP (103.2± 2.77 mg GAE/g dw) and TF (132.4±2.69 mg RU/g dw) were obtained from methanol extract of pomegranate peel.

Our results are similar to that of Elfalleh et al.[21] who reported that the methanol extracts of different parts of the pomegranate gave the highest values of total polyphenols, flavonoids and anthocyaninns compared to aqueous extract. Also, Sultana et al.[10] and Turkmen et al.[22] reported that solvents with different polarity had significant effect on polyphenol content and antioxidant activity. Moreover, the most effective effect of methanol in extracting phenolic components from different plant materials was concluded by different authors[12,22,23,24].The present results strongly support that phenolic are important components of pomegranate plant, and some of their pharmacological effects could be attributed to the presence of these valuable constituents.

It is well known that, the concentration of phenolic content usually higher than the concentration of flavonoid in most cases, but in this study we found an inverse trend. The present results are in partial similar to that of Elfalleh et al.[21] who found a higher total flavonoid content (12.77 ± 0.23 mg/g dw) in the aqueous extract of the pomegranate leaves than the total polyphenol (9.85 ± 0.82 mg/g dw). Moreover, different authors found a higher concentrations of flavonoid more than phenolic level such as Sultana et al.[10] who found a higher concentration of total flavonoid (1.68 g/100g dw) in absolute methanolic extract of *Moringa oleifera* root more than the total phenolic (0.22 g/100g dw). Besides, phenolic compounds undergo a complex redox reaction with the phosphotungstic and phosphomolybdic acids present in the Folin-Ciocalteu (FC) reagent. However, Nazck and Shahidi[25] reported that Follin-Ciocalteau reagent was not specific and can detects all phenolic groups found in the samples including those found in the extractable proteins. Correspondingly some chemical groups of proteins, organic acids, and sugars present in the extracts can also react with FC reagent and therefore can interfere with the results[26].

Total anthocyanin content: Figure 1 shows anthocyanin contents of each fruit parts. The peel extract showed the highest anthocyanin content (3.8 mg/g fw) followed by whole fruit (1.9 mg/g fw), seeds (1.7 mg/g fw), and flesh

(0.14 mg/g fw) respectively. Anthocyanin is natural pigments which possesses pharmacological properties and is used by humans for nutraceuticals and therapeutic purposes[27]. However, Noda et al.[28] isolated three major anthocyanidins: delphinidin, cyanidin and elargonidin from pomegranate juice which have high antioxidant activity. Several studies have revealed that anthocyanin extracts are responsible for the high antioxidant activities of fruit and other food extracts[29]. It is well known that, anthocyanins are water-soluble flavonoid compounds that produce colours ranging from orange and red to various shades of blue and purple, and have a critical role in the quality of many fresh and processed fruit, vegetables, and plants[30]. They attract much interest for its health function such as their potential antioxidant ability[31].

HPLC analysis of main phenolic compounds in peel methanolic extract: Phenolic compounds can be defined as substances possessing an aromatic ring bearing one or more hydroxy substituents, including their functional derivatives[32]. Because of the enormous diversity of phenolic compounds in different plant extracts, it is difficult to identify every compound, but it is not difficult to identify major and important phenolic compounds. HPLC analysis of major phenolic compounds in some medicinal plants have been developed[24,33].

The previous results presented in Table (1) confirmed that pomegranate peel methanolic extract exhibited the highest amount of total phenolic and total flavonoid as compared to the other parts. Thus, to understand the phenolic nature of the peel methanolic extract, HPLC analysis was carried to identify major phenolic compounds in pomegranate peel methanolic extract. The components of pyrogallol, chlorogenic acid, coumaric acid, ferulic acid, cinnamic acid, benzoic acid, catechin, rutin, acacetin, genistein and kaempferol have been identified in peel methanolic extract (Table 2) by comparisons to the retention time and UV spectra of authentic standards. Many authors proved the presence of different phenolic compounds in pomegranate fruits by HPLC analysis. In this concern, Hmid et al.[34] reported the presence of gallic, chlorogenic, caffeic, ferulic, ellagic acids, catechin, epicatechin, phloridzin, quercetin and rutin in pomegranate juices. Also, Middha et al.[35] revealed the presence of some major phenolic compounds such as gallic acid and ellagic acids in addition to punicalagin as a major ellagitannin in *Punica granatum* fruit peel**.** On the other hand, Cai et al.[33] revealed the presence of quercetin and vanillic acid in pomegranate peel. Moreover, Mansour et al.[36] proved the presence of gallic acid, ellagic acid, caffeic acid, p-coumaric acid, quercetin, and vanillic acid in pomegranate peel.

HPLC analysis in our study revealed that, chlorogenic acid was the most abundant phenolic acid in peel methanolic extract, science it constituted 11.915% of the total extracted compounds followed by the principal flavonoid compound: rutin (4.828%). Besides, the other phenolic acids: coumaric acid, pyrogallol, ferulic acid, benzoic acid and cinnamic acid were constituted 1.297%, 0.529%, 0.077%, 0.025% and 0.018% of the total extracted compounds, respectively. In addition to the other flavonoid compounds: acacetin, kaempferol, catechin and genistein were constituted 0.024%, 0.024%, 0.019% and 0.013% of the total extracted compounds, respectively. The present results are in agreement with that of Palafox-Carlos et al.[37] who reported that chlorogenic acid was the most abundant phenolic compound found by HPLC in mango fruit pulp with concentration of (28 mg/100 g dw), and suggests that this compound probably maintain the phenolic content and the antioxidant activity of mango fruit. Also, Hatipoglu et al.[38] reported that chlorogenic acid and caffeic acid have been identifies as the major phenolic acids in the methanolic extract of *Hyssopus officinalis* by HPLC analysis**.** Alternatively, catechin and rutin were the major flavonoid compounds were found in *Gardenia jasminoides* extract by HPLC analysis[24]. Also, rutin was the predominant identified compound in *Melia azedarach* leaf extract, followed by kaempferol-3-O-β-rutinoside and chlorogenic acid[39]**.** Catechin and rutin both showed beneficial effects such as antioxidant, anti-ageing and may prevent cardiovascular complecations[40]. Their beneficial effects are attributed to their ability to reduce oxidative stress, lipid peroxidation, free radical generation and low density lipoprotein (LDL) cholesterol-oxidation[40].

Antioxidant activities

DPPH radical scavenging activity: DPPH antioxidant assay is developed based on the ability of 2, 2-diphenyl-1-picryl-hydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for a visible deep purple color of DPPH in alcoholic solution and the color intensity can be measured at absorbance 515 nm.[27]. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance.

The quality of the antioxidants in the extracts was determined by the IC_{50} values, which means denoting the concentration of the sample required to scavenge 50% of the DPPH free radicals (Figure 2). The lower IC_{50} value means stronger scavenging DPPH free radicals. Among the pomegranate parts, the peel methanolic extract exhibited the highest DPPH scavenging activity, with the lowest IC_{50} value 14.75 µg/ml. However, the IC_{50} of flesh, seeds and whole fruit methanolic extracts and BHA values were found to be 17.96, 128.27, 18.59, and 5.39 µg/ml, respectively. Nevertheless, ethyl acetate extracts showed lower DPPH scavenging activity compared to methanolic extracts, for example, the IC_{50} value of ethyl acetate extracts of peel , flesh , seeds and whole fruits , were respectively 25.86 ,72.48 , 333.94 and 100.71 µg/ml. In terms of DPPH radical inhibition and despite used solvents, tested pomegranate parts were classified as follows: peel > flesh >whole fruit> seeds. In the present study the DPPH scavenging activity of pomegranate fruit different parts is associated with their TP and TF contents; thus, the methanolic extract of peel showed the highest DPPH scavenging activity compared to other parts. The present results also are in agreement with

Singh et al.[41] who reported that the methanol extract of pomegranate peel showed the highest DPPH scavenging activity among ethyl acetate and water extracts and attributed its higher activity to its higher percentage of total phenolic content. Moreover, the DPPH radical scavenging activity has been shown to be directly related with the total phenolic content present in the extracts as suggested by other previous reports[28,42].

Although, the highest DPPH scavenging activity of peel extract may be attributed to its higher content of phenolic compounds: chlorogenic acid, rutin, coumaric acid and pyrogallol (Table 2). Our results are in accord with the previous results of Terashima et al.[43] who reported the DPPH• radical scavenging activity of chlorogenic acid, quercentin and rutin phenolic compounds. Also, Antioxidant properties of chlorogenic and caffeic acids are well known and high concentration of these phenolic acids were thought to be responsible for the radical scavenging activity of *Hyssopus officinalis* extract[38].

It can be concluded that the extracts obtained using high polar solvent (methanol) were considerably more effective radical scavengers than those using less polarity solvent (ethyl acetate), indicating that antioxidant or active compounds of different polarity could be present in pomegranate parts. With change in solvent polarity its ability to dissolve especial group of antioxidant compounds alters and influences the antioxidant activity estimation[13]. Moreover, Reddy et al.[44] stated that total tannins and purified constituents (e.g., ellagic acid and punicalagins) of pomegranate fruit possessed antioxidant activity and strongly inhibited ROS generation with IC_{50} value of 0.33 to11.0 µg/ml.

Ferric reducing antioxidant power (FRAP): In the present study, pomegranate peel methanolic extracts exhibited the highest reducing power activity followed by flesh, whole fruit and seeds as shown in Table (3), at 100 µg/ml, the values were: 166.9±2.86 , 159.6±1.38 , 143.9±2.91 and 20.4±1.89 mg/g dw respectively.

The highest reducing power activity of peel followed by flesh may be attributed to their higher contents of total phenolic and total flavonoid as presented in Table (1). Therefore the reducing powers of pomegranate fruit parts are probably due to the action of hydroxyl group of the phenolic compounds which might act as electron donors. In this concern Gil et al.[45] stated that the antioxidant activity of pomegranate fruit has been attributed to the high level of phenolic compounds. Also, Rabeta and Nur Faraniza[23] concluded that higher total phenolic content gave higher ferric reducing antioxidant power values in the leaves and fruits of *Garcinia atrovirdis* and *Cynometra cauliflora*. Moreover, Uddin et al.[24] stated that higher ferric reducing ability of the methanol extract of *Gardenia jasminoides* might be due to the high concentration of phenolic compounds. The main phenolic compounds: chlorogenic acid, rutin, coumaric acid and pyrogallol in peel extract (Table 2) may be responsible for its highest ferric reducing antioxidant power. In this concern, the ferric reducing antioxidant power in pomegranate juices was evaluated and this antioxidant activity may be attributed to the presence of gallic, chlorogenic, caffeic, ferulic, ellagic acids, catechin, epicatechin, phloridzin, quercetin and rutin[34]. It is well known that, ferric reducing antioxidant power is widely used in evaluating antioxidant activity of plant polyphenols. Principally, FRAP assay treats the antioxidants in the sample as reductant in a redox-linked colorimetric reaction[26]. Antioxidant compounds which act as reducing agent exert their effect by donating hydrogen atom to ferric complex and thus break the radical chain reaction[46]. Also, the results of Table (3) showed that reducing power values were higher in methanol extracted parts compared to ethyl acetate extraction. This showed that methanol solvent was more efficient in extracting antioxidants in plant materials compared to ethyl acetate. A study by Rabeta and Nur Faraniza[23] showed that methanol extractions gave higher ferric reducing antioxidant power values compared to water extraction in the leaves and fruits of *Garcinia atrovirdis* and *Cynometra cauliflora*.

The reducing power of seeds part was the lowest among all parts (Table 3). As given in Table (1), the total phenolic content of peel part was nearly seven times higher than seeds. Additionally, the flavonoid contents were also higher compared to flesh and seeds. All these results clearly indicated that peel part contain more antioxidant activities than flesh and seeds. Our data was in agreement with Guo et al.[47] and Li et al.[48] they found that fruit peel of mango, kiwifruit, guava, and orange among others contains higher concentration of phenolic, flavonols, and antioxidant activities than pulp and seed extracts. These differences could be explained by fruit growing condition, or analytical method, such as the solvent used to prepare the pomegranate extract.

CONCLUSIONS

The present results revealed that the high polar solvent (methanol) had efficient effect on total phenolic, total flavonoid contents and antioxidant activities of pomegranate fruit different parts compared to ethyl acetate as a low polar solvent. Also, this study indicated that the methanolic extract of peel part possessed the highest phenolic, flavonoid, anthocyanin contents and antioxidant activities compared to the other fruit parts. The highest antioxidant activities of peel extract may be attributed to the presence of chlorogenic acid, rutin, coumaric acid and pyrogallol as main phenolic compounds which have been identified by HPLC analysis. This suggests that the peel extract of pomegranate fruit can be potentially used as a source of natural antioxidant agent. Further studies are recommended including extra isolation and structure elucidation of bioactive pure compounds from peel extracts to establish which components offers the best antioxidant activity.

REFERENCES

1. Jang IC, Jo EK, Bae MS, Lee HJ, Jeon GI, Park E. Antioxidant and antigenotoxic activities of different parts of persimmon (*Diospyros kaki* cv. Fuyu) fruit. J Med Plants Res 2010; 4:155-160.

2. Prior RL, Cao G. Antioxidant phytochemicals in fruits and vegetables: Diet and health implications. HortScience 2000; 35(4):589-592.
3. Othman A, Ismail A, Ghani AN, Adenan I. Antioxidant capacity and phenolic content of cocoa beans. Food Chem 2007; 100:1523-1530.
4. Balasundram N, Sundram K, Sammar S. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chem 2006; 68:191-203.
5. Salaheddin ME, Kader AA. Post-harvest physiology and storage behaviour of pomegranate fruits. Sci Hort 1984; 24:287-298.
6. Machado TB, Leal CR, Amaral AC, Santos KR, Silva MG, Kuster RM. Antimicrobial ellagitannin of *Punica granatum* fruits. J Braz Chem Soc 2002; 13:606-610.
7. Bhowmik D, Gopinath H, Kumar BP, Duraivel S, Aravind G, Kumar KPS. Medicinal uses of *Punica granatum* and its health benefits. J Pharmacog Phytochem 2013; 1(5):28-35.
8. Loren DJ, Seeram NP, Schulman RN, Holtzman DM. Maternal dietary supplementation with pomegranate juice is neuroprotective in an animal model of neonatal hypoxic-ischemic brain injury. Pediatr Res 2005; 57:864-858 .
9. González ME, Moreno DA, García-Viguera C. A new drink rich in healthy bioactives combining lemon and pomegranate juices. Food Chem 2009; 115:1364-1372.
10. Sultana B, Anwar F, Ashraf M. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. Molecules 2009; 14:2167-2180.
11. Mohammedi Z, Atik F. Impact of solvent extraction type on total polyphenols content and biological activity from *tamarix aphylla*. Int J Pharm Bio Sci 2011; 2:609-615.
12. Mohamed AA, Ali SI, El-Baz FK. Antioxidant and antibacterial activities of crude extracts and essential oils of *Syzygium cumini* leaves. PLoS One 2013; 8(4):e60269.
13. Zhou K, Yu L. Effects of extraction solvent on wheat bran antioxidant activity estimation. Lebensm Wiss Technol 2004; 37:717-721.
14. Kaur C, Kapoor HC. Antioxidant activity and total phenolic content of some Asian vegetable. Inter J Food Sci Technol 2002; 37:153-161.
15. Dewanto V, Wu X, Adom KK, Liu RH. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. J Agric Food Chem 2002; 50:3010-3014.
16. Martinez AE, Favret EA. Anthocyanin synthesis and lengthening in the first leaf of barley isogenic lines. Plant Sci 1990; 71:35-43.
17. Cheng GW, Breen PJ. Activity of phenylalanine ammonialyase (PAL) and concentrations of anthocyanins and phenolics in developing strawberry fruit. J Amer Soc Hort Sci 1991; 116:865-969.
18. Khalil MY, Moustafa AA, Naguib NY. Growth, phenolic compounds and antioxidant activity of some medicinal plants grown under organic farming condition. World Journal of Agricultural Sciences 2007; 3(4):451-457 .
19. Tenpe CR, Aman U, Amol B, Yeole YG. *In vitro* antioxidant and free radical scavenging activity of *Jasminum sambac* Linn. leaves. Phcog Mag 2008; 4(15):124-129.
20. Zhao GR, Xiang ZJ, Ye TY, Yuan YJ, Guo ZX. Antioxidant activities of *Salvia miltiorrhiza* and *Panax notoginseng*. Food Chem 2007; 99:767-774.
21. Elfalleh W, Hannachi H, Tlili N, Yahia Y, Nasri N, Ferchichi A. Total phenolic contents and antioxidant activities of pomegranate peel, seed, leaf and flower. J Med Plants Res 2012; 6:4724-4730.
22. Turkmen N, Sari F, Velioglu YS. Effect of extraction solvents on concentration and antioxidant activity of black and black mate polyphenols determined by ferrous tartrate and Folin-Ciocalteu methods. Food Chem 2006; 99:838-841.
23. Rabeta MS, Nur Faraniza R. Total phenolic content and ferric reducing antioxidant power of the leaves and fruits of *Garcinia atrovirdis* and *Cynometra cauliflora*. Int Food Res J 2013; 20(4):1691-1696.
24. Uddin R, Saha MR, Subhan N, Hossain H, Jahan IA, Akter R, Alam A. HPLC-analysis of polyphenolic compounds in *Gardenia jasminoides* and determination of antioxidant activity by using free radical scavenging assays. Adv Pharm Bull 2014; 4(3):273-281.
25. Naczk M, Shahidi F. Extraction and analysis of phenolics in food. J chromatogr A 2004; 1054(1):95-111.
26. Huang D, Ou B, Prior RL. The chemistry behind antioxidant capacity assays. J Agric Food Chem 2005; 53:1841-1856.
27. Kong JM, Chia LS, Goh NK, Chia TF, Brouillard R. Analysis and biological activities of anthocyanins. Phytochemistry 2003; 64(5):923-933.
28. Noda Y, Kaneyuki T, Mori A, Packer L. Antioxidant activities of pomegranate fruit extract and its anthocyanidins: delphinidin, cyanidin, and pelargonidin. J Agric Food Chem 2002; 50:166-171.
29. Rogez H, Pompeu DR, Akwie SNT, LaRondelle Y. Sigmoidal kinetics of anthocyanin accumulation during fruit ripening: A comparison between açai fruits (*Euterpe oleracea*) and other anthocyanin-rich fruits. J Food Compost Anal 2011; 24:796-800.
30. Varasteh F, Arzani K, Barzegar M, Zamani Z. Changes in anthocyanins in arils of chitosan-coated pomegranate (*Punica granatum* L. cv. Rabbab-e-Neyriz) fruit during cold storage. Food Chem 2012; 130:267-272.
31. Hou Z, Qin P, Zhang Y, Cui S, Ren G. Identification of anthocyanins isolated from black rice (*Oryza sativa* L.) and their degradation kinetics. Food Res Int 2013; 50:691-697.

32. Strack D, 1997. Phenolic metabolism. In: Dey, P.M., Harbourne, J.B. (Eds.), Plant Biochemistry. Academic Press, San Diego, CA, pp. 387-416.
33. Cai Y, Luo Q, Sun M, Corke H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci 2004; 74:2157-2184.
34. Hmid I, Elothmani D, Hanine H, Oukabli A, Mehinagic E. Comparative study of phenolic compounds and their antioxidant attributes of eighteen pomegranate (*Punica granatum* L.) cultivars grown in Morocco. Arab J Chem 2013; In press.
35. Middha SK, Usha T, Pande V. HPLC evaluation of phenolic profile, nutritive content, and antioxidant capacity of extracts obtained from *Punica granatum* fruit peel. Adv Pharmacol Sci 2013; ID 296236.
36. Mansour E, Ben Khaled A, Lachiheb B, Abid M, Bachar K, Ferchichi A. Phenolic compounds, antioxidant, and antibacterial activities of peel extract from Tunisian pomegranate. J Agr Sci Tech 2013; 15:1393-1403.
37. Palafox-Carlos H, Yahia EM, González-Aguilar GA. Identification and quantification of major phenolic compounds from mango (*Mangifera indica*, cv. Ataulfo) fruit by HPLC-DAD-MS/MS-ESI and their individual contribution to the antioxidant activity during ripening. Food Chem 2012; 135:105-111.
38. Hatipoglu G, Sökmen M, Bektas E, Daferera D, Sökmen A, Demir E, Sahin H. Automated and standard extraction of antioxidant phenolic compounds of *Hyssopus officinalis* L. ssp. angustifolius. Ind Crops Prod 2013; 43:427-433.
39. Aoudia H, Oomah BD, Zaidi F, Zaidi-Yahiaoui R, Drover JCG, Harrison JE. Phenolics, antioxidant and anti-inflammatory activities of *Melia azedarach* extracts. International Journal of Applied Research in Natural Products 2013; 6(2):19-29.
40. Augustyniak A, Bartosz G, Cipak A, Duburs G, Horakova L, Luczaj W, et al. Natural and synthetic antioxidants: an updated overview. Free Radic Res 2010; 44(10):1216-62.
41. Singh R, Murthy KC, Jayaprakasha J. Studies on the antioxidant activity of Pomegranate (*Punica granatum*) peel and seed extracts using *in vitro* models. J Agric Food Chem 2002; 50:81-86.
42. Ghasemzadeh A, Jaafar HZE, Rahmat A. Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (*Zingiber officinale* Roscoe). Molecules 2010; 15(6):4324-4333.
43. Terashima M, Kakuno Y, Kitano N, Matsuoka C, Murase M, Togo N, Watanabe R, Matsumura S. Antioxidant activity of flavonoids evaluated with myoglobin Method. Plant Cell Rep 2012; 31:291-298.
44. Reddy M, Gupta S, Jacob M, Khan S, Ferreira D. Antioxidant, antimalarial and antimicrobial activities of tannin-rich fractions, ellagitannins and phenolic acids from *Punica granatum* L. Planta Med 2007; 73:461-467.
45. Gil MI, Tomás-Barberán FA, Hess-Pierce B, Holcroft DM, Kader AA. Antioxidant activity of pomegranate juice and its relationship phenolic composition and processing. J Agric Food Chem 2000; 48:4581-4589.
46. Singh N, Rajini PS. Free radical scavenging activity of an aqueous extract of potato peel. Food Chem 2004; 85:611-616.
47. Guo C, Yang J, Wei J, Li Y, Xu J, Jiang Y. Antioxidant activities of peel, pulp and seed fractions of common fruits as determined by FRAP assay. Nutr Res 2003; 23(12):1719-1726.
48. Li Y, Guo C, Yang J, Wei J, Xu J, Cheng S. Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Food Chem 2006; 96 (2):254-260.