Phenolic Compounds and Antioxidant Activities of Pomegranate Peels

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***Abstract*—Methanol and aqueous extracts of pomegranate peels (Punica granatum L.) from two Egyptian varieties (Wardey and Manfalouty) were screened for phenolic compounds and antioxidant activities. Amounts of phenolic compounds in methanol peels extracts were higher than those in water extracts. The relative contents of phenolic compounds displayed variability as Manfalouty contained higher percent of phenolic compounds for protocatchoic acid, P-cumaric acid, chlorogenic acid, catechin, epicatechin, ellagic acid. However, Wardey peel contained higher percentage of phenolic compounds for vanillic acid, Caffeic acid and ferulic acid. Antioxidant activities of peel extracts by β-carotene-linoleate model system showed that inhibition values of methanol both peel extracts exhibited higher values than water extracts.**

***Index Terms*—pomegranate peels, methanol extract, water extract, phenolic compounds, antioxidant activities**

I. Introduction

Pomegranate (*Punica granatum* L.) has gained commercial importance in food and health industries due to increasing scientific evidence linking its consumption to better health outcomes [1], [2]. The peel makes up ~ 50% of the fruit [3]. Pomegranate marc is normally used as cattle feeds with low value or directly disposed in the field that could cause environmental problem. However, pomegranate marc could be a good raw material for producing natural antioxidants because of its high content of antioxidants [4].

Polyphenols have exert beneficial benefits [5], having free radical scavenging capacity and antioxidant ability [6]. Pomegranate Peel exhibited high antioxidant activity in various in vitro models [1]. The peel of the pomegranate has been extensively used in folk medicine [7].

Although several studies have shown that pomegranate peels proved to be important source of phenolic compounds, with several health benefits, its use remained very limited [8].

The aim of the present study was designed to determine individual phenolic compounds and evaluate the antioxidant potential of methanol and aqueous extracts of pomegranate peels for two Egyptian varieties, Manfalouty and Wardey.

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II. Materials and Methods

A. Materials

1) Plant materials

Pomegranate (*Punica granatum* L.) fruits namely, Manfalouty and Wardey varieties were obtained from Horticulture Department Farm, Horticulture Research Institute, Agricultural Research Center, Giza, Egypt. Fruits were manually peeled and the collected peels were rinsed with distilled water, cut into small sections and dried in oven at 40 ℃ for 48h, then ground by a mill, sieved to obtained particle size of 20 meshes and maintained in air-tight plastic bags in desiccators at room temperature.

B. Methods

1) Preparation of extracts

Five-gram of finely-powdered dried pomegranate peels of each varieties were separately extracted with methanol and cold water. Extract was obtained by shacking water bath pomegranate peel samples with either methanol (1:10, w/v) or distilled water at 25 ℃ at 20 rpm for 24h, followed by centrifugation at 5000g for 15 min and filtered through Whatman No.41 filter paper. Residue re-extracted twice and the extracts were evaporated to dryness at 40 ℃. Dried extracts were then re-dissolved in adequate amounts of either methanol or water to obtain a concentration of 50mg/ml of each, and then stored at 4 ℃ till used.

2) Gross chemical analysis

Moisture, ash, ether extract, and crude fiber were determined according to the methods in [9].

3) Determination of total phenolics

The concentration of phenolic compounds in the extracts was determined as described by [10] and results were expressed as tannic acids equivalents. The extracts (200ml) were dissolved in a mixture of methanol and water (6:4 v/v). Sample (0.2ml) was mixed with 0.1ml of tenfold diluted Folin-Ciocalteu reagents and 0.8ml of 7.5% sodium carbonate solution. After standing for 30 min at room temperature, the absorbance was measured at 765 nm using spectrophotometer.

4) Determination of phenolic compounds

HPLC as the preferred technique for both separation and quantification of phenolic compounds, purified

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phenolic were applied to an HPLC instrument utilizing a reversed phase C18 column (RP-C18), Photo Diode Array detector (PDA) and polar acidified organic acids [11]. Phenolic compounds of extracts were prepared according to [12]. Sample was washed with water, dried 55 ℃ and milled to give powder. Dried ground peels (15g) were placed in a Whatman cellulose thimble. Sample was washed with water, dried 55 ℃ and milled to give powder. Sample was extracted in a Soxhlet extraction system using methanol for four hours (degree boiled of methanol 64.4 ℃), then methanol was removed by rotary. The obtained extracted were kept at 4 ℃ until analysis [13].

The crude extract solutions obtained were filtered through Whatman No.41 filter paper for removal of peel particles, 3g of samples added to 10ml methanol (80%, v/v) and homogenize in ultra- sonic bath. The column temperature was maintained at 30 ℃ at 280nm, [14]. A constant flow rate of 1ml/min was used with two phase: (A) water containing 2.5% acetic acid, (B) methanol containing 2.5% acetic acid.

Simple polyphenols were identified by comparison of their retention times with standards. They were quantified by comparing the peak area against the standard curve for the reference solutions containing that compound. All samples calculation was done using the following equation:

$$CS = CS1 \text{ X } (PS/PS1)$$

where CS is the concentration of the sample, CS1 is the concentration of the standard, PS is peak area of sample and PS1 is the peak area of standard.

5) Antioxidant assay using β-carotene - linoleate model system

Antioxidant activity of pomegranate peel extracts for each variety was evaluated using β-carotene –linoleate model system described by [10]. 0.2mg of β-carotene in 0.2ml of chloroform, 20mg of linoleic acid and 200mg of Tween-40 (polyoxyethylene sorbitol palmitate) were mixed. Chloroform was removed at 40 ℃ under vacuum. The resulting mixture was dissolved with 10ml of distilled water and was mixed well for 1-2 minutes. To this solution, 40ml of oxygenated water was added. Four milliliter aliquots of the emulsion were pipetted into different test tube containing 0.2ml of extracts (50 and 100 ppm) and butylated hydroxyanisol (50 and 100 ppm) in ethanol. Butylated Hydroxyanisol (BHA) was used for comparative purpose. A control containing 0.2ml of ethanol and 4ml of the above emulsion was prepared. The tubes were placed at 50 ℃ in a water bath and the absorbance at 470nm was taken at zero time (t=0). Measurement of absorbance was continued till the color of β-carotene disappeared in the control tubes (t=120m) at an interval 15 min. A sample prepared as above without β-carotene served as blank.

The Antioxidant Activity (AA) of the extracts was calculated in terms of bleaching β-carotene using this following formula, AA = 100 {1- (A0-At)/A00 – A0t}, where A0 and A00 are the absorbance values measured at zero time of the incubation for test sample and control, respectively. At and A 0t are the absorbance measured in the test sample and control, respectively, under incubation for 120 min. The results were expressed on basis of preventing bleaching of β-carotene.

6) Statistical analysis

All measurements were taken in triplicate and expressed as means ± S.D. The statistical analysis was performed using SPSS for Windows (SPSS, Inc.). P values less than 0.05 was considered to be significant [15].

III. Results and Discussion

A. Gross Chemical Composition of Pomegranate Peels

The data given in Table I represented the chemical composition of the two studied pomegranate varieties peels (Wardey & Manfalouty).

TABLE I. Gross Chemical Composition of Pomegranate Peels (on Dry Weight Basis)

Varieties	Moisture %	Ash%	Crude oil%	Crude fiber %
Wardey	6.35	2.61	3.28	15.52
Manfalouty	7.71	2.59	2.22	12.03
$LSD_{0.05}$	0.02	0.02	0.01	0.01

The studied pomegranate varieties peels (Wardey & Manfalouty) contained 6.35 to 7.71% moisture, 3.28 and 2.22% crude oil, 2.61 to 2.24% ash and 15.52 and 12.03% crude fibers, respectively. Statistical analysis of data proved that all the above mentioned contents in the two varieties were significantly different ($p<0.05$). Higher contents of crude oil, ash, and crude fibers were found in Wardey peels than in Manfalouty.

The chemical analysis of pomegranate peels showed significant differences among varieties [16].

B. Extracted Yield and Total Phenolic Content (TPC)

The extracted yields of pomegranate peels using two extraction methods and total phenolic contents of pomegranate peels extracts from the two studied varieties, as determined by Folin-Ciocalteu method are shown in Table II.

TABLE II. Effect of Extraction Method on Yield and Total Phenolic Contents of Pomegranate Peels

Varity	Yield (% dry weight of pomegranate peels)	
	Methanol extract	Aqueous extract
Manfalouty	48.2	17.1
Wardey	33.3	12.5
	Total phenolic content mg(ETA)/100g extract	
Manfalouty	113.26	32.6
Wardey	74.94	16.2

The yield of methanol extract was 48.2% for Manfalouty peels and 33.3% for Wardey peels. While, the yield of water extracts of Manfalouty and Wardey peels were 17.1% and 12.5%, respectively.

The obtained results confirmed those in [17] demonstrating that total pomegranate peel extracted with methanol gave higher yield compared to water and attributed this to the polarity differences between solvent, and consequently the solubility of the solute into the solvent.

The amounts of phenolic compounds in methanol extracts from the pomegranate peels were higher than those of water peel extracts. The responses of the extracts might arise from the variety and/or quantity of phenolic found in the two different extracts of pomegranate peels. However, in case of Manfalouty peel extracts, both the water and methanol showed the larger amounts of phenolic contents than those of Wardey peel extracts as illustrated in Table II.

The variations in phenolic contents of the extracts might arise from the variety and/or quantity found in the different peel extracts. The higher total phenolic contents in both methanol and water extracts of Manfalouty peels might be ascribed to their higher extraction yield. The antioxidants have been mainly constituted by phenolic compounds and have strong potential in scavenging free radicals [18].

The extraction of phenolic compounds from the fruit was commonly achieved with methanol or aqueous methanol as previously mentioned in [19]. Indeed a suitable extraction procedure should be developed and improved to recover as many antioxidants as possible before an extract rich in natural antioxidants could be further explored for possible application in health promoting supplements in food industry [5].

The obtained results assured those reported in literature [20]-[22], reporting that the yield of extractable compounds from the peel of pomegranate in methanol extract was higher than in water extract.

C. Quantification of Individual Phenolic Compounds in Pomegranate Peel

The identified phenolic compounds in methanol pomegranate peels extract of Manfalouty and Wardey varieties, quantified using HPLC are presented in Table III.

As evident from the data in Table III that ellagic, chlorogenic acid, catechin, epicatechin, protocatchoic acid, vanillic acid, Caffeic acid, P-Cumaric acid and ferulic acid were the main phenolic constituents in the two varieties (Manfalouty and Wardey). However, the relative contents of these compounds displayed variability.

TABLE III. POLYPHENOLS COMPOSITION IN TWO SAMPLES BY HPLC QUANTIFICATION ANALYSIS (MG/100G)

Phenolic compounds	Manfalouty peels	Wardey peels
Protocatchoic acid	58	52.9
Vanillic acid	33	43
Caffeic acid	17	22
P-Cumaric acid	10	2.9
Chlorogenic acid	493	327
Ferulic acid	144	155
Catechin	50	27.7
Epicatechin	56	47.7
ellagic acid	12	11

Manfalouty peels contained higher percent of phenolic compounds for protocatchoic acid, *P*-cumaric acid, chlorogenic acid, catechin, epicatechin, ellagic acid. However, Wardey peel contained higher percentage of phenolic compounds for vanillic acid, Caffeic acid and ferulic acid. The contents of polyphenol compounds considerably varied with the variety of pomegranates [23]. Variations of the individual phenolic compounds content between different accessions of Tunisian pomegranate peels indicated that gallic acid, was the major phenolic compound, followed by ellagic acid, caffeic acid and P-coumaric acid, while vanillic acid and querecetin were present only in small quantities [24].

D. Antioxidant Activity

The antioxidant activity through β-carotene - linoleate model system of pomegranate fruit peel extracts at 50 and 100 μg/ml concentrations were compared with Butylated Hydroxyanisol (BHA) illustrated in Fig. 1.

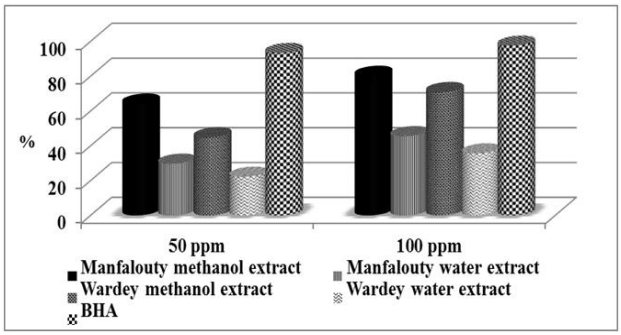

Figure 1. Antioxidant activities of pomegranate peel extracts (Manfalouty and Wardey) and BHA by β-carotene - linoleate model system (% inhibition of bleaching of β-carotene).

Pomegranate peel extracts prepared by different solvents exhibited various degree of antioxidant activity as measured using the β-carotene - linoleate model system (the bleaching of β-carotene in the absence of an antioxidant is a free mediated phenomenon resulting from linoleic acid). The inhibition values of both pomegranate peel water and methanol extracts, and the standard BHA increased with increasing concentration of the extracts. At 100 μg/ml concentrations, these values for water peel and methanol Manfalouty extracts reached 45.5 & 80.21%, and for those of Wardey extracts were 35.61 & 70.87%, respectively compared to that of BHA (97.5%). The methanol peel extracts of the two pomegranate varieties under study showed stronger antioxidant activities than water extracts. The fact that the pomegranate methanol extracted higher antioxidant activity than the water extracts might be explained by their higher total phenolic contents (Table II).

Both methanol peels extracts of the two varieties demonstrated similar effect on the inhibition of linoleic acid oxidation. But, the antioxidant activity of peel extracts of Manfalouty varieties was higher than that of Wardey variety. The obtained results assured those of [22] on the higher antioxidant activity of methanol as pomegranate peel extract demonstrated higher DPPH radical scavenging activity of pomegranate peel compared to water extract. Pomegranate peel extracts also showed varied antioxidant activity among Tunisian cultivars and between solvents (methanol and water) and was highly correlated with the total phenolics [24].

IV. Conclusion

Methanol extracts of the two varieties showed higher yields than those of water extractThe antioxidant activities of peel extracts of Manfalouty variety were higher than Wardey variety.

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